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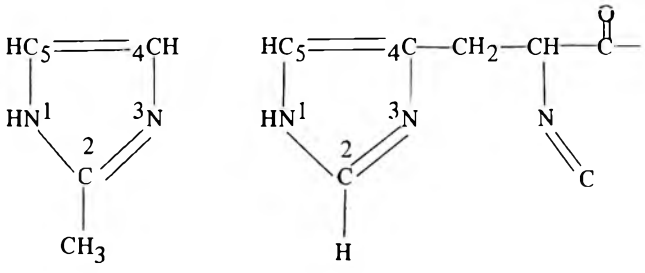
Letters

Cooked meat color

I would like to comment on a statement made by Professor G.G. Giddings in his authoritative review on "The basis of color in muscle foods" (Giddings, 1977). When discussing the nature of the cooked meat haemoprotein he states, "Since mere methylation places the imadazole group in the latter category (i.e. sterically hindered) it is unlikely that two denatured protein-bound imadazoles can occupy the two axial heme co-ordination sites." I would like to point out that the merely methylated compound referred to is 2-methyl imadazole (Wagner and Kassner, 1975) in which the methyl group is adjacent to the binding nitrogen atom while in a protein-bound imadazole group the substitution is removed from the binding nitrogen atom at position 1 although that at postition 3 is sterically hindered.



2-methyl imadazole protein-bound imadazole

Thus I do not believe that steric considerations make diimadazole complexes of ferric haematin unlikely pigments in cooked meat. This view is further substantiated by the fact that the haematin iron in some cytochromes (b and c_3) is co-ordinated to two protein-bound imadazole groups (Mathews et al., 1971; Dobson et al., 1974) and haemoglobin may form this type of complex on denaturation (Peisach and Blumberg, 1971).

–*David A. Ledward,* Food Science Labs., Dept. of Applied Biochemistry & Nutrition, University of Nottingham, Sutton Bonington, Loughborough, Leics., England. LE12 5RD.

Dr. Ledward's points are well taken and, I feel, further underscore the uncertainty surrounding the precise structure(s) of cooked meat pigment, which research such as his should eventually clarify. It would seem intuitively that steric constraints would play a far greater role in the case of denatured proteins than in that of methyl substitutions on 'free' imidazoles. Further, I have reservations as to the appropriateness of native specialized hemeproteins, with two intramolecular imidazoles as axial ligands according to a precise molecular design, being invoked as analogs of denatured protein diimidazole complexes. Steric considerations aside, I feel that the chemistry of the cooked meat pigment argues most strongly against the latter model in systems wherein the relatively heat resistant myoglobin is virtually completely 'denatured'.

–*G. G. Giddings,* Senior Technologist, Marine Products, Fundación Chile, Avda. Santa María 06500, Santiago, Chile, S.A.

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Peisach, J. and Blumberg, W.E. 1971. The specific compounds formed during the reversible and irreversible denaturation of haemoglobin and its constituent chains. Proc. 1st Inter-American Symp. Hemoglobins, Caracas, 1969. "Genetic, Functional, and Physical Studies of Hemoglobins," p. 199. Karger, Basel.

Wagner, G.C. and Kassner, R.J. 1975. Spectroscopic properties of ferrous heme complexes of sterically hindered ligands. Biochim. Biophys. Acta. 392: 319.

Proper containers, processing time and temperature critical for home canning

In reference to the paper by Fields et al. (1977), no evidence is presented to substantiate the authors' emphasis that the presence of viable microorganisms may have been accounted for by improper containers (p. 932). Since the authors determined the vacuum before jars were opened, it is important to know if these containers more frequently had faulty closures (not vacuum-sealed). The dimensions might also have been compared to those of the recommended containers to determine whether or not a difference in heating rate to the center would be probable. Additional evidence might have been gained by grouping the two container types and comparing microbiological recoveries for products processed the same time and method. Lacking this substantiating evidence, the problem appears to be underprocessing (i.e. not using recommended methods) or recontamination through use of the open kettle method.

I would agree with the authors that education of homemakers is important. However, it is also important that our recommedations are based on fact and that stress is put on the critical steps, processing time and temperature.

–*Margy Woodburn,* Dept. of Foods & Nutrition, Oregon State University, Corvallis OR 97331

This is in response to Dr. Woodburn's letter concerning the paper of Fields et al. (1977).

In her letter in the last paragraph, she stated that it is important that recommendations are based upon facts and stress is put on the critical steps, processing time and temperature. Of course this statement implies that the authors were

neither dealing with facts nor stressing processing conditions which is not true.

Our paper points out the great variation in time and temperature of processing (Table 3), the use of nonrecommended types of jars (Table 2), the existence of tomato samples with high pH values (Table 5) and the types of bacteria isolated (Table 7). All of these factors are important. In Dr. Woodburn's letter, she was referring to improper containers as those with faulty seal only (with no vacuum). Aside from faulty closures, the nonrecommended types of jars may not have the thermal stability that the recommended jars have, such that the home canners would have the tendency to underprocess the product to prevent breakage of the jars. Both of these characteristics are the ones we refer to in improper or nonrecommended types of jars.

Specific recommendations were given in conclusions, not on page 932. Some samples had no vacuum or little vacuum. The use of improper containers along with improper processing conditions are linked together on page 932. What we saw was a combination of both factors. There was no doubt that there was underprocessing. Underprocessing is stressed in the introduction to the paper.

I would hope that all Departments of Foods and Nutrition personnel would recommend only those jars and lids that are made for the purpose of canning. We feel that this is just as important as other steps in the canning process.

—*Marion L. Fields.* Dept. of Food Science & Nutrition College of Agriculture, 1—74 Agriculture Building, Columbia MO 65201

REFERENCE

Fields, M.L., Zamora, A.F. and Bradsher, M. 1977. Microbiological analysis of home-canned tomatoes and green beans. J. Food Sci. 42: 931—934.

On the calculation of viscoelastic constants from relaxation curves

In recent years a great deal of interest in food rheology has been developed especially because of the latter implications in texture evaluation. In solid foods, the viscoelastic behavior has frequently been characterized by rheological constants derived from relaxation tests. Regardless of how the data have been presented (in terms of forces, stresses or moduli) the source of information has been fitting experimental relaxation curves by equation of the kind (Mohsenin, 1970):

$$F(t) = \sum_{i=1}^{n} a_i \exp(-b_i t) \qquad (1)$$

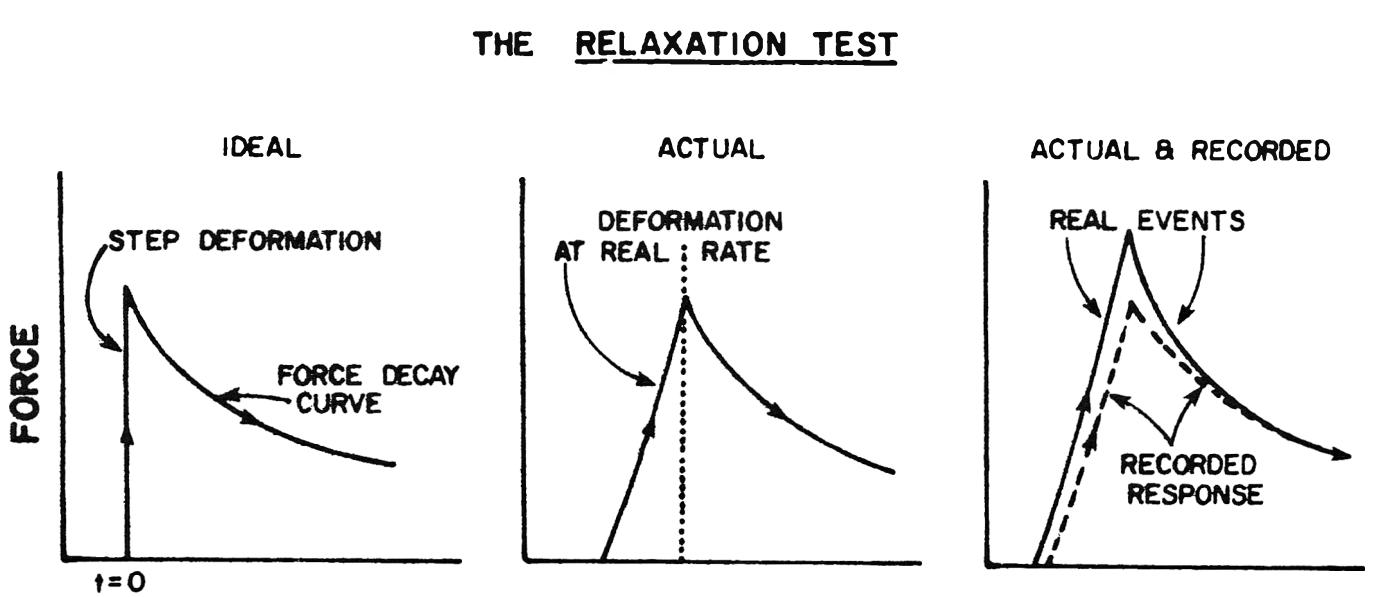
where F(t) is the decaying force level at time t and a_i and b_i the characteristic constants derived from a Maxwellian model.

One of the properties of Eq (1) is that it can practically fit any kind of smoothly descending curve of the type encountered in relaxation tests. Provided there is a sufficient number of terms (normally 2—4 are sufficient) the fit will have a high statistical correlation coefficient also. The latter may tempt to arrive at the erroneous conclusion that the constants so calculated are a true representation of the material's viscoelastic properties. This applies to both the number of terms and the magnitude of the constants.

The actual relaxation test is done by imposing a strain on the specimen (usually by a kind of universal testing machine) and recording the force decay with time. Though in real food materials a considerable stress relaxation is expected to have already occurred during the deformation state (Shama and Sherman, 1973), the initial stages of the tests are frequently characterized by a very steep and rapid decay of the force (Morrow and Mohsenin, 1966; Mohsenin, 1970; Peleg and Calzada, 1976).

Under such circumstances, the recorder time response and the mechanical deceleration of the crosshead become significant factors as pointed out and discussed by Voisey and Kloek (1975).

Since many common universal testing machines are equipped with relatively slow recorders (full scale time of about 1 sec), the initial part of the recorded curve is a destorted representation of the real relaxation (see figure). (When the deformation itself is done at a high crosshead speed, uncertainty about the correct initial force level is also added.)



Fitting such an experimental curve may result in terms that are significantly influenced by instrumental artifacts.

It should also be added that food materials may have a nonlinear rheology and memory (Mohsenin, 1970; Shama and Sherman, 1973; Clark et al., 1971). This implies that the constants obtained by relaxation tests depend by definition on the deformation history and the strain level. As a result, any numerical values of viscoelastic constants determined by fitting Eq (1) to a single relaxation curve should be looked upon with caution because they are not independent physical constants of the material. In other words, even if they were determined in a proper experimental technique they will vary with the test conditions and would not be an appropriate basis for quantitative prediction of rheological behavior.

—*M. Peleg*, Dept. of Food & Agricultural Engineering, University of Massachusetts, Amherst, MA 01003

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Voisey, P.W. and Kloek, M. 1975. Instron recorder pen response. J. Texture Studies 6: 379.

Thermal Processing Calculations

I have read through the series of papers on thermal processing recently published in the Journal of Food Science (Lenz and Lund, 1977a, b, c) with great interest. The papers show an excellent way for combined applications of principles for heat conduction as well as for chemical reaction kinetics and findings discussed in the paper give much needed refinements in the mathematical evaluations of heat sterilization processes. However, as one of the researchers whose published works were cited, I would like to make the following clarifications on some statements given in the papers.

Accreditation for application of a 12-point numerical integration formula

I greatly appreciated accreditation to our published works on page 998 of the paper, The lethality-Fourier number method: Experimental verification of a model for calculating average quality factor retention in conduction-heating canned foods (Lenz and Lund, 1977b). However, it was stated erroneously in the paper that a mass average sterilizing value was estimated by the above named integration formula by Timbers and Hayakawa (1967) and by Hayakawa (1969). The first of these two citations should either be deleted or revised to Hayakawa (1976) because of the following reason. If one closely examines pages 17 through 24 of Food Technology 21(8), one easily observes that there are expanded abstracts of two papers printed on these pages:

Part 1. Comparison of existing procedures (Timbers and Hayakawa, 1967)
Part 2. Development of a new method (Hayakawa, 1969)

Since the use of the numerical integration formula is presented only in Part 2, even though the presentation is fairly brief, proper citation should refer to this part.

Classification and general characteristics of published procedures for thermal process evaluation

Table 1 of another paper, The lethality-Fourier number method: Experimental verification of a model for calculating temperature profiles and lethality in conduction-heating canned foods (Lenz and Lund, 1977a), shows an interesting way of classifying published procedures for thermal process evaluations. It is quite unfortunate that this classification is based on somewhat superficial evaluations of the procedures. According to a generally accepted definition, formula methods are those which have built-in means for predicting time-temperature relationships of foods undergoing heat processes. Therefore, all methods except general methods, which are listed in Table 1, are formula methods. To develop these methods, higher empirical or theoretical formulas were used for estimating food temperatures. Therefore, the following classification is more proper compared with the one shown in your paper.

General method
Formula method
- Theoretical – Flambert and Deltour
  Teixeira et al.
  Stumbo (mass average)
- Empirical – Ball and Olson
  Stumbo (critical point)
  Herndon et al.
  Hayakawa

In my opinion, formula methods based on the application of empirical formulas are much easier and more flexible to utilize for thermal process evaluation in comparison with the theoretical formula methods. The empirical formula methods are applicable to almost any types of foods and to any shapes of containers because there were almost no restrictive assumptions imposed for the development of the empirical formulas. However, as you know quite well, the theoretical heat conduction equations, on which most theoretical formula methods were based, were derived analytically or numerically by imposing several restrictive assumptions on the thermophysical properties of food, shape of food, and boundary conditions applicable to food surface. Therefore, there are great limitations in the applications of those procedures based on the use of the heat conduction equations.

As you might be aware of, most empirical formula methods are simple to use and are applicable to the estimation of a variety of situations which include problems with a broken heating curve, divided process, liquid food, solid-liquid mixture, and noncylindrical food containers.

The simple analysis of a theoretical formula, which has been frequently used for estimating food temperature during the heating phase of a thermal process, shows that the intercept coefficient, j value, of the heating curve determined by this formula is approximately equal to 2.04 although there are slight deviations from this value dependent upon the selection of a linear line portion on the curve. This signifies that most theoretical formula methods do not estimate accurately the lethal effect of thermal processes when j values deviate considerably from 2.04.

Incidentally, major existing procedures for process evaluation are examined in detail in my review paper (Hayakawa, 1977) presented at the 37th Annual Meeting of IFT, Philadelphia, PA.

Others

It was attributed on p. 991 that observed variations in food temperatures determined experimentally were caused solely by temperature measuring devices and by biological variability of raw materials. However, in my opinion, there should be the following sources of variations in the experimental data in addition to the above named two sources: blending operation, formulation of samples, weight of sample per one can, and operation of retort. Even though samples were prepared carefully, it would be almost impossible to eliminate variations

caused by these additional factors unless a proper statistical design of experiments was utilized.

–*Kan-ichi Hayakawa*, Dept. of Food Science, Cook College, Rutgers–The State University of New Jersey, P.O. Box 231, New Brunswick, NJ 08903

Thank you for your letter commenting on the series of papers which appeared in the Journal of Food Science (Lenz and Lund, 1977a, b, c). I would like to take this opportunity to respond to your comments.

First, with regard to the citation attributing the 12-point integration procedure (p. 998) to Timbers and Hayakawa (1967), you are quite correct. That citation should be:

> Hawakawa, K.I. 1967. Mass average sterilizing value for thermal process. Part 2. Development of a new method. Food Technol. 21(8): 21.

Second, with regard to the classification of the methods for calculating the sterilizing value of thermal processes. we were not attempting to classify the various procedures. When we refer to *the* Formula Method (with a capital F), the general inference is to Ball's development. You are quite right that the remaining methods can then be classified into empirical and theoretical heat transfer considerations.

Your point about the utility of empirical methods and the less restrictive assumptions is well taken but we have shown in the paper that the theoretical formula predicts temperatures to within the accuracy of replication of actual experimental data. This is the only real criterion which a method must meet. Certainly the empirical method will continue to be the method of choice simply because of simplicity of use and applicability to conduction and convection heating. With regard to j_h values, the fact that the value is theoretically 2.04 and in reality is often markedly different from that value does not eliminate the theoretical conduction equation from consideration. The variation in thermal diffusivity and thermocouple location lead to variability in determination of j_h from actual heat penetration data. Again, the important criterion is whether the method predicts the temperature within the variability actually experienced in measurement. In this case, we have shown this to be the situation and conclude that the theoretical formula is applicable.

Third, you are quite right in pointing out that there are several additional variables which influence variability associated with heat penetration measurements. We lumped all of those variables into what we call "biological variability." This was perhaps a poor choice of nomenclature since this variability does indeed include that attributed to product formulation, filling procedure and canning operation. This, however, does not affect the outcome of the study or its applicability.

–*Daryl Lund*, Dept of Food Science, Babcock Hall, 1605 Linden Drive, Madison, WI 53706

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Hayakawa, K. 1977. Critical review on methods for the quantitative evaluation of heat processes. (Abstract #293) Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA June 5–8, 1977.

Lenz, M.K. and Lund, D.B. 1977a. The lethality-Fourier number method: Experimental verification of a model for calculating temperature profiles and lethality in conduction-heating canned foods. J. Food Sci. 42: 989.

Lenz, M.K. and Lund, D.B. 1977b. The lethality-Fourier number method: Experimental verification of a model for calculating average quality factor retention in conduction-heating canned foods. J. Food Sci. 42: 997.

Lenz, M.K. and Lund, D.B. 1977c. The lethality-Fourier number method: Confidence intervals for calculated lethality and mass-average retention of conduciton-heating, canned foods. J. Food Sci. 42: 1022.

Timbers, G.E. and Hayakawa, K. 1967. Mass average sterilizing value for thermal process. Food Technol. 21: 17.

Authors retract reference to Canadian grading system

Because of criticisms raised by Drs. W.R. Usborne and H.T. Fredeen and Mr. W.R. Jennings, we wish to retract all references to the Canadian grading system published in our recent paper "Prediction of beef quality by three grading systems," by J.L. Garcia-de-Siles, J.H. Ziegler and L.L. Wilson (*J. Food Sci.* 42: 711–715, 1977). The systems included were the previous USDA (1965), the revised USDA (1975) and the Canadian standards imposed in 1972.

The major problem encountered resulted from our attempt to impose the carcass muscling descriptions found in the Canadian standards on carcasses which were part of a 9-yr study at Penn State. In personal communication with Dr. Usborne at the Reciprocal Meats Conference, 1974, Dr. Jose Garcia-de-Siles understood that it would be permissible to use an absolute M. longissimus area in assigning Canadian grades to carcasses for which we had only data and which were not available for subjective application of the developed degree of muscling as required in the Canadian guidelines. Dr. Garcia-de-Siles' understanding was that a minimum 71.0 cm^2 M. longissimus area for the weights of cattle included in our study was compatible with the official Canadian regulations considering that both grades A and B require "muscling that has no marked deficiency, as evidenced by a medium to large Longissimus dorsi muscle relative to the carcass weight . . ." [Re: Canada Department of Agriculture, 1972. Beef carcass grading resulations. Canada Gazette Part 2, 106(18): 1685.] Since the analyses we performed were done by computer, it required some mathematical instructions to apply the Canadian system. It was "assumed" to be logical that a meat-type cattle with a medium Longissimus dorsi muscle would have a minimum area of 12.9 cm^2 (2.0 $in.^2$ per hundred weight of carcass) or at least it would have a M. longissimus area as large as the mean for the population being studied. The mean for all cattle carcasses in our study was 71.1 cm^2.

Technically our use of the existing Canadian grading standards in the paper was in error. However, we feel confident that the results reported for the categories labeled "Canadian grades" (Table 5 and 6) were accurate using those standards which we applied. This does mean, however, since the entire consideration of muscling is based on a subjective evaluation of "marked deficiencies," the Canadian system cannot be applied to sets of data which have been accumulated prior to its introduction or in situations in which carcasses are not graded in accordance with the specific provisions of the Canadian standards. However, to apply the Canadian grading system to carcasses over a period of time to determine the relative contributions of any characteristic measured or subjectively evaluated and degree of muscling, a scoring system would have to be developed for "marked deficiencies" in musculature.

Also, in this study, we employed our Meats Laboratory quality scoring system in order to categorize texture, firmness, lean color and fat color. Although we did not use the precise terminology of the Canadian standards, we did feel perfectly capable of equating our "cherry red" with the Canadian "bright red colour" and our "casty" with a fat "slightly tinged with reddish or amber colour." Perhaps in this instance too we have erred because of significant differences in the cattle populations of the two countries involved of which we are not aware.

Conformation as reported in Tables 5 and 6 was in no way used to determine the Canadian grade to which a carcass was assigned. Conformation for each carcass was determined as described in the 1965 USDA standards. The results were reported for informational purposes only. From our paper one can readily observe that carcasses scoring lower in conformation were found in the C_1 and C_2 grades which gives credence

to the contention that the carcasses in those grades were inferior in muscle development.

We apologize for any confusion we may have caused by imposing the minimum restriction of 71.0 cm^2 M. longissimus dorsi on Canadian grades A and B. However, we feel that the data reported are unique with regards to depth and thoroughness of the information obtained on cattle of known breeding, feeding and intensive management background, and we believe that our study still provides useful information. As a result of the intensive management program, these cattle and their resulting carcasses should not be expected to yield the same distribution of carcass grades in either the U.S. or Canada as would result from grading total commercial slaughter.

In addition, we heartily encourage other researchers, both in Canada and the U.S., to develop objective measurements of muscling so that grading standards can be applied to existing sets of data rather than to include additional subjective evaluations which provide no factual basis for study and analysis.

—*J.L. Garcia-de-Siles, J.H. Ziegler* and *L.L. Wilson*, Dept. of Food Science, The Pennsylvania State University, 15 Meats Laboratory, University Park, PA 16802

ERRATUM NOTICE

J. Food Sci. 42(5): 1153–1155 (1977). Processing effect on proximate composition and mineral content of meats of blue mussels (Mytilus edulis) by B.M. Slabyj and P.N. Carpenter. On page 1154, the footnote to Table 1 was inadvertently omitted. Should read: [a]Average of nine determinations except dry weight with six determinations. Numbers in parentheses indicate standard deviation. Please change accordingly.

W. E. TOWNSEND, A. A. KLOSE and B.G. LYON
Animal Products Utilization & Marketing Research Lab.
USDA Richard B. Russell Agricultural Research Center, ARS
P.O. BOX 5677, Athens, GA 30604

CHEMICAL AND SENSORY CHANGES IN FREEZE-DRIED CHICKEN AND PORK DURING HIGH TEMPERATURE, OXYGEN-FREE STORAGE

ABSTRACT

Chemical and sensory changes were determined in cooked diced chicken, and raw and cooked sliced pork that were freeze dried to residual moisture levels of less than 2% and stored in sealed tin cans under nitrogen at −40, 28, 38 and 48°C for 1, 3 and 6 months. Orsat analysis of headspace gas confirmed the absence of oxygen and no carbon dioxide developed during storage. Level of glucose in general decreased with increasing temperature and time of storage. Lactic acid content was 50 to 100-fold that of glucose on a molal basis and exhibited no detectable change over 6 months at temperatures up to 48°C. No appreciable change in the level of ϵ-amino nitrogen was apparent. Reducing substances, expressed as ascorbic acid, increased with temperature and time of storage. There were no marked changes in percent rehydration due to storage temperature and time for cooked diced chicken, and raw and cooked sliced pork. Sensory evaluation, by triangle tests, of the differences between control samples at −40°C and samples at 28, 38 and 48°C detected deterioration in cooked chicken at 48°C for 1 month and thereafter; and at 38°C for 3 months and thereafter. For raw pork, significant deterioration was detected at 38 and 48°C at 3 months and thereafter. For cooked pork significant deterioration was detected at all storage times and temperatures, compared to the control.

INTRODUCTION

MANY FREEZE-DRIED FOODSTUFFS are available to the Armed Services, and/or civilian consumers. Complete meals can now be served by use of dehydrated foods only. However, despite all of the advantages of freeze drying (reduction in weight, inhibition of bacterial growth, savings in storage and transportation), and the fact that freeze-dried meat products initially are of high quality (Anglemier et al., 1960), they may deteriorate during storage, and rehydration and texture are often inadequate.

Numerous publications (Regier and Tappel, 1956a; Harper and Tappel, 1957; Tappel et al., 1957; Ballantyne et al., 1958) have described the effects of freeze-drying of meat and poultry on quality and stability. Tappel et al. (1957) reported that precooked freeze-dried poultry rehydrated poorly, was tougher and had a drier texture than the control meat. Similar results were reported by Bele et al. (1966) who studied the effect of freeze-drying on the quality of cooked and raw breast and thigh chicken meat.

According to Tuomy and Felder (1964), freeze-dried cooked sliced pork had been considered for inclusion in military operational rations, but its use was questioned because of toughness and lack of a typical cooked pork flavor. They also reported that both cooked and raw freeze-dried pork were sensitive to browning. Sharp (1957a) stated that browning was due to the Maillard reaction, involving reducing sugars, protein, and amino acid constituents. Thomson et al. (1962) stated that browning reactions can account for all of the undesirable characteristics, except rancidity, observed during the storage of freeze-dried meats.

Freeze-dried meat and poultry with improved storage stability and acceptability after rehydration and cooking are needed by the military and would have many civilian uses. As a step toward achieving such products, our research aimed to further define and correlate the major physical, chemical, and sensory changes that occur during the storage of precooked, diced freeze-dried chicken, and raw and precooked sliced freeze-dried pork under conditions of very low oxygen and moisture.

EXPERIMENTAL

Preparation and freeze drying of product

Chicken. Freshly slaughtered commercial whole broiler breasts packed in ice were purchased from a local poultry plant. Breasts were cooked in 88°C water to an internal temperature of about 77°C, chilled in ice overnight, deboned, tempered to about −3°C and diced to 1.25 × 1.25 × 0.9 cm size with the largest surface area perpendicular to the direction of the fibers.

Raw and cooked pork. Fresh bone-in loins were purchased from a local meat packing plant and returned to the laboratory for further processing. The longissimus muscle was trimmed of excess fat, tempered to −3°C and sliced in 1 cm thick slices perpendicular to fiber direction. For cooked pork, the trimmed longissimus muscle was stuffed in a moisture proof fibrous casing, cooked in 88°C water to an internal temperature of 77°C, chilled overnight at 2.0°C, and sliced in 3 mm thick slices perpendicular to fiber direction.

Initial moisture contents were determined on representative samples of the three types of products. All products were placed on stainless steel trays and blast frozen at −40°C.

Two lots of each product (22–27 kg) were freeze dried in a Vac-U-Dyne Pilot Freeze-Dryer (Model VPFD-CX) operating at a pressure of less than 600 microns. Heat was applied to the dryer trays by direct platen contact, with the platen temperature maintained at 47°C throughout the sublimation process. Drying times of 40–44 hr were employed to insure products with less than 2% moisture throughout the piece. Upon completion of drying, the chamber vacuum was released with high purity nitrogen and samples of dried product were quickly removed for determination of residual moisture.

Packaging and storage

A standard amount (480g diced chicken; 520g of raw or cooked pork) of the dried product from each lot was weighed into No. 10 tin cans. Cans were sealed with a Rooney Semi-automatic Can Sealer after evacuation and flushing three times with high purity nitrogen. When it was found that the action of the can sealer did not insure absence of oxygen, the cans were punctured, placed in a desiccator, evacuated and flushed with nitrogen three times. Cans under slight positive pressure to prevent re-entry of air were resealed with solder and examined for leaks by submerging the cans in water under vacuum. The cans were randomly divided into groups for storage at three temperatures: 28°C, 38°C, 48°C and −40°C for controls. A randomly selected can from each lot and each storage temperature was opened for analysis after 1, 3 and 6 months of storage.

Analytical methods

All chemical analyses and physical measurements were performed in duplicate or triplicate. Moisture content of raw, cooked, and freeze-dried product was determined by standard AOAC methods (1965).

Glucose was extracted from the freeze-dried product by the procedure of Henrickson et al. (1955), and the content of glucose determined by a modification of the glucose-oxidase method of Fleming and Pegler (1963).

Lactic acid was determined by a method (Sigma Technical Bulletin No. 826-UV, Sigma Chemical Company, 1974) based on the enzymatic oxidation of lactic acid to pyruvic acid mediated by NAD and LDH.

Table 1—Glucose content (mg/100g dry product) of freeze-dried chicken and pork stored for 1, 3 and 6 months[a]

Product	Storage temperature and time											
	−40°C (Control)			28°C			38°C			48°C		
	1-mo	3-mo	6-mo	1-mo	3-mo	6-mo	1-mo	3-mo	6-mo	1-mo	3-mo	6-mo
Cooked diced chicken												
Lot I	25	40	34	37	21	12	16	5	3	6	1	0
Lot II	43	46	50	50	19	21	17	10	4	6	1	0
Raw sliced pork												
Lot I	51	49	60	45	53	29	37	–	6	6	–	7
Lot II	50	38	60	43	31	23	43	–	15	16	–	5
Cooked sliced pork												
Lot I	80	61	78	78	81	81	48	71	61	17	28	3
Lot II	90	78	76	74	31	66	30	13	15	5	7	3

[a] Values are means of three determinations from three 5-g samples. Significant effects, as determined by analysis of variance were for chicken: Temperature, Time, Lot, Temp X Time, all $P < 0.01$; and for cooked pork: Temperature $P < 0.01$, Lot $P < 0.01$, and Temp X Lot $P < 0.05$. Data for raw pork were not analyzed, because of missing data.

Content of ϵ-amino nitrogen groups was determined by the trinitrobenzene sulfonic acid (TNBS) method for available lysine (Kakade and Liener, 1969).

Reducing substances, expressed as ascorbic acid, were determined by a modification of the 2,6-dichlorophenol-indophenol method of Loeffler and Ponting (1942).

Headspace gas of each can was analyzed just prior to opening the cans of stored product for sensory evaluation. Samples were taken and pressures were determined with a Beckman Headspace Sampler. Oxygen and carbon dioxide contents of headspace gas were determined by standard Orsat procedures, which involve measurement of the volume percent absorbed by sodium hydroxide (CO_2) and by alkaline pyrogallate (O_2).

Rehydration and preparation of samples for sensory evaluation

Samples were rehydrated according to Military specifications. About 750 ml of distilled water was brought to a boil, 100g dry freeze-dried chicken added, and heat provided for continuous gentle boiling. After 20 min, water was poured off and chicken allowed to drain and cool for 5 min before weighing. About 18g of the rehydrated samples was used as an individual portion for sensory tests.

Slices of raw pork were rehydrated in water (28–38°C) for 20–25 min, drained, weighed, and grilled 2 min on each side in electric skillets set at 193°C. Cooking oil (100 ml) was used to coat the skillets. Slices were cut into pieces about 2.5 cm square. A test portion consisted of 2 or 3 squares.

Slices of cooked freeze-dried pork were rehydrated in water at 94–100°C for 20–25 min, turning once after 10 min. Slices were cut into pieces about 2.5 cm square. A test portion consisted of two or three squares.

Percent rehydration (gross rehydrated weight as a percentage of the gross initial weight before freeze drying) was calculated from the data obtained during reconstitution of the product for sensory evaluation and from moisture contents before and after freeze drying. Calculations followed the formula used by Osman and Morse (1960), which included a correction for the moisture content of the freeze-dried product.

Sensory evaluation

The triangle test (Amerine et al., 1965), used with an option for the panelists to indicate the basis for their selection, was used to determine whether the freeze-dried products stored at three temperatures differed from the control sample stored at −40°C. Ten trained and screened panelists evaluated the test products after 1, 3 and 6 months storage, with two sets of samples at each session, and 2 sessions each day (4 reps.).

For the 1 and 3-month storage samples, triangle tests combined with a descriptive term (appearance, texture, juiciness, etc.) were used as a basis for selection and characterization of the odd sample. For the 6-month storage samples, triangle tests combined with scoring of seven parameters were used to evaluate effects of storage temperature and time. For each set of samples, panelists selected the odd sample and then scored the odd sample and same samples for tenderness, mealiness, fibrousness, wetness, darkness, off-flavor, and desirability, on an intensity scale from 0 (none or not applicable) to 4 (extremely). Scoring data were recorded and then analyzed by the "t" test for paired observations only for the panelists who correctly identified the odd-sample, since the scores by panelists who failed to distinguish the odd-sample were obviously ambiguous and spurious. Triangle test data were interpreted from the tables of Roessler (Amerine et al., 1965).

RESULTS & DISCUSSION

Chemical changes

Within the accuracy of the Orsat apparatus (≤0.2%), no carbon dioxide or oxygen could be detected in the headspace of any of the cans of product for any storage temperatures and storage times. The zero oxygen level in the headspace confirmed the adequacy of nitrogen packing and can tightness. The lack of carbon dioxide production in this study contrasts with the results of Sharp (1957a) who noted production of carbon dioxide in freeze-dried precooked pork stored in nitrogen at high (60%) relative humidities, and as reported by Hodge (1953) in a review of browning reactions in model systems. The lack of carbon dioxide production may have been due to the low residual moisture content or to other conditions that inhibited the Strecker degradation, i.e., conversion of α-amino acids to aldehydes containing one less carbon, with the liberation of carbon dioxide.

Before freeze-drying, cooked chicken contained 73%, raw pork 70%, and cooked pork 63% moisture. The mean residual moisture content of the six lots of freeze-dried product (cooked chicken, raw pork, cooked pork), immediately after removal from the freeze dryer, was 0.75% and ranged from 0.57–0.92. The mean moisture content of all products taken from cans over the 6 month period was 1.09%, with a standard deviation of 0.29. This small increase could reflect absorption of atmospheric moisture in transfer from drier to can.

Substantial, increasing losses of glucose (Table 1) occurred with increasing temperatures and time of storage for cooked chicken, raw and cooked pork, with little or none remaining after 6 months at 48°C. Sharp (1957a) reported that the initial concentration of free fermentable sugar in samples of dehydrated cooked pork, equilibrated at 60% RH, was 0.26% of which 0.18% was glucose. After 60 days at 37 and 50°C, no free sugar could be detected in the meat.

The rates of loss of glucose in the freeze-dried chicken over the 6-month storage period were used to calculate the temperature coefficient and corresponding energy of activation. The temperature coefficient Q_{10} was 2.8, equivalent to an energy of activation of about 20 Kcal/mole and agrees well with the results of Regier and Tappel (1956b) who reported an appar-

Table 2—Reducing substances, as μg equivalent ascorbic acid per gram dry product of freeze-dried, chicken and pork stored for 1, 3 and 6 months[a]

	Storage temperature and time											
	−40°C (Control)			28°C			38°C			48°C		
Product	1-mo	3-mo	6-mo	1-mo	3-mo	6-mo	1-mo	3-mo	6-mo	1-mo	3-mo	6-mo
Cooked diced chicken												
Lot I	–	11	28	14	29	44	35	46	58	39	67	71
Lot II	11	18	30	18	–	52	41	59	71	51	77	103
Raw sliced pork												
Lot I	0	2	0	0	2	0	0	23	53	11	17	139
Lot II	0	0	0	0	3	2	0	25	52	11	138	128
Cooked s iced pork												
Lot I	0	1	5	0	–	15	5	14	83	12	95	117
Lot II	0	0	2	0	0	7	9	1	42	7	41	68

[a] Values are means of six determinations. Significant effects, as determined by analysis of variance were for chicken: Temperature, Time and Lot $P < 0.01$; for raw pork: Temperature $P < 0.01$, Time $P < 0.05$; for cooked pork: Temperature $P < 0.01$, Time $P < 0.01$, Lot $P < 0.05$, Temp X Time $P < 0.01$.

ent activation energy of 25 Kcal/mole for the deterioration of freeze-dried beef during storage at several temperatures.

For cooked chicken, all main effects (temperature, time, lot) were highly significant ($P < 0.01$), and interactions were also significant. The important effects were temperature and the temperature x time interaction. Results were similar for cooked pork, but the influence of time was relatively less than that for chicken. Data for raw pork were not analyzed because of missing data.

Lactic acid content of freeze-dried samples was essentially the same for all temperatures and times of storage. Overall means, in mg lactic acid per gram of dry product, were 22.7 for cooked chicken, 28.8 for raw pork, and 21.4 for cooked pork. There was a significant ($P < 0.01$) difference between the two lots of chicken, 20.3 vs 25.2. The absence of storage related change in lactic acid content does not rule out its participation in deteriorative reactions, because the molal content of lactic acid was about 100 times the initial molal content of glucose (estimated from 1 month, −40°C values), and small changes, e.g., less than 1%, in lactic acid would not be detected by the analytical method. The question of lactic acid involvement has been raised by Lewis et al. (1949) who reported that brown pigments formed from interaction of glucose and nitrogen free hydroxy acids, and by Sharp (1957a) who found that additions of lactic acid to a mixture of insoluble pork protein and glucose did not affect browning.

The ϵ-amino nitrogen content was from 100–200 times the initial glucose content on a molal basis, and displayed no statistically significant differences due to lot, or to temperature and time of storage. The overall mean ϵ-amino nitrogen contents under all conditions were, in millimoles per gram dry weight, 0.48 for cooked chicken, 0.45 for raw pork, and 0.42 for cooked pork.

Changes in the content of reducing substances, expressed as μg equivalents of ascorbic acid, a known reductone, are shown in Table 2. For freeze-dried chicken, level of reducing substances increased significantly ($P < 0.01$) as a function of storage temperature and time, and also differed significantly between lots. For raw freeze-dried pork slices, there were significant differences between temperatures ($P < 0.01$) and between times ($P < 0.05$). For cooked pork, differences were significant between temperatures ($P < 0.01$), times ($P < 0.01$), and lots ($P < 0.05$); temperature by time interaction also was significant ($P < 0.01$). The maximum molal content of reducing substances formed corresponds to 15–30% of initial molal glucose content. The development of reductone-like reducing substances is a well known characteristic of sugar-amine browning reactions (Stadtman, 1948).

Color changes

Panelists easily detected darkening in the 48°C samples of cooked chicken and cooked pork compared to the −40°C (Control) samples after 1 month. Darkening was detected in the 38°C samples of all three products after 3 months, and in

Table 3—Triangle tests, correct judgments over total judgments[a]

	Storage temperature and time								
	−40°C vs 28°C			−40°C vs 38°C			−40°C vs 48°C		
Product	1-mo	3-mo	6-mo	1-mo	3-mo	6-mo	1-mo	3-mo	6-mo
Cooked diced chicken	12/32	17*/32	13/36	14/32	17*/32	31***/34	17*/32	29***/32	34***/34
Raw sliced pork	17*/34	15/32	18*/36	15/32	23***/32	21**/36	15/32	28***/32	35***/36
Cooked s iced pork	18**/32	18**/32	16/36	16*/32	24***/32	30***/36	19**/32	28***/32	33***/34

[a] Levels of Significance: *5%, **1%, ***0.1%

Table 4—Mean scores and differences, and significance of differences between paired scores for freeze-dried cooked chicken after 6 months of storage[a]

Temperature effect	Sensory parameter						
	Tenderness	Mealiness	Fibrousness	Wetness	Darkness	Off-flavor	Desirability
28°C	2.38	1.62	1.15	2.08	1.08	0.46	2.23
−40°C	2.54	1.69	1.31	2.15	0.92	0.54	1.92
Difference	−0.16	−0.07	−0.16	−0.07	+0.16	−0.08	+0.31
Significance[b]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
38°C	1.84	1.58	1.81	2.23	1.97	0.61	1.52
−40°C	2.84	1.48	1.10	2.35	0.77	0.45	2.10
Difference	−1.00	+0.10	+0.71	−0.12	+1.20	+0.16	−0.58
Significance	≠	N.S.	≠	N.S.	**	N.S.	≠
48°C	1.47	1.74	2.03	2.09	2.62	1.09	1.09
−40°C	2.29	1.62	1.65	2.32	0.71	0.44	1.97
Difference	−0.82	+0.12	+0.38	−0.23	+1.91	+0.65	−0.88
Significance	**	N.S.	N.S.	N.S.	**	**	≠

[a] Rehydrated chicken scored by nine panelists in two sessions with two replicates per session. 0 = none, 4 = extreme.
[b] Significance of differences between temperatures tested by t test for paired observations. N.S. = nonsignificant, *significant at 5% level, **significant at 1% level, ≠ significant (1%) overall, but only significant in one of two sessions treated separately.

the 28°C samples of raw pork after 6 months. The reactions leading to color changes obviously had high temperature coefficients. These findings confirm those of Sharp (1957b) who found that brown discoloration increased with temperature of storage. Similar observations were reported by Regier and Tappel (1956a) for beef stored at 35.5 and 54.4°C.

Rehydration

There was no significant change in percent rehydration of the freeze-dried products due to storage temperature or time. Mean rehydration values (percent rehydration based on gross rehydrated weight as a percentage of the gross initial weight) over all temperatures and times were 76% for cooked diced chicken, 86% for raw pork and 70% for cooked pork.

The limited rehydration capacities of the products were results of cooking and/or freeze drying treatments, so it is in these processing areas where improvements in rehydration should be sought.

The relatively low rehydration values cannot be attributed to inferior orientation of muscle fiber to cut surfaces (Anglemier et al., 1960) because both chicken dices and pork slices were cut with the fibers perpendicular to the greatest surface, the optimum orientation for rehydration.

Sensory evaluation

When the cans were opened for rehydration tests and panel evaluation of the product, a stale and somewhat rancid odor was noticed in samples stored at the higher temperatures, and its intensity increased with storage time. Burnett et al. (1955) reported similar off-odors for cooked freeze-dried pork, and Chipault et al. (1961) for freeze-dried chicken.

Table 3 shows panel results of the triangle tests for cooked diced freeze-dried chicken, raw and cooked freeze-dried pork slices. For chicken, sensory evaluation of the −40°C control samples and samples at the three experimental temperatures detected deterioration in the 48°C samples at 1 month and thereafter; and in the 38°C samples at 3 months and thereafter. The 38 and 48°C samples were less tender, more fibrous, darker and drier than the −40°C samples. Significant differentiation of 28 and −40°C was established at 3 months, but not at 1 and 6 months, so an overall effect for 28°C seems questionable.

Scores for cooked chicken of panelists who successfully differentiated the −40°C controls from experimental temperature samples at 6 months are summarized and evaluated in Table 4. No significant effect for any of the seven qualities was observed for 28°C vs −40°C. Samples at 38°C were significantly darker than −40°C controls, and there were good indications (significant at 1% for overall data, but only significant in one of two sessions) that they were less tender, more fibrous, and less desirable than −40°C samples. Samples at 48°C were significantly less tender, darker, and more off-flavored than controls, and there was a good indication that they were less desirable than −40°C controls.

For raw freeze-dried pork slices (Table 3), there were easily detectable and highly significant changes at 38 and 48°C at 3 months and thereafter. Based on scoring data, the 28°C raw pork samples stored for 6 months were not significantly different from the controls (Table 5). The 38°C samples were significantly darker and less desirable than −40°C controls, and there were good indications that they were less tender, more fibrous, and drier. The 48°C samples were significantly less tender, more fibrous, drier, darker, more off-flavored and less desirable than the controls. It was difficult to detect differences in the raw freeze-dried pork samples because grilling after rehydration masked much of the color difference and dried both the treated and control samples.

Significant changes (Table 3) were detected in the cooked freeze-dried pork slices at 1, 3 and 6 months for all three temperatures, and the degree of significance increased with temperature. On the basis of the scoring data at 6 months (Table 6), compared to the −40°C controls, there were no significant effects for 28°C samples, but there was an indication of greater darkness in the 38°C samples. The 48°C samples were significantly more fibrous, drier, darker, more off-flavored, less desirable and tended to be less tender and less mealy than the controls.

CONCLUSIONS

THE RESULTS of this investigation indicate that storage at excessive (38, 48°C) temperatures for 3–6 months caused serious deterioration of freeze-dried chicken and pork at 1% moisture and 0% oxygen levels. Color, tenderness, and flavor were affected.

Rehydration capacity of freeze-dried cooked chicken and raw and cooked pork at 1% moisture content was not influenced by storage in nitrogen at temperatures as high as 48°C and for periods as long as 6 months. Improvements in rehydration should be sought in pretreatment of raw products, e.g. presoaking in sodium chloride or phosphate solutions, and freeze-drying steps.

Table 5—Mean scores and differences, and significance of differences between paired scores for freeze-dried raw pork slices after 6 months of storage[a]

Temperature effect	Sensory Parameter						
	Tenderness	Mealiness	Fibrousness	Wetness	Darkness	Off-flavor	Desirability
28°C	1.78	0.83	2.11	0.83	2.00	0.83	1.22
−40°C	1.78	0.83	1.78	1.00	1.61	1.17	1.33
Difference	0	0	+0.33	−0.17	+0.39	−0.34	−0.11
Significance[b]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
38°C	1.05	0.76	2.67	0.81	2.19	1.00	0.62
−40°C	2.10	0.90	1.86	1.48	0.95	0.62	1.90
Difference	−1.05	−0.14	+0.81	−0.67	+1.24	+0.38	−1.28
Significance	≠	N.S.	≠	≠	**	N.S.	**
48°C	0.80	0.83	3.29	0.31	3.66	1.97	0.20
−40°C	2.40	0.94	1.86	1.71	1.09	0.23	2.17
Difference	−1.60	−0.11	+1.43	−1.40	+2.57	+1.74	−1.97
Significance	**	N.S.	**	**	**	**	**

[a] Rehydrated and cooked pork scored by nine panelists in two sessions with two replicates per session. 0 = none, 4 = extreme.
[b] Significance of differences between temperatures tested by t test for paired observations. N.S. = nonsignificant, *significant at 5% level, **significant 1% level, ≠ significant (1%) overall, but only significant in one of two sessions treated separately.

ϵ-Amino nitrogen content was 100 to 200-fold that of glucose on a molal basis, and no change was detected over 6 months at temperatures up to 48°C. Loss of 1 mole amino nitrogen per 1 mole of glucose probably could not have been established within the accuracy of the analytical methods. The molal ratio (100–200) of ϵ-amino nitrogen to glucose found in some meats indicates that the molal ratios (0.5–5) used in most model system studies are unrealistic and might lead to erroneous conclusions about the behavior of natural products.

Reducing substances, as measured by 2,6-dichlorophenolindophenol, accumulated to 15–30% of initial glucose level on a molal basis after 6 months at 48°C and might be a good chemical indicator of deterioration.

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Table 6—Mean scores and differences, and significance of differences between paired scores for freeze-dried cooked pork slices after 6 months of storage[a]

Temperature effect	Sensory parameter						
	Tenderness	Mealiness	Fibrousness	Wetness	Darkness	Off-flavor	Desirability
28°C	1.53	1.27	2.13	1.20	1.93	1.13	1.13
−40°C	1.87	1.20	1.80	1.47	1.47	0.87	1.53
Difference	−0.34	+0.07	+0.33	−0.27	+0.46	+0.26	−0.40
Significance[b]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
38°C	1.87	1.60	1.67	1.33	2.10	1.07	1.23
−40°C	2.13	1.37	2.10	1.37	1.17	0.73	1.53
Difference	−0.26	+0.23	−0.43	−0.04	+0.93	+0.34	−0.30
Significance	N.S.	N.S.	N.S.	N.S.	≠	N.S.	N.S.
48°C	1.06	0.97	2.88	0.97	3.30	1.00	0.64
−40°C	2.06	1.39	1.52	1.64	0.88	0.36	1.82
Difference	−1.00	−0.42	+1.36	−0.67	+2.42	+0.64	−1.18
Significance	≠	≠	**	**	**	**	**

[a] Rehydrated cooked pork scored by nine panelists in two sessions with two replicates per session. 0 = none, 4 = extreme.
[b] Significance of differences between temperatures tested by t test for paired observations. N.S. = nonsignificant, *significant at 5% level, **significant at 1% level, ≠ significant (1%) overall, but only significant in one of two sessions treated separately.

KI SOON RHEE
Consumer Research Center, Texas Agricultural Experiment Station
Texas A&M University, College Station, TX 77843

FACTORS AFFECTING OXYGEN UPTAKE IN MODEL SYSTEMS USED FOR INVESTIGATING LIPID PEROXIDATION IN MEAT

ABSTRACT

Measurements of linoleate oxidation catalyzed by nonheme iron or metmyoglobin (MetMb) have been frequently used as a model systems approach to investigate the mechanism of lipid peroxidation in meat. The objective of this study was to determine systematically the effects of Tween 20 (a nonionic surfactant), phosphate (a buffer component), MetMb (a heme iron catalyst), and Fe^{2+}-EDTA (a nonenzymic nonheme iron catalyst) on linoleate oxidation. The oxidation was determined by polarographic measurements of oxygen tension. Tween 20 slightly enhanced MetMb catalysis of linoleate oxidation at pH 7.6 and markedly increased Fe^{2+}-EDTA catalysis at pH 5.6. Phosphate accelerated MetMb catalysis but inhibited Fe^{2+}-EDTA catalysis. The rate of MetMb catalysis was not proportional to MetMb concentration even within a narrow range whereas the rate of Fe^{2+}-EDTA catalysis increased linearly with the concentration of Fe^{2+}-EDTA (1:1) complex. Appropriate model conditions for the two catalytic systems have been proposed for effective utilization of the model systems in future research. The choice of Fe^{2+}-EDTA as a nonenzymic nonheme iron catalyst has been explained based on its catalytic activity and practical considerations in the use of polarographic measurements of oxygen uptake for linoleate oxidation.

INTRODUCTION

LIPID PEROXIDATION is a major cause of quality deterioration in meat and meat products. Much effort has been devoted to identification of catalysts responsible for the peroxidation. It is the traditional view that hemoproteins are the major catalysts of lipid oxidation in meat and meat products (Tappel, 1952, 1953a; Younathan and Watts, 1959). In contrast, Sato and Hegarty (1971) and Love and Pearson (1974) proposed that nonheme iron plays a major role in accelerating lipid oxidation in cooked meat. Kwoh (1971), however, presented an evidence for the presence of both types of catalytic activity in meat, although heme was reported to be the dominant catalyst.

Measurements of linoleate oxidation catalyzed by inorganic iron (representing nonenzymic nonheme iron), metmyoglobin (MetMb), or tissue homogenates have been used as a model systems approach to investigate the mechanism of lipid peroxidation in meat and fish (Fischer and Deng, 1977; Kendrick and Watts, 1969; Kwoh, 1971; Lee et al., 1975). Lipid oxidation was determined by polarographic measurements of oxygen tension decrease due to the reaction between substrate (linoleate) and catalyst(s) present. The relative importance or predominance of either inorganic iron or MetMb as lipid oxidation catalyst was determined by comparing the effects of certain additives on inorganic iron catalyzed or MetMb catalyzed oxidation of linoleate with those on tissue (meat muscle or fish flesh) homogenate catalyzed oxidation of the fatty acid substrate.

Ferrous or ferric ions are very weak catalysts of the oxidation of unsaturated fatty acids but their catalytic activity can be greatly increased in two ways, either by conversion of the iron to an iron chelate compound or by addition of a reducing agent (Wills, 1965). Kwoh (1971) incorporated Fe^{2+} in the nonheme iron model system as a complex of Fe^{2+} and EDTA in 1:1 ratio—iron chelates of EDTA have been used to produce free radicals in various oxidative reactions (Norman and Smith, 1964; Orr, 1967; Udenfriend et al., 1954). Our preliminary experiments had shown that the rate of catalysis by Fe^{2+} alone is not fast enough to give rise to measurable amounts of oxygen uptake within a relatively short period, i.e., less than 10 minutes, of polarographic measurements. The catalysis by MetMb is fast, but MetMb in high concentrations inhibits linoleate oxidation (Kendrick and Watts, 1969). Whereas linoleic acid was emulsified with Tween 20 in all of the model system studies conducted to investigate lipid peroxidation in meat and fish, a recent study on lipid oxidation at the lipid-water interface showed that a small amount of Tween 20 (0.2%) can almost completely inhibit the oxidation catalyzed by inorganic iron or heme (Morita et al., 1976). Another important variable in the model systems is buffer components. Wills (1965) reported that linoleate (emulsion prepared without Tween 20) oxidation catalyzed by Fe^{3+} plus ascorbic acid or by hemoglobin was inhibited by high concentration of phosphate buffer.

A systematic study of the variables involved in the two catalytic systems would be highly desirable for effective utilization of model systems approach to investigate lipid peroxidation in meat. This paper reports the results of such a study.

EXPERIMENTAL

EMULSIONS OF LINOLEIC ACID (Sigma Chemical Company, two different lots of approximately 99% purity with no detectable peroxides) were prepared as described by Kwoh (1971) but using different concentrations of phosphate buffer and Tween 20—linoleic acid was solubilized by the addition of KOH and Tween 20. Fe^{2+}-EDTA system was at pH 5.6 and MetMb system at pH 7.6, since Fe^{2+}-EDTA is more active at acidic pH while MetMb is more active at alkaline pH (Kwoh, 1971). Linoleate emulsion was slightly whitish at pH 5.6, but was optically clear at pH 7.6.

MetMb (Sigma Chemical Company, horse heart, salt-free and lyophilized) was dissolved in phosphate buffer. Fe^{2+}-EDTA (1:1) solution was prepared in distilled water, for it turned opaque and grayish when made in phosphate buffer. Distilled, deionized water was used throughout this study.

Oxygen consumption in model systems was measured with a Beckman Fieldlab oxygen analyzer (Model 1008) coupled to a Linear recorder (Model 260). The recorder chart speed was set at 0.5 inch/min. The detailed procedure was as follows. A 50-ml Erlenmeyer flask with a Teflon magnet was positioned on a thick layer of paper-asbestos pad on a magnetic stirrer. Then, 20 ml of catalyst solution were quickly mixed with 40 ml of emulsion in a 250-ml Erlenmeyer flask and immediately transferred to the 50-ml flask. The sensor was promptly inserted into the neck of the flask, and the recorder was started.

The rates of oxygen uptake were linear after the first few minutes for Fe^{2+}-EDTA catalysis, but were curvilinear when MetMb was the catalyst. Therefore, the oxygen uptake for Fe^{2+}-EDTA catalysis was calculated from the recording by measuring the linear slope over a 3-min period whereas that for MetMb catalysis was calculated using the initial 3-min portion of the recording. Oxygen consumption was expressed as mm Hg pO_2/min. The oxygen uptake values of duplicate determinations were close with an average coefficient of variation of 3.4% (range: 0–5.8% with 10 sets of samples) for Fe^{2+}-EDTA catalysis and 2.0% (range: 0–7.1% with 14 sets of samples) for MetMb catalysis.

Neither the emulsion nor the catalysts oxidized at a measurable rate when tested separately. All the oxygen uptake studies were conducted at room temperature (24–25°C).

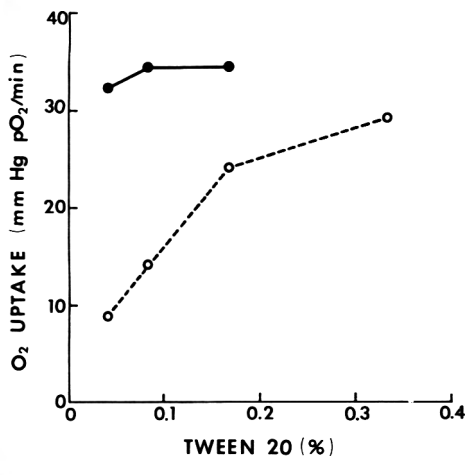

Fig. 1—Effect of Tween 20 concentration on linoleate oxidation catalyzed by Fe^{2+}-EDTA and MetMb. Linoleic acid, 6 × 10^{-3}M; MetMb, 5 × 10^{-7}M; Fe^{2+}-EDTA (3.75 × 10^{-4}; 3.75 × 10^{-4} M); phosphate, 0.02M; ●—● MetMb catalysis; ○- - -○ Fe^{2+}-EDTA catalysis.

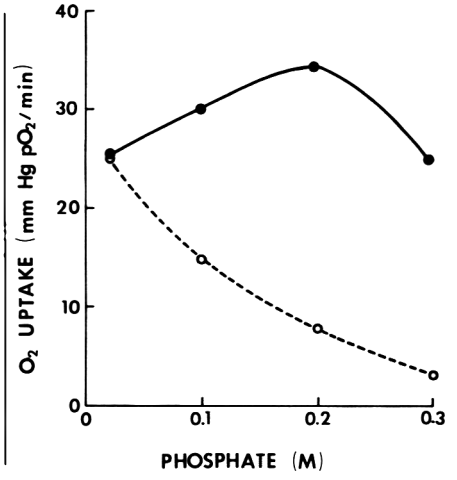

Fig. 2—Effect of phosphate concentration on iinoleate oxidation catalyzed by Fe^{2+}-EDTA and MetMb. Linoleic acid, 6 × 10^{-3}M; MetMb, 5 × 10^{-7}M; Fe^{2+}-EDTA (3.75 × 10^{-4}M; 3.75 × 10^{-4}M); Tween 20, 0.167% (w/v). ●—● MetMb catalysis; ○- - -○ Fe^{2+}-EDTA catalysis.

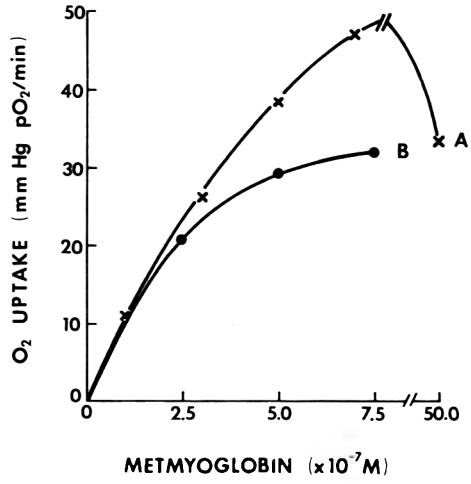

Fig. 3—Effect of MetMb on the rate of linoleate oxidation. Linoleic acid, 6 × 10^{-3}M; Tween 20, 0.167% (w/v). (A) 0.2M phosphate; (B) 0.05M phosphate.

RESULTS & DISCUSSION

Effect of Tween 20

The rate of Fe^{2+}-EDTA catalyzed oxidation of linoleate emulsion increased markedly in a linear manner with Tween 20 concentration up to 0.167% (w/v) and thereafter at a reduced rate (Fig. 1). The rate of MetMb catalyzed oxidation increased only slightly when Tween 20 concentration increased from 0.042% to 0.084%, leveling off at higher concentrations (Fig. 1). This finding is in contrast with the inhibitory effect of Tween 20 reported by Morita et al. (1976) for lipid oxidation at the lipid-water interface. There is a major difference between the two systems: lipid, catalyst, and Tween 20 were all solubilized in emulsion in this study whereas the system used by Morita et al. consisted of two phases (lipid and aqueous) with oxidation occurring at the interface.

The different effects of Tween 20 on Fe^{2+}-EDTA and MetMb systems may be explained by the physical states of linoleate emulsion at the pH's of the two systems. At pH 7.6 adopted for MetMb system, the emulsion was clear and transparent with the lowest Tween 20 concentration tested, 0.042%. At pH 5.6 employed for Fe^{2+}-EDTA system, the emulsion was not completely clear even with 0.333% of Tween 20, although the cloudiness of the emulsion decreased with increasing amounts of Tween 20. As fatty acid emulsions are less stable at acidic pH's (Wills, 1965), Tween 20 in increasing concentrations could have enhanced the oxidation of linoleate by reducing emulsion droplet size and thus by increasing the interaction between the catalyst and the lipid substrate.

Certain surfactants are known to form aggregates with proteins, but the nature of the interaction between the hemoprotein MetMb and Tween 20 is not known. The results of this study, however, indicate that Tween 20 per se may have little effect on the catalytic activity of MetMb.

Effect of phosphate

The effect of phosphate was examined by preparing linoleate emulsion in different concentrations of phosphate buffer (pH 5.6 for Fe^{2+}-EDTA system; pH 7.6 for MetMb system). Figure 2 shows the results. The rate of oxygen uptake of Fe^{2+}-EDTA catalysis decreased steadily with the increase in phosphate concentration. The rate of MetMb catalysis, however, increased with phosphate concentration up to 0.2M and decreased thereafter. Wills (1965) reported that linoleate oxidation catalyzed by Fe^{3+} plus ascorbic acid or by hemoglobin was unaffected by 0.02M phosphate but inhibited when phosphate concentration was greater than 0.1M. It should be noted that, in his study, linoleate emulsion was prepared without Tween 20, hemoglobin was used instead of MetMb, the oxidation occurring in an hour or longer period was measured by the manometric method, and the reaction temperature was much higher, i.e., 37°C.

The inhibition of Fe^{2+}-EDTA catalysis of linoleate oxidation by phosphate is likely due to complex formation of Fe^{2+} with phosphate, thereby rendering Fe^{2+} ineffective as catalyst. It is not known why phosphate at concentrations lower than 0.2M accelerates MetMb catalyzed oxidation of linoleate, while a higher concentration was inhibitory. It is only hypothesized that phosphate at appropriate concentrations may protect MetMb from destruction in the free radical chain reaction system of lipid peroxidation, or may act as a ligand for MetMb, the ligand somehow enhancing the catalytic activity of MetMb. Phosphate at concentrations higher than 0.2M may exert unfavorable effects, in some unknown ways, on the interaction between MetMb, Tween 20, and linoleate.

Effect of MetMb and Fe^{2+}

Varying concentrations of MetMb were tested in two different final concentrations of phosphate, 0.05 and 0.2M. The rate of oxygen uptake increased curvilinearly with MetMb concentration within 10^{-7} molar concentrations (Fig. 3). The progressive decline of the rate increase at higher MetMb concentrations, as manifested by the curvilinear response, was more pronounced with 0.05M phosphate than with 0.2M, the optimum phosphate concentration for MetMb system. Further increase of MetMb concentration to 5 × 10^{-6}M inhibited linoleate oxidation even with 0.2M phosphate.

The decline of the rate of linoleate oxidation with increasing concentrations of MetMb may be partly attributable to the reduction of free linoleate accessible to MetMb catalysis. Nakamura and Nishida (1971) reported that 1 mole of hemoglobin was associated with 880 moles of potassium linoleate and that induction or inhibition of linoleate oxidation occurred when the molar ratio of hemoglobin to potassium linoleate in the medium was 1:3800, or when 23% of the potassium linoleate added was associated with hemo-

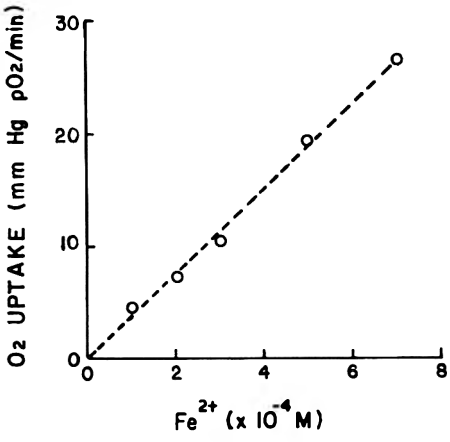

Fig. 4—Effect of Fe^{2+}-EDTA (1:1) on the rate of linoleate oxidation. Linoleic acid, 6 × 10^{-3} M; Tween 20, 0.167% (w/v); phosphate, 0.02M.

globin, 77% being in the free form. In the present study, the molar ratio of MetMb to linoleate was 1:1200 at the highest MetMb concentration tested, 5 × 10^{-6} M (see "curve A" in Fig. 3). If 1 mole of MetMb was associated with 880 moles of linoleate, 73% of linoleate in the system was bound to MetMb at 5 × 10^{-6} M MetMb.

The observed response to MetMb concentration may be also ascribed to the interaction of MetMb with lipid hydroperoxides. Hemoproteins in excess have been considered either to catalyze the breakdown of lipid peroxides into inactive products (Banks et al., 1961) or to combine with lipid peroxides, as they are produced, to form relatively stable compounds, thus retarding the initiation of new reaction chains (Kendrick and Watts, 1969). The inhibition of linoleate oxidation by high concentrations of hemoproteins has been also demonstrated by Hirano and Olcott (1971) and Lewis and Wills (1963).

The effect of varying Fe^{2+} concentration was different from the effect for MetMb. When used as an equimolar mixture of Fe^{2+} and EDTA, linearity was demonstrated over the entire Fe^{2+} concentration range studied (0–7 × 10^{-4} M), which was much higher than the concentration range tested with MetMb (Fig. 4).

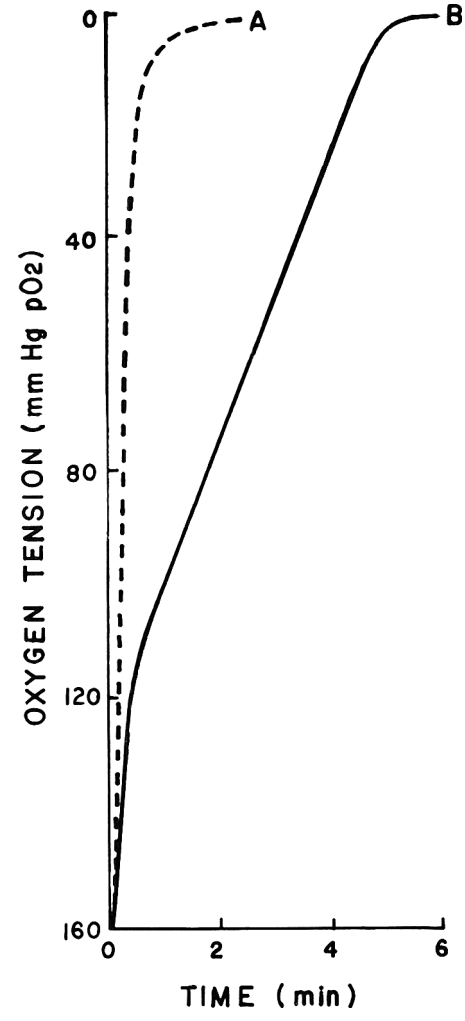

Fig. 5—Effect of the time of mixing Fe^{2+} and EDTA solutions on the oxygen consumption measured for linoleate oxidation catalyzed by Fe^{2+}-EDTA. Linoleic acid, 6 × 10^{-3} M; Fe^{2+}-EDTA (3.75 × 10^{-4}: 3.75 × 10^{-4} M); Tween 20, 0.167% (w/v); phosphate, 0.02M. (A) Fe^{2+} and EDTA solutions mixed immediately before recording; (B) Fe^{2+} and EDTA solutions mixed 30 minutes before recording. Chart speed, 0.5 inch/min.

Remarks on Fe^{2+}-EDTA catalysis and MetMb catalysis

Fe^{2+}-EDTA (1:1) complex was suggested as a nonheme iron model for free radical formation studies in model systems prior to application in foods (Liu, 1970), although this particular iron complex may not occur in food systems. The main advantage of Fe^{2+}-EDTA complex as a nonenzymic nonheme iron catalyst is its ability to catalyze fatty acid peroxidation at constant rate and its high catalytic activity. The activity of Fe^{2+} alone is too low to allow the use of polarographic methods for oxygen consumption. A combination of Fe^{3+} and ascorbic acid was used by Wills (1965) to catalyze linoleic acid oxidation measured by the manometric method, which required one to several hours. However, when it was tested for the polarographic method using the oxygen analyzer, there was no measurable amount of oxygen uptake in a 10 min recording period.

It should be mentioned that Fe^{2+} and EDTA have to be mixed in advance, preferably longer than 30 min before recording oxygen uptake. If they are mixed immediately before recording, oxygen available in the 50-ml Erlenmeyer flasks is all used up within 1 min (Fig. 5). The exhaustion of oxygen within this short period does not reflect oxygen uptake due to peroxidation of linoleic acid catalyzed by Fe^{2+}-EDTA. Rather, it may be attributable to oxygen consumed for generating free radicals when Fe^{2+} is mixed with EDTA.

The nonlinear recording pattern of MetMb catalysis mentioned under "Experimental" requires further explanation. The curvilinear pattern with MetMb catalysis at pH 7.6 has been found to be due to the high pH at which progressive destruction of MetMb (Tappel, 1953b) is more pronounced. Experiments being conducted in our laboratory at the time of this writing have shown that at pH 7.0 the oxygen uptake pattern of MetMb catalyzed oxidation is linear after an initial induction period. Although Kwoh (1971) did not clarify—in terms of actual polarographic recording pattern—the relation between the rate of oxygen uptake and pH of MetMb system, she did mention that MetMb catalyzed linoleate oxidation was much faster at pH 7.8 than at lower pH's but the rate of oxygen uptake decreased as the oxidation progressed at pH 7.8. Tappel (1953b) observed a curvilinear relationship between oxygen absorption measured by a Warburg manometer and reaction time for hemin catalyzed oxidation of colloidal linoleate at 0°C. The use of pH's not higher than 7.0 is recom-

mended for the polarographic measurement of oxygen uptake for MetMb catalysis. The catalytic activity of MetMb is less at lower pH's, but determination of the rate of oxygen tension decrease is simpler with the linear recordings.

Finally, the following conditions seem to be appropriate for the two model systems when the concentration of potassium linoleate is fixed at 6×10^{-3}M: (1) MetMb system—pH 7.0; 0.1–0.2% Tween 20; 0.2M phosphate, $2–5 \times 10^{-7}$M MetMb; and (2) Fe^{2+}-EDTA (1:1) system—pH 5.5 or 5.6; 0.2–0.5% Tween 20, 0.02M phosphate, $2–6 \times 10^{-4}$M Fe^{2+}. The model system approach is useful not only for identifying catalysts responsible for lipid peroxidation in a given muscle food, as it has been used by many investigators (Fischer and Deng, 1977; Kwoh, 1971; Lee et al., 1975), but may also be a time-saving tool for pre-testing or screening new antioxidants for the muscle food when the catalysts have been identified.

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LEANNE E. HEARNE,[1] MARJORIE P. PENFIELD and GRAYCE E. GOERTZ
Dept. of Food Science, Nutrition & Food Systems Administration
College of Home Economics and Agricultural Experiment Station
The University of Tennessee, Knoxville, TN 37916

HEATING EFFECTS ON BOVINE SEMITENDINOSUS: SHEAR, MUSCLE FIBER MEASUREMENTS, AND COOKING LOSSES

ABSTRACT

Progressive changes in Warner-Bratzler shear values of meat cores and in dimensions and integrity of muscle fibers from beef semitendinosus cores heated in tubes in a water bath to simulate oven roasting of top round roasts at 93 and 149°C to endpoint temperatures of 40, 50, 60 and 70°C were evaluated. Slower heating and higher endpoint temperatures resulted in greater cooking losses. Warner-Bratzler shear values and muscle fiber diameters decreased from 40 to 50 to 60°C. Sarcomere length of muscle fibers decreased from 60 to 70°C. Muscle fibers disintegrated as internal temperature increased. Fiber disintegration was greater at the faster rate of heating for cores heated to 60°C than at the slower rate. A faster rate of heat penetration might result in greater degradation of muscle fibers at a lower temperature. An increase in fiber disintegration with heating of cores from 60 to 70°C suggested an increase in tenderness, but changes in shear values were not apparent in this temperature range. It appears that other factors oppose the tenderizing effect of increased disintegration of muscle fibers at higher temperatures.

INTRODUCTION

CHANGES IN TENDERNESS of meat that occur during heating are complex and related primarily to two structural components of the muscle tissue—the muscle fibers and connective tissue fibers. The nature and extent of changes in these two components present opposing effects on tenderness. Draudt (1972) and Laakkonen et al. (1970) have theorized that the heat-induced change of fibrous connective tissue to granular connective tissue has a tenderizing effect, whereas the hardening of the myofibrillar proteins has a toughening effect.

Various heating rates, endpoint temperatures, and combinations of heating rate and endpoint temperature have been used in studies of the effects of heat treatment on the structure of muscle fibers in relation to tenderness of the meat. Because heat treatments used in reported studies have varied, relationships between the heat-induced changes in the muscle fibers and the resulting tenderness of the meat have not been clearly defined.

Several studies have suggested that a slower rate of heat penetration results in increased collagen solubilization without excessive hardening of muscle fibers (Cover, 1943; Bramblett et al., 1959; Bramblett and Vail, 1964; Bayne et al., 1969, 1971). Results of studies on heat-induced changes in connective tissue in relation to tenderness have varied. Bayne et al. (1971) and Paul et al. (1973) found no relationship between shear values and changes in connective tissue during heating of meat samples. In contrast, Penfield and Meyer (1975) reported an association between decreasing Warner-Bratzler (WB) shear values and increasing percentage of collagen solubilized as the internal temperature of meat cores increased. Dubé et al. (1972) attributed increases in shear values during heating to the hardening of muscle fibers, and the observed plateau in shear values at higher temperatures was associated with the conversion of collagen to gelatin. Few studies however, have made direct comparisons of heat-induced changes in muscle fibers and in the tenderness of the meat as measured by subjective and objective methods.

The objectives of this study were: (1) to investigate progressive changes in the structure of muscle fibers and in the shear values of the meat cores heated at two rates to four endpoint temperatures; and (2) to related changes in fiber diameter, sarcomere length, and disintegration of muscle fibers to the changes in tenderness of the meat cores as measured by the Warner-Bratzler shear.

EXPERIMENTAL

Source and characteristics of meat

Seven USDA Choice rounds from Angus heifer carcasses were obtained (11/6/75) from a local packing company. The carcasses met the following specifications: USDA Choice grade; 227–295 kg carcass; 1.0–1.3 cm of backfat; maturity Type A; and typical small marbling level. The ST muscle from each round was excised after 4 days aging at 1°C. Muscle pH was determined (Rogers et al., 1967); values ranged from 5.25–5.48 with a mean of 5.41. Muscles were wrapped and frozen at −15°C for no longer than 14 wk.

Preparation and samples and physical testing

Each frozen ST muscle was cut across the muscle fibers into 5.7 cm sections. The meat was allowed to thaw slightly to permit the removal of 18 cylindrical cores, 2.5 cm × 5.7 cm, cut parallel to the muscle fibers. Cores were placed in 50 ml Pyrex centrifuge tubes, randomly assigned to heating treatments and stored in the refrigerator (2–3°C) up to 24 hr before heating.

The procedure used in heating cores to simulate oven roasting of 2 kg top round roasts at 93°C for approximately 9.5 hr (slow rate) or at 149°C for approximately 2.5 hr (fast rate) was described previously by Penfield and Meyer (1975). After heating and cooling of the cores, appropriate weights were taken to determine cooking losses of the meat cores.

Following overnight refrigeration, each core was sheared three times with a Warner-Bratzler shear. Shear values for the two cores from a heat treatment were averaged prior to statistical analysis.

Four sections of a core remained after shearing. Two center sections were designated for histological work: the two remaining sections were ground once through a grinder attachment of a Hobart mixer (5 mm holes) and mixed thoroughly. Fat was extracted from ground samples and the residue dried; percent nonfat dry weight (NFDW) was calculated (Penfield and Meyer, 1975).

Histological procedures

A center section of each meat core was fixed in a 10% formalin and physiological salt solution for at least 24 hr. A fiber suspension was prepared for fiber diameter and sarcomere length measurements according to a modification of procedures of Tuma et al. (1962) and viewed under a Bausch & Lomb Dynazoom Phase Contrast Microscope equipped with an eye piece micrometer. Sarcomere length was determined by counting the number of A bands of each fiber along 110 microns of a micrometer at 970× magnification under oil immersion (Hegarty and Allen, 1975). Counts were made to the nearest half sarcomere on 25 randomly selected fibers. Fiber diameter of 25 randomly selected fibers was measured with the micrometer at 100× magnification.

Small blocks, approximately 3 mm^3, of fixed raw and cooked samples were sectioned on an AO Spencer 880 Freezing Clinical Microtome. Tissue sections, 20–25 μm thick, were stained with Sudan III fat stain and Harris hematoxylin tissue stain, mounted in glycerine

[1] Current address: Tennessee State University, Nashville, TN 37203

jelly, and viewed at 430× magnification. Photographs corresponding to five degrees of disintegration were selected and used to judge tissue sections for extent of disintegration. Values for disintegration ranged from 1, no disintegration, to 5, extreme disintegration. Five slides were selected from each heat treatment, coded by a nonobserver and scored by two observers for degree of disintegration. Scores from the two observers were not significantly different ("t" = 1.126; df = 266) and were averaged for statistical analysis.

Statistical analysis

The design of the experiment was a split-plot design with seven replications. Each animal (muscle) constituted a plot. Analysis of variance and orthogonal comparisons were used to study the functional relationship between heating rate and endpoint temperature for measurements that were taken. When significance was found, the Student-Newman-Keuls Test (Sokal and Rohlf, 1969) was applied.

RESULTS

Shear value

Shear values were similar for cores heated at the two rates (Table 1). Endpoint temperature influenced shear value ($P < 0.001$) and was independent of heating rate. The effect of endpoint temperature on shear values is illustrated by endpoint temperature means (Table 1). A significant ($P < 0.01$) curvilinear trend was noted. A small, but significant ($P < 0.01$), decrease in shear value occurred with heating of cores from 40 to 50°C. A greater decrease occurred with heating from 50 to 60°C, but changes in shear values were not apparent with heating from 60 to 70°C. Similar changes in shear values as influenced by endpoint temperature were found by Penfield and Meyer (1975) and Laakkonen et al. (1970). Dubé et al. (1972) found WB shear values increased with increasing cooking temperature above 60°C for bovine meat cylinders. In contrast, Paul et al. (1973) found that endpoint temperature did not influence shear values of bovine ST strips heated from 58 to 82°C.

Considerable variation in shear values was found for cores heated at either rate to 40 and 50°C (standard deviations ranged from 1.6–3.7). Less variability in shear values was found for cores heated to 60 and 70°C at the two rates (standard deviations ranged from 0.4–0.9). Because of this variability in shear values at the lower internal temperatures, the effect of heating rate on shear values is not clear. Shear variability at different endpoint temperatures and its subsequent effects on results of studies of progressive heat-induced changes in meat need further investigation.

Fiber diameter, sarcomere length, and fiber disintegration

Fiber diameter and sarcomere length were influenced by endpoint temperature ($P < 0.001$) but not by heating rate (Table 1). Fiber diameter decreased ($P < 0.01$) with heating from 40 to 50 to 60°C, but little change occurred from 60 to 70°C. Sarcomere length remained unchanged until cores were heated at either rate from 60 to 70°C. Curvilinear ($P < 0.01$) trends in fiber diameter and sarcomere length data also were found.

Disintegration was defined, for purposes of this study, as the loss of structural integrity attributed to cracks, breaks and granulated areas in the muscle fibers. Fiber disintegration values were similar for the two heating rates but increased ($P < 0.001$) with an increase in endpoint temperature (Table 1). An increase ($P < 0.01$) in fiber disintegration value occurred with each observed increase in internal temperature reached by the cores. The effect of heating rate on fiber disintegration was dependent on endpoint temperature ($P < 0.01$). The fast rate of heating resulted in a greater fiber disintegration value than did the slow rate at an endpoint temperature of 60°C. These results suggested that changes responsible for degradation of fibers are not only temperature dependent as generally accepted but are dependent on the rate of heat penetration.

Table 1—Effects of heating rate and endpoint temperature on heating time, cooking loss, shear value, fiber diameter, sarcomere length and disintegration of bovine semitendinosus cores[a]

Source of variation	Shear value (kg/2.5–cm core)	Fiber diam (μm)	Sarcomere length (μm)	Fiber disintegration value[b]	Cooking time (min)	Drip cooking loss (%)	Evaporative cooking loss (%)	Total cooking loss (%)	Nonfat dry wt (%)
Rate[c]									
Slow	10.9a	57.1a	2.1a	3.1a	–	21.7a	7.3b	29.0b	30.2b
Fast	11.6a	58.4a	2.1a	3.2a	–	21.0a	2.0a	23.0a	27.3a
Endpoint(°C)[d]									
40	16.6c	69.0c	2.2b	2.1a	–	12.4a	0.6a	12.9a	24.9a
50	14.4b	58.5b	2.2b	2.9b	–	16.6b	1.3ab	17.9b	24.7a
60	6.9a	52.6a	2.2b	3.6c	–	24.7c	2.8b	27.5c	28.0b
70	7.0a	51.0a	1.8a	4.1d	–	31.7d	14.0c	45.6d	37.2c
Rate × endpoint (°C)[d]									
Slow 40	15.7	67.6	2.2	1.9a	125	12.5a	0.6a	13.1a	24.8ab
50	15.0	59.1	2.2	3.1c	172	18.6b	1.6ab	20.2b	25.3ab
60	6.5	51.6	2.2	3.2c	248	27.0d	4.0bc	31.1d	29.3c
70	6.4	50.2	1.9	4.1d	581	28.6d	23.1d	51.7f	41.3e
Fast 40	17.5	70.3	2.2	2.3ab	79	12.2a	0.5a	12.6a	25.0ab
50	13.9	57.8	2.3	2.8bc	97	14.6a	1.0a	15.6a	24.2a
60	7.3	53.6	2.1	3.9d	115	22.4c	1.6ab	24.0c	26.8b
70	7.5	51.9	1.8	4.0d	165	34.7e	4.9c	39.6e	33.2d

[a] Means of seven replications
[b] 1, no disintegration, to 5, extreme disintegration
[c] Rate means within a column followed by a common letter are not significantly different as determined by F test ($P < 0.05$)
[d] Endpoint or rate × endpoint means within a column followed by a common letter are not significantly different as determined by Student-Newman-Keuls test ($P < 0.01$)

Heating rates, cooking losses and NFDW

The meat heating times for cores are shown in Table 1. The total heating time for cores to reach 70°C was 3½ times longer with the slow rate than with the fast rate.

Heating rates influenced evaporative and total cooking losses (Table 1). Cores heated at the slow rate had greater ($P < 0.001$) evaporative and total cooking losses than cores heated at the fast rate.

Endpoint temperature affected drip, evaporative and total cooking losses ($P < 0.001$). Drip loss increased ($P < 0.01$) with each observed increase in internal temperature. Evaporative loss was greater at 60°C than at 40°C.

The effect of heating rate on drip, evaporative, and total cooking losses was dependent on endpoint temperature. Drip loss at 40°C was unaffected by rate. The slow rate of heating resulted in greater ($P < 0.01$) drip loss than did the fast rate at endpoint temperatures of 50 and 60°C and less drip at 70°C. Evaporative loss was affected by heating rate only at 70°C. The longer time of heating at the slow rate resulted in greater ($P < 0.01$) evaporative loss for the slow 70°C cores. In addition, it permitted the evaporation of drip losses which accounts for the lower drip loss for the slow 70°C cores. Bayne et al. (1971) found greater cooking losses for semimembranosus roasts cooked in a 93°C oven than paired roasts cooked at 149°C. The roasting of beef at low oven temperatures would be expected to require longer cooking times and to result in greater cooking losses.

Heating rate and endpoint temperature influenced the nonfat dry weight of meat cores (Table 1). Differences in the heating times resulted in differences of NFDW because of moisture losses from the meat. Changes in NFDW were not apparent with heating from 40 to 50°C but increased as cores were heated from 50 to 60 to 70°C. Slow 60 and 70°C cores had larger ($P < 0.01$) NFDW values than cores heated at the fast rate of 60 and 70°C which reflects a greater loss of moisture at the slow rate.

DISCUSSION

RELATIONSHIPS between changes in fiber diameter and sarcomere length and changes in shear value are difficult to interpret. Shear value, fiber diameter and sarcomere length decreased with heating from 40 to 70°C. Decreases in shear value paralleled decreases in fiber diameter. Shear value and fiber diameter decreased with heating from 40 to 50 to 60°C and remained unchanged with heating to 70°C. Sarcomere length remained unchanged with heating between 40 and 60°C, and then decreased with heating from 60 to 70°C. Bouton and Harris (1972) suggested that the bunching up of connective tissue fibers caused by sarcomere shortening and the increases in cooking losses at higher temperatures are factors in addition to muscle fiber coagulation and connective tissue solubilization that might influence shear value. Shear values for cores heated from 60 to 70°C did not decrease but remained unchanged. The decrease in muscle fiber hardening in this temperature range might have influenced shear value. Decreases in fiber diameter from 40 to 60°C partially reflect increases in cooking losses. Cooking losses at higher temperatures might increase as sarcomeres shorten and force out fluids.

Increased disintegration of muscle fibers was associated with an increased number of cracks, breaks, and granulation in the fibers and a decrease in shear value. Schmidt and Parrish (1971) noted that the effect of increasing internal temperature on structural components of muscle was related to changes in tenderness and shear resistance. Progressive shrinkage and fragmentation of endomysial connective tissue began at 50°C, and myofibrillar proteins coagulated and hardened at 60°C and above. They concluded that maximum tenderness occurs when the connective tissue has fragmented without the coagulation of the myofibrillar proteins.

Fiber disintegration values indicated a progressive increase in fiber disintegration with increases in internal temperature. As fiber disintegration increased with heating of cores from 60 to 70°C, shear values did not appreciably change even though the increase in fiber disintegration values of muscle fibers heated to 70°C suggests an increase in tenderness. Increased disintegration may influence components of meat texture other than shearability. The loss of fluids, the coagulation of myofibrillar proteins, and the shortening of sarcomeres at this temperature might influence tenderness to a greater extent than the disintegration of the muscle fibers.

Other factors appear to be influencing shear values. Dubé et al. (1972) implied from their findings that shear measurements might be affected by the amount and toughness of connective tissue to a greater extent than by sarcomere shortening. Significant increases ($P < 0.01$) in percent solubilized collagen, as reported by Penfield and Meyer (1975), were accompanied by decreased WB shear values for beef ST cores heated from 50 to 60°C. However, differences in solubilized collagen did not completely explain changes in tenderness. Findings reported by Paul et al. (1973) suggested that muscle becomes more dense and compact because of increased coagulation of the muscle fiber proteins with increased heating. This increased coagulation might have a greater impact on the changes in tenderness than the solubilization of connective tissue at higher temperatures.

Draudt (1972) suggested that heating meat samples between 60 and 74°C should demonstrate a maximum in the hardening reaction of muscle fibers with little effect on tenderness attributed to collagen solubilization. A faster heating rate resulted in increases in breaking strength value for ST cores heated from 60 to 70°C but not for cores heated at a slower rate (Penfield et al., 1976). At the faster rate, limited time for connective tissue solubilization but high enough temperatures to cause hardening of muscle fibers might have accounted for decreased tenderness. The higher but not statistically different shear values for fast 70°C cores than for slow 70°C cores in this study (Table 1) might have been the beginning of such a hardening trend. It is possible that a significant hardening of muscle fibers caused by coagulation of myofibrillar proteins might have been observed if cores had been heated above 70°C.

The denaturation of the myofibrillar proteins influences the disintegration of muscle fibers. Further study of the effects of heat on the denaturation of myofibrillar proteins in relation to the hardening of muscle fibers is needed to better understand heat-induced changes in tenderness of meat. The effects of endpoint temperature, as well as heating rate, on tenderness of meat are not explained adequately by changes in fiber diameter, sacromere length and fiber disintegration. It becomes increasingly apparent that changes in tenderness of meat are complex and reconfirms the opinion that many factors influence changes in the tenderness of meat during heating.

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LEANNE E. HEARNE,[1] MARJORIE P. PENFIELD and GRAYCE E. GOERTZ
Dept. of Food Science, Nutrition & Food Systems Administration
College of Home Economics and Agricultural Experiment Station
The University of Tennessee, Knoxville, TN 37916

HEATING EFFECTS OF BOVINE SEMITENDINOSUS: PHASE CONTRAST MICROSCOPY AND SCANNING ELECTRON MICROSCOPY

ABSTRACT

Progressive structural changes in heated bovine semitendinosus (ST) muscle fibers were studied using phase contrast microscopy and scanning electron microscopy. ST cores were heated at two rates which simulated the oven roasting of top round roasts at 93 and 149°C to endpoint temperatures of 40, 50, 60 and 70°C. Increased heating of muscle fibers resulted in the disintegration of Z-line structure; increases in intermyofibrillar spaces; shortening of sarcomeres; cracks and breaks in the myofibrils at the Z-line; and fragmentation or granulation of the myofibrils. The slower rate of heating resulted in extensive granulation and fragmentation of the muscle fibers, whereas the faster rate resulted in more cracks and breaks in fibers. The effect of heating rate on the disintegration of muscle fibers suggested that the rate of heat penetration might influence the type and extent of disintegration of muscle fibers. Heating to 70°C resulted in extensive fragmentation of muscle fibers. Heat-induced changes in the structure of muscle fibers are discussed in relation to possible influence on the tenderness of heated meat.

INTRODUCTION

ALTERATIONS in the structural components of muscle tissue caused by heat influence the tenderness of the meat (Schmidt and Parrish, 1971). Changes in the microscopic appearance of muscle tissue reflect the changes occurring in the muscle fibers and connective tissue fibers during heating. The nature and extent of these changes present opposing effects on tenderness. The conversion of fibrous connective tissue to granular connective tissue has a tenderizing effect, and the hardening of the myofibrillar proteins has a toughening effect.

Changes in microscopic appearance of heated bovine muscle fibers include fiber shrinkage; sarcomere shortening; cracks and breaks occurring at the Z-line; granulation within a relatively intact endomysial reticulum; disintegration of Z-line structure; and thick and thin filament coagulation (Doty and Pierce, 1961; Paul, 1965; Hostetler and Landmann, 1968; Giles, 1969; Schmidt and Parrish, 1971; Dubé et al., 1972; Jones et al., 1977). These researchers and others have suggested that the tenderness of meat should initially reach an optimum because of connective tissue fragmentation, but further heating should result in a decrease in tenderness because of the hardening of muscle proteins and the loss of fluids.

Changes in muscle fibers from bovine semitendinosus (ST) cores heated to endpoint temperatures of 40, 50, 60 and 70°C were related to Warner-Bratzler shear values by Hearne et al. (1978). Shear value and fiber diameter decreased from 40 to 50 to 60°C; sarcomere length decreased from 60 to 70°C; and fiber disintegration steadily increased with increases in internal temperature. An increase in disintegration of muscle fibers heated to 70°C was not accompanied by a decrease in shear values. The coagulation of myofibrillar proteins and loss of fluids at this temperature were suggested as factors that might have a greater effect on tenderness than the disintegration of muscle fibers.

Light microscopes and transmission electron microscopes have been used in many studies to observe the structure of muscle fibers. Recently, the scanning electron microscope has been used in the study of the structure of muscle fibers. Although the resolution of the SEM is not as great as the TEM, the SEM has as its major advantages a greater depth of field which permits a three-dimensional view of the sample and an ease of sample preparation. Because of the recent use of the SEM as a research tool in the study of muscle fibers, standardized preparation techniques have not been established. In addition, interpretation of the structural components of muscle tissue observed in SEM photomicrographs has been conflicting among researchers.

Research on the microstructure of bovine muscle tissue, as viewed with the SEM, has focused primarily on the structure of raw muscle as affected by postmortem changes. Heat-induced changes in the microstructure of bovine muscle fibers have received limited study (Schaller and Powrie, 1972; Cheng and Parrish, 1976; Jones et al., 1977). Structural changes in heated muscle fibers observed with the SEM have been theoretically related to the tenderness of the meat. Yet, these studies have not measured tenderness either objectively or subjectively in order to accurately relate structural changes to changes in measurements of meat tenderness. Many of the experimental procedures used in heating meat samples do not simulate methods used in meat cookery.

This study was designed to investigate progressive structural changes in heated bovine muscle fibers using phase contrast microscopy (PCM) and scanning electron microscopy.

EXPERIMENTAL

Materials and heating

Two USDA Choice rounds from Angus heifer carcasses (227–295 kg) were obtained (11/6/75) from a local packer. The ST muscle from each round was excised after 4 days aging at 1°C and stored at −15°C up to 14 wk.

Sample preparation and method of heating were described in detail by Hearne et al. (1978). Meat cores, 2.5 cm in diameter, placed in tubes were heated in a water bath at rates comparable to the oven roasting of top round roasts at 93 and 149°C to four endpoint temperatures: 40, 50, 60 and 70°C.

Phase contrast microscopy

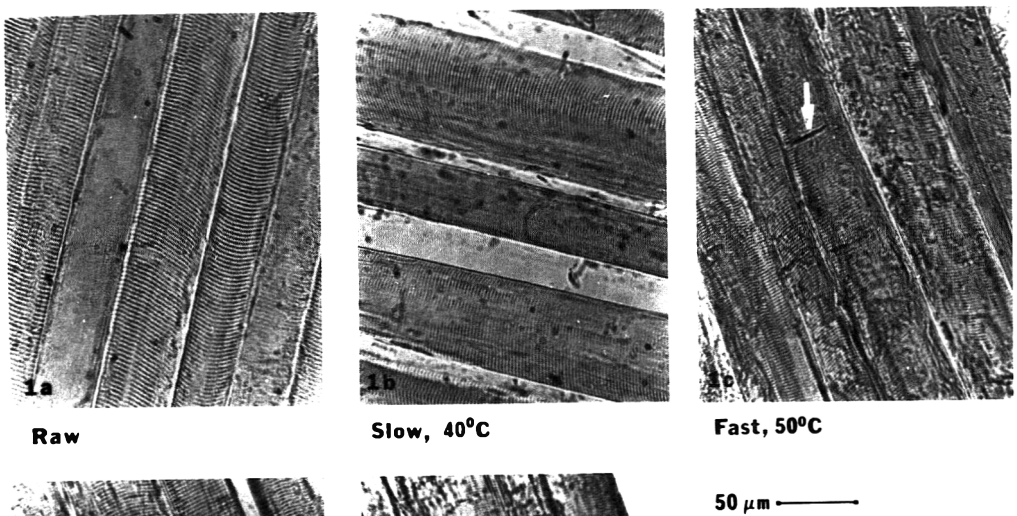
A center section of each meat core and three randomly selected raw samples from each ST muscle were fixed in a 10% formalin and physiological salt solution for at least 24 hr. Meat samples were sectioned (20–25 μm) and the tissue sections were stained with Harris hematoxylin stain and Sudan III fat stain and mounted in glycerine jelly. Slides were observed at 430× magnification with a Bausch & Lomb Dynazoom Phase Contrast Microscope. Photographs were taken with a Kodak Color Snap 35 Camera on the microscope.

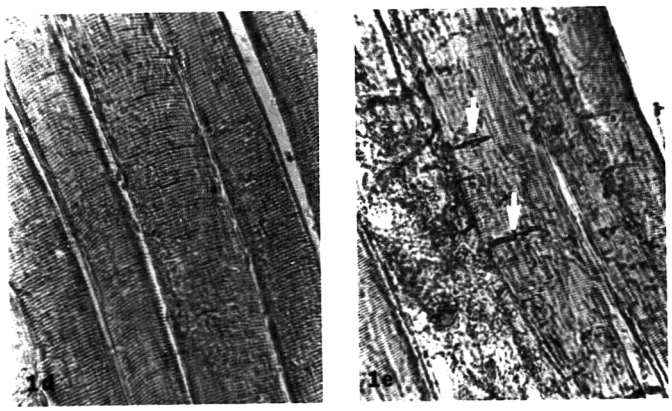
Scanning electron microscopy

A modification of procedures outlined by Beidler (1970) was used for preparation of samples for observation under the SEM. A center section from the cores for each heat treatment and a randomly selected raw sample from each muscle were fixed in 10% formalin and physiological salt solution for at least 24 hr. Ten individual random samples from each heat treatment and raw sample were cut into 3 × 3 × 1 mm blocks and placed in 0.1M phosphate buffer solution (pH 7.0) prior to critical point drying. One block was randomly selected from each group of individual samples and serially dried in solutions of 20, 50, 75, 90 and 100% acetone.

Following dehydration, samples were critical point dried in carbon dioxide. Dried tissue samples were glued to specimen stubs with DAG 45, a graphite suspended in isopropyl alcohol, and stored in a desiccator

[1] Current address: Tennessee State University, Nashville, TN 37203





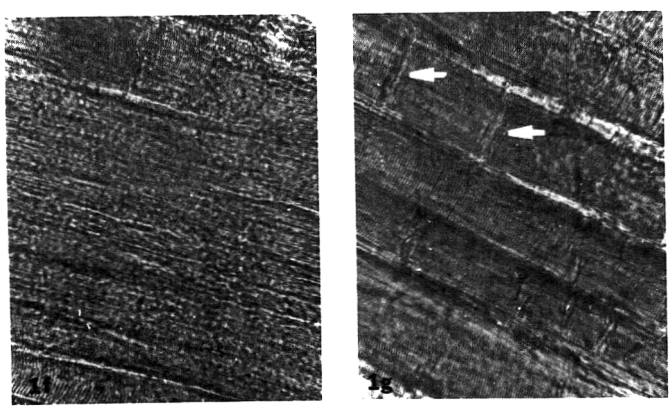


Fig. 1a–g—Phase contrast photomicrographs of tissue sections from raw bovine ST muscles and ST cores heated at two rates to four endpoint temperatures. 1a—Raw fibers; intact fibers. 1b—Slow, 40° C; banding pattern clear and distinct. 1c—Fast, 50° C; cracks in muscle fibers (arrow). 1d—Slow, 60° C; granulation within relatively intact muscle fibers. 1e—Fast, 60° C; increased number of cracks in muscle fibers (arrows). 1f—Slow, 70° C; heavy granulation in muscle fibers with loss of banding pattern. 1g—Fast, 70° C; numerous cracks and complete breaks in fibers (arrows).

until viewed with the scanning electron microscope. Dried samples were coated with palladium-gold (40:60; approximately 500–600 A layer) in a vacuum evaporator. Samples were examined with an AMR 900 Scanning Electron Microscope at 21 Kv. Random photomicrographs were taken from each block with a Polaroid camera equipped on the microscope. Photomicrographs from each block were examined, and a descriptive analysis of the muscle fibers was reported. Differences in endpoint temperature and heating rate effects were assessed by comparing scanning electron photomicrographs. Average sarcomere length of muscle fibers was measured from the photomicrographs, but the loss of the Z-line structure at the higher internal temperatures made it difficult to obtain an accurate measurement.

RESULTS & DISCUSSION

Phase contrast microscopy

Raw meat sections from beef ST muscles exhibited intact muscle fibers with clear and distinct banding patterns typical of skeletal myofibrils as shown in Figure 1. In several cases fibers from raw and heated beef ST muscles showed passive contraction, or kinking, of muscle fibers. Few, if any, cracks (partial breaks) or complete breaks in muscle fibers were present in sections from ST cores heated at either rate to 40°C (Fig. 1b). The banding pattern of the muscle fibers remained unchanged. At both heating rates, cores heated to 50°C showed muscle fibers containing some cracks and breaks (Fig. 1c). Most fibers remained intact and showed few signs of disintegration.

Sections from cores heated to 60°C contained cracks and breaks in the muscle fibers and considerable granulation within the fibers as illustrated in Figures 1d and 1e. Sarcomeres became less distinct, and the structural integrity of the muscle fibers diminished. Sarcomeres appeared to be shortened, and muscle fibers were more compact. Breaks and cracks in the muscle fibers occurred in the I band.

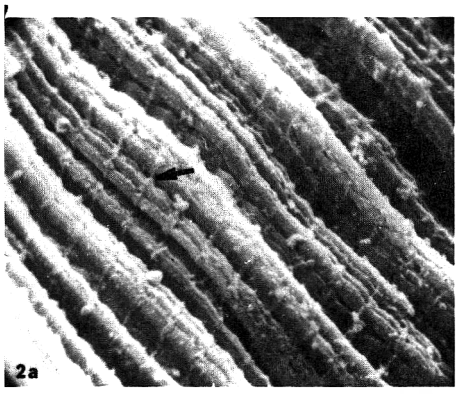
Heating cores to 70°C caused further disintegration of the muscle fibers. In some cases, extreme granulation resulted in a loss of the typical banding pattern of muscle fibers (Fig. 1f) and complete breaks in the muscle fibers (Fig. 1g).

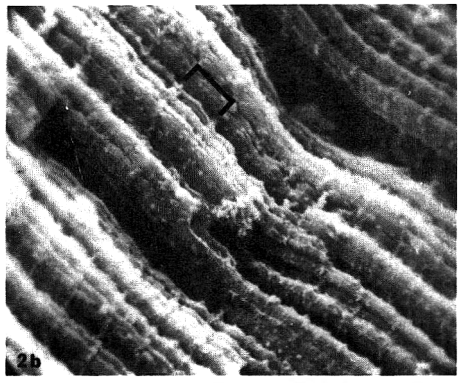
Some differences in the effects of the two heating rates were observed. Obvious signs of disintegration were not visible until samples were heated to 60°C and above. Disintegration observed in the tissue sections from cores heated at the slow rate to 60 and 70°C showed extensive granulation within the muscle fibers (Fig. 1d, f). At the fast rate, tissue sections from cores heated to 60 and 70°C showed less granulation but more cracks and breaks than the slowly heated cores (Fig. 1e, g). A large number of cracks and breaks occured in the muscle fibers heated at the fast rate; the banding pattern remained intact in most cases. Paul (1963) observed increases in cracks, breaks and granulation that appeared to be progressive with longer heating. The heavy granulation within fibers heated at the slow rate to 70°C might be related to the dry, mealy characteristic of meat cooked for long periods. The length of time that muscle fibers are heated at temperatures at which myofibrillar proteins are coagulated apparently influences the extent and type of disintegration of muscle fibers.

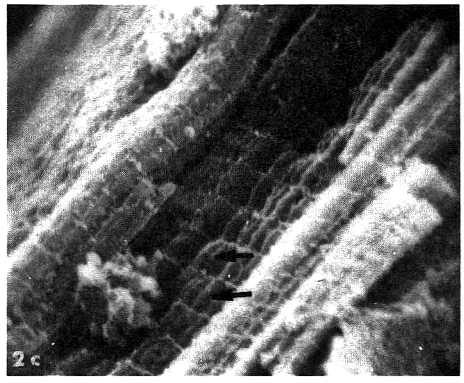
Scanning electron microscopy

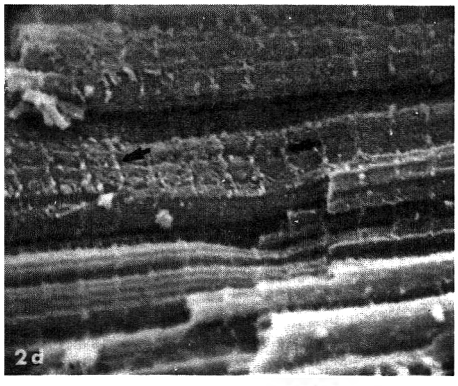
Scanning electron photomicrographs were taken at a magnification of 5000× because it provided the clearest observation of the structural details of the myofibrils and a direct comparison among heating treatments. The myofibrils were observed in areas in which the sarcolemma was removed during sample preparation or heating.

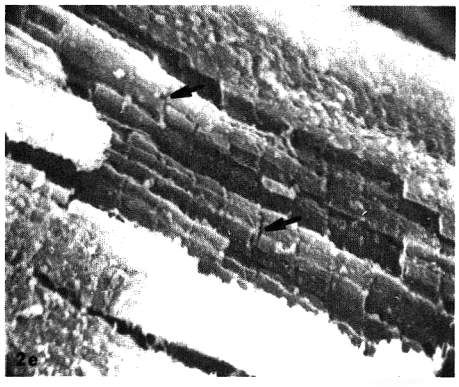
Myofibrils from raw muscle samples, in most cases, were intact (Fig. 2a, b). Broken fibers at the Z-line might be a result

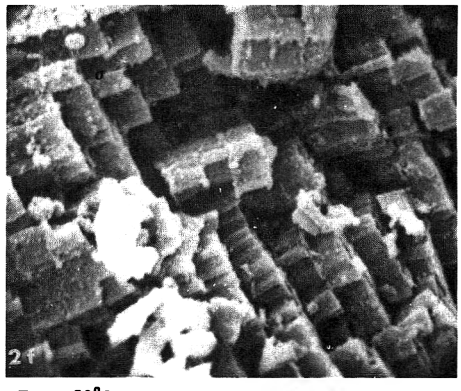


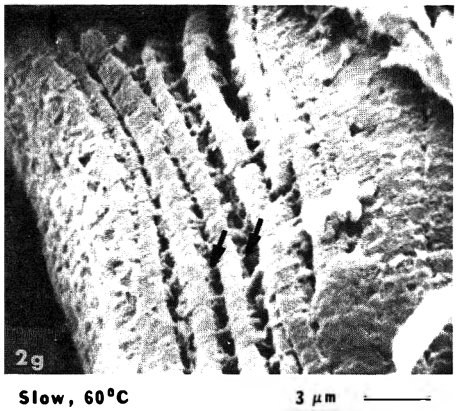


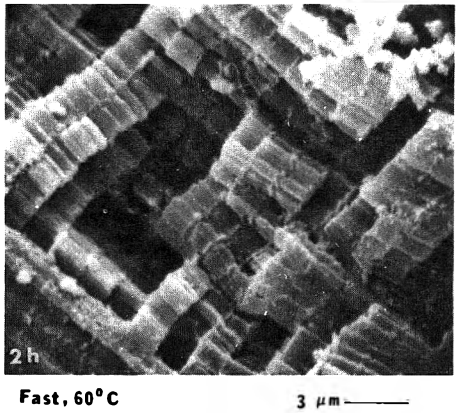


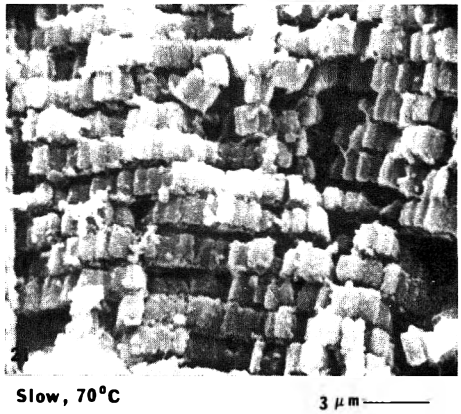












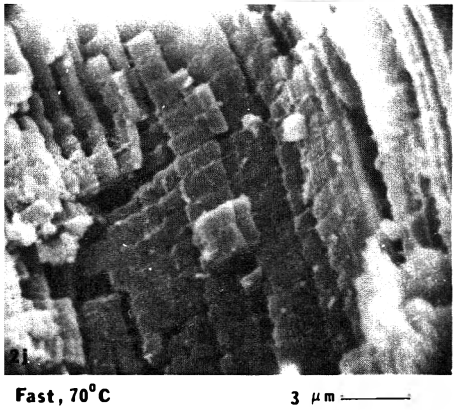


Fig. 2a–j—Scanning electron photomicrographs of bovine muscle fibers from raw ST muscles and ST cores heated at two rates to four endpoint temperatures. 2a—Raw fibers; transverse elements (arrow). 2b—Raw fibers, sarcomere indicated. 2c—Slow, 40°C; bulging of sarcomeres in A band area (arrows). 2d—Fast, 40°C; small cracks in I band area. 2e—Slow, 50°C; breaks in myofibrils (arrows). 2f—Fast, 50°C; Z-line structures no longer apparent. 2g—Slow, 60°C; intermyofibrillar spaces (arrows). 2h—Fast, 60°C; blocks consisting of individual sarcomeres. 2i—Slow, 70°C; heavy fragmentation of myofibrils. 2j—Fast, 70°C; shortened sarcomeres and fragmented myofibrils.

of preparation techniques or postmortem aging changes (Davey and Dickson, 1970). Sarcomere length measured from the photomicrographs was approximately 2.2 μm. Apparently, sample preparation did not cause fiber shrinkage because the average sarcomere length of fibers from a suspension of the fixed tissue was also 2.2 μm (Hearne et al., 1978). Transverse elements, regarded as part of the sarcoplasmic reticulum and T system that overlays the Z-line, were visible but not prominent.

Samples of muscle heated to 40°C at both rates still exhibited transverse elements, but in some cases there were signs of disintegration of these Z-line structures. Small cracks were observed in the I band near the Z-line as indicated by the arrows in Figure 2d. At this magnification, it was not possible

to identify whether the point of breakage in the I band was at the I band-Z-line junction or I band-A band junction. Jones et al. (1977) observed that breakage in the myofibrils of heated ST cubes occurred at the A band-I band junction or at the former I band area. Shortening of the sarcomeres to approximately 1.8 μm was accompanied by the bulging of the sarcomere in the center where the A band is located (Fig. 2c). This physical change in the myofibrillar structure is related possibly to the denaturation of the myofibrillar proteins. Hamm (1966) noted that coagulation of myofibrillar proteins begins at about 35°C. Between 35 and 50°C coagulation is caused by the aggregation of unfolded protein molecules and followed by the formation of unstable bonds. Coagulation continues between 50 and 70°C with the formation of more stable bonds. Hegarty and Allen (1975) postulated that the initial shortening of the sarcomere when heated resulted from the shortening of the A band.

Heating to 50°C caused further disintegration of the Z-line structure and actual breaks in the myofibrils occurred at the Z-line (Fig. 2e, f). Breaks in the myofibrils possibly resulted from I band disintegration, sarcomere shortening, and disintegration of the Z-line structures. The shortened sarcomeres measured approximately 1.7 μm. Schmidt and Parrish (1971) also observed through transmission electron microscopy the extensive degradation of the Z-line structures and sarcomere shortening in beef LD muscle heated to 50°C.

Intermyofibrillar spaces increased with the heating of samples to 60°C as indicated by the arrows in Figure 2g. Increases in intermyofibrillar spaces might have resulted from the shrinkage of the myofibrils and the disintegration of the Z-line structures which caused weakened lateral attachments. Weakening of lateral attachments might be related to increases in tenderness of meat samples heated to 60°C (Hearne et al., 1978). Increased fragmentation of the myofibrils resulted in blocks consisting of an individual sarcomere (Fig. 2h). At 60°C, sarcomere length was 1.6 μm.

Heavy fragmentation of the myofibrils resulted with heating to 70°C (Fig. 2i, j). The granulation, or loss of structural integrity, within the muscle fibers at 60 and 70°C as observed with the PCM corresponds to the fragmentation of the muscle fibers observed in SEM photomicrographs. Sarcomere length continued to decrease with increases in internal temperature and measured approximately 1.3 μm at 70°C.

Differences in the effects of heating rate on myofibrillar structure were difficult to observe from the photomicrographs taken at this magnification. An increase in the number of photomicrographs taken at various magnifications might have shown more differences between the two heating rates. But, some possible differences were observed in the myofibrillar structures from these photomicrographs. Myofibrils heated at the fast rate to 50°C (Fig. 2f) tended to be more fragmented than those heated at the slow rate. A faster rate of heat penetration apparently resulted in disintegration of muscle fibers at lower temperatures. Extreme fragmentation of the myofibrils heated at the slow rate to 70°C can be seen in Figure 2i. The total heating time at the slow rate was approximately 3½ times longer than at the fast rate, and the heating time from 60 to 70°C was over four times longer at the slow rate. Greater coagulation of myofibrillar proteins might occur at the slow rate because of the longer time of heating in a temperature range at which coagulation proceeds. An increase in myofibrillar coagulation might be related to increases in fragmentation, sarcomere shortening and fluid loss.

Methodology used in SEM sample preparation and viewing caused little structural damage to muscle fibers. The major problem in the viewing of a sample was the build-up of an electrical charge on a sample. A thick initial coating of palladium-gold or the recoating of the sample with the metal alloy helped to eliminate charging and to secure any loose fibers.

Structural changes in muscle fibers were apparent at 40°C in the scanning electron photomicrographs. This suggests that small changes might occur early in the heating of muscle fibers, but these changes are relatively small and likely insignificant to tenderness. Progressive increases in the disintegration of muscle fibers with increasing internal temperature from 40 to 70°C were evident in tissue sections observed with the PCM. Similarly, SEM photomicrographs showed increases in fragmentation and in intermyofibrillar spaces of the muscle fibers and the shortening of sarcomeres with increased heating. The extensive degree of fragmentation shown in muscle fibers heated to 70°C suggests an increase in tenderness because of increased fragmentation of the muscle fibers. Yet, previous studies reported that shear values of meat cores showed little change, if any, with the heating of cores from 60 to 70°C (Hearne et al., 1978; Penfield et al., 1976; Penfield and Meyer, 1975). At 70°C, the loss of fluids and the coagulation of myofibrillar proteins might influence tenderness more than factors such as fragmentation of muscle fibers or collagen solubilization. Study of the microstructural changes in connective tissue fibers with heating in relation to tenderness of the meat might provide additional information to explain changes in the tenderness of heated muscle tissue. Whether microscopic changes in muscle tissue structure are related to differences in components of tenderness perceived during mastication other than shearability or ease of fragmentation is a topic that needs to be investigated. Many unknowns still remain in the study of factors influencing tenderness of cooked meat.

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CHIN-SHENG CHENG[1] and F. C. PARRISH JR.
Muscle Biology Group, Departments of Animal Science and Food Technology
Iowa State University, Ames, IA 50011

EFFECTS OF POSTMORTEM STORAGE CONDITIONS ON MYOFIBRILLAR ATPase ACTIVITY OF PORCINE RED AND WHITE SEMITENDINOSUS MUSCLE

ABSTRACT

This study was carried out to determine the effects of postmortem storage time, temperature and pH on myofibrillar proteins of red and white muscle. Myofibrils were isolated from (1) the red and white portion of semitendinosus muscle postmortem stored at 2°C and (2) at-death red and white portions and suspended and stored in unbuffered 0.15M KCl at 2°C and in buffered 0.15M KCl (pH 5.5 and 7.0) at 2° and 25°C. To determine the effect of storage conditions on myofibrillar proteins, ATPase activity was assayed at different ionic strengths and with different modifiers. Assays of myofibril ATPase activity from postmortem muscle showed that (1) myofibrils from the white portion had greater ATPase activity than those from the red portion, (2) Ca^{2+}-modified activity from both portions increased and (3) Mg^{2+}-EGTA-modified activity increased from the white portion, but remained unchanged from the red portion, during postmortem storage. These changes could be due to modifications of the regulatory protein components of muscle by calcium-activated factor activity. For those myofibrils isolated from at-death muscle and incubated under simulated storage conditions, a precipitous decrease occurred in Ca^{2+}- and Mg^{2+}-(low ionic strength) and Ca^{2+}- and EDTA-(high ionic strength) modified ATPase activity of myofibrils stored in 0.15M KCl, pH 5.5, at 25°C. Otherwise, little change occurred in these activities under other simulated conditions of storage (i.e., 2°, pH 5.5 and 7.0; and 25°, pH 7.0) with the exception that EGTA modified activity (indicates loss of Ca^{2+} sensitivity) increased from the white portion at 25° and 2°C, pH 7.0, and from the red portion at 25°C, pH 7.0. Hence, a high storage temperature of 25°C has more detrimental effect on the integrity of myofibrillar proteins, as measured by changes in ATPase activity, than does a low pH of 5.5, or fiber type.

INTRODUCTION

A MAJOR CHANGE in the myofibrillar proteins during postmortem storage is the modification of the actin-myosin interaction. Some lines of evidence for the modification of the actin-myosin interaction are: (1) the increased Ca^{2+}-Mg^{2+}-modified adenosine triphosphatase (ATPase) activity of actomyosin (Fujimaki et al., 1965; Robson et al., 1967) and of myofibrils (Goll and Robson, 1967); (2) the increased sensitivity or weakening of the actin-myosin complex to dissociation by ATP (Fujimaki et al., 1965; Okitani et al., 1967); (3) turbidity development of myosin B (Arakawa et al., 1970a); and (4) the increase in protein extractability during postmortem aging (Chaudhry et al., 1969; Davey and Gilbert, 1968; Penny, 1968). Abnormal conditions of high carcass musculature temperature and rapid drop in pH, such as described by Briskey (1964) for PSE pork, could bring about further modifications in myofibrillar protein interactions during postmortem storage.

Porcine semitendinosus muscle can be separated on a gross morphological basis into a dark (red) and light (white) portion (Beecher et al., 1965a). Fiber types, however, are mixed in these two portions, with the light portion having about 80% white fibers and the dark portion having slightly less than 50% red fibers (Beecher et al., 1965b). These portions also have different rates of glycolysis with the light portion having the more rapid rate (Beecher et al., 1965a), and different amounts of ATPase activity with myofibrils and actomyosin isolated from white muscle having greater ATPase activity than those from red muscle (Seidel et al., 1964). Barany et al. (1965) showed that the Ca^{2+}- and EDTA-activated ATPase of myosin from white muscle was more alkaline stabile and, from red muscle, more acid stabile. Also, fiber type may have some unique characteristics that affects ATPase activity and Ca^{2+} sensitivity. Furukawa and Peter (1971) have reported that troponin from red muscle binds less Ca^{2+} than does troponin from white muscle. On the other hand, α-actinin from red and white muscle has very similar properties (Suzuki et al., 1973).

ATPase activity has been used to monitor the effect of postmortem storage on myofibrillar protein integrity by Goll and Robson (1967) and Penny (1968). More recently Suzuki and Goll (1974) measured ATPase activity to determine the effect of calcium activated factor on changes in actin-myosin interaction and myosin. Because little is known about the effect of postmortem storage conditions on myofibrils of different fiber types, this study was carried out by using ATPase activity as a criterion of myofibrillar protein integrity.

MATERIALS & METHODS

FOUR MARKET-WEIGHT (about 100-kg) normal, healthy pigs were slaughtered and sampled in this study. Semitendinosus muscle was excised from one side of the carcass 10 min after exsanguination and separated on a gross morphological basis into light (white) and dark (red) portions. These portions served as the at-death sample (30 min after exsanguination). The other semitendinosus was excised 24 hr postmortem from the companion side which was stored at 2°C. The white and red portions were separated, and each of the portions were cut into three longitudinal strips for further postmortem storage at 2°C. These muscle strips were then wrapped and stored in polyethylene film and randomly used for 1-, 3- and 7-day postmortem muscle samples.

Myofibrils from at-death and postmortem muscle were prepared by mincing in a precooled grinder and then homogenizing with 10 volumes (v/w) of 0.25M sucrose, 1 mM EDTA, 0.05 Tris · HCl, pH 7.6, solution in a Waring Blendor for 20 sec. The suspension was sedimented at 1,000 × G for 10 min, and the sedimented myofibrils were resuspended in 5 volumes of sucrose solution and recentrifuged at 1,000 × G for 10 min. After the supernatant was decanted, the sedimented myofibrils were homogenized in 5 volumes of 0.05M Tris, 1 mM EDTA, pH 7.6, solution in a Waring Blendor for 10 sec. Then, the suspension was passed through a polyethylene strainer (18 mesh) to remove connective tissue and centrifuged as described before. The sediments were washed twice more by suspending in 5 volumes of 0.15M KCl and centrifuging at 1,000 × G for 10 min. Finally, the sedimented myofibrils were resuspended in 5 volumes of 0.15M KCl solution. All preparative steps were conducted at 2°C with precooled solutions.

At-death myofibrils were stored in combinations of pH 5.5 or 7.0 and 2° or 25°C for 1-, 3- and 7-day storage, and at each time period, myofibrils were assayed in duplicate for ATPase activity. A total volume of 20 ml myofibril suspension containing a final concentration of 7.0 mg protein/ml, 0.15M KCl, 0.1M Tris-acetate, pH 5.5 or 7.0, and 0.1 mM sodium azide was stored in a 25-ml flask covered with parafilm.

ATPase assay of myofibrils from at-death and postmortem muscle was conducted according to the procedure described by Goll and Robson (1967). The conditions of the assay are specified in the results section. ATPase activity was expressed as μmoles of phosphorus liberated in 1 min by 1 mg of protein.

[1] Present address: Dept. of Food Science, North Carolina State University, Raleigh, NC 27607

Protein concentration was measured by the biuret procedure (Gornall et al., 1949) as modified by Robson et al. (1968).

Analysis of variance and Duncan's new multiple-range test were carried out according to the methods described by Snedecor and Cochran (1967).

RESULTS & DISCUSSION

Ca^{2+}-, Mg^{2+}- and EGTA-modified ATPase activity of myofibrils was assayed at low ionic strength to determine the effect of postmortem storage on myofibrillar proteins and fiber type. Figure 1 graphically shows that the Mg^{2+}-modified ATPase activity of myofibrils isolated from postmortem muscle stored at 2°C and myofibrils isolated from at-death muscle and stored in 0.15M KCl at 2°C was greater in white than in red myofibrils at 1 and 7 days postmortem storage. The difference in enzymic activity between white and red myofibrils was significant ($P < 0.01$), and the activity at-death

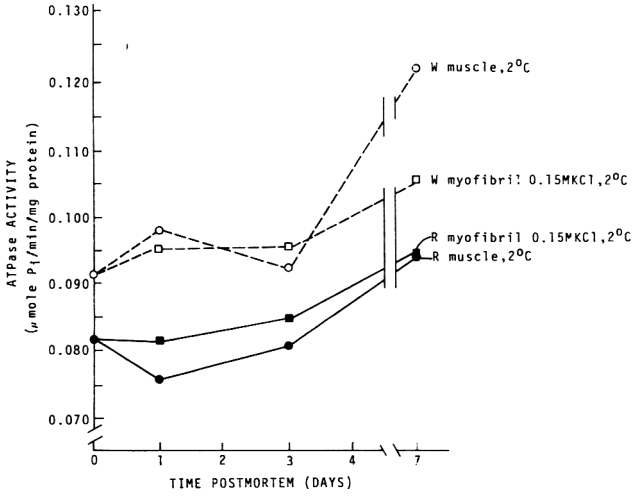

Fig. 1—Mg^{2+}-modified ATPase activity (low ionic strength) of myofibrils isolated from red and white portions of at-death porcine semitendinosus and stored in 0.15M KCl at 2°C, and of myofibrils isolated from red and white porcine semitendinosus portions stored at 2°C for 1, 3 and 7 days postmortem. Assay conditions: 0.5–0.7 mg protein/ml, 1 mM $MgCl_2$, 0.05 mM $CaCl_2$, 1 mM ATP, 0.05M KCl, 0.05M Tris-acetate, pH 7.0, incubated at 25°C for 10 min. (R) = red; (W) = white.

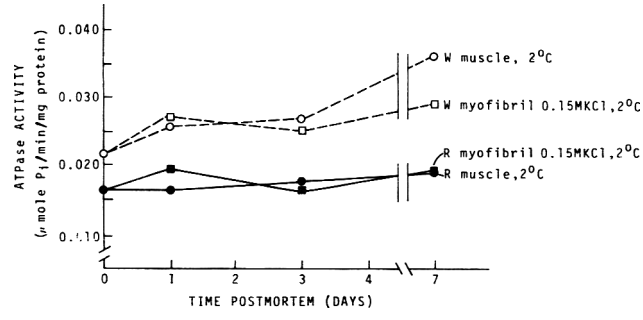

Fig. 2—EGTA-modified ATPase activity (low ionic strength) of myofibrils isolated from red and white portions of at-death porcine semitendinosus and stored in 0.15M KCl at 2°C, and myofibrils isolated from red and white porcine semitendinosus portions stored at 2°C for 1, 3 and 7 days postmortem. Assay conditions: 0.5–0.7 mg protein/ml, 0.1 mM EGTA, 1 mM $MgCl_2$, 1 mM ATP, 0.05M KCl, 0.05M Tris-acetate, pH 7.0, incubated at 25°C for 10 min. (R) = red; (W) = white.

was significantly different from that at 1 and 7 days. The ATPase activity of myofibrils isolated from at-death muscle and stored in 0.15M KCl at 2°C paralleled ATPase activities of myofibrils from muscle stored at 2°C. Ca^{2+}-modified ATPase activity, although not shown, was similar to that of the Mg^{2+}-modified ATPase activity. This increase in Ca^{2+}- and Mg^{2+}-modified ATPase activity of myosin B and myofibrils prepared from postmortem rabbit and bovine muscle has been reported by others (Fujimaki et al., 1965; Goll and Robson, 1967; Robson et al., 1967). In addition, means and standard errors for all assays were similar to those obtained by Goll and Robson (1967).

Both troponin and α-actinin, two regulatory protein components of the myofibril, are able to modify the actin-myosin interaction in vivo (Ebashi and Ebashi, 1964), and this may perhaps be an explanation for an increase in ATPase activity of myofibrils from postmortem stored muscle. If proteolysis of troponin occurred during postmortem storage of muscle, the inhibitory effect that this protein has on the actin-myosin interaction, and on Mg^{2+}-modified ATPase activity, would be removed. In addition, Z-disk degradation during postmortem storage may release α-actinin to more favorably interact with actin and, thus, cause the characteristic α-actinin-induced increase in the Mg^{2+}-modified ATPase activity of actomyosin (Arakawa et al., 1970c). Hence, changes in either α-actinin or troponin could cause the increased ATPase activity of myofibrils. Arakawa et al. (1970a), however, could obtain little evidence to substantiate that postmortem changes in α-actinin and the tropomyosin-troponin complex per se were the primary cause of postmortem modification in the actin-myosin interaction.

Insofar as the effect of proteolysis on ATPase activity is concerned, our increase in Ca^{2+}- and Mg^{2+}-modified ATPase results are similar to the effects of brief tryptic treatment on Mg^{2+}-modified ATPase and Ca^{2+} sensitivity of myosin B from various muscle types in guinea pigs. Furukawa and Peter (1971) have reported that Mg^{2+}-modified ATPase from both white and red vastus muscle of guinea pig were activated by brief trypsin treatment, whereas the Mg^{2+}-modified ATPase of the soleus was not activated by this treatment. They also found that brief trypsin treatment abolished the Ca^{2+} sensitivity of myosin B from red and white vastus muscle. Goll et al. (1971) reported several similarities between the effects of brief trypsin treatment and the effects of postmortem storage on the subcellular components of muscle cells. Indeed, a study recently reported by Suzuki and Goll (1974) showed that a calcium-activated sarcoplasmic factor (CASF), a proteolytic enzyme isolated from rabbit skeletal muscle, caused a 20–25% increase in Mg^{2+}-modified ATPase activity of myofibrils. They attributed the increase in Mg^{2+}-modified ATPase activity to CASF degradation of troponin, and a decrease in Mg^{2+}-modified ATPase activity to possible CASF destruction of α-actinin, a protein of the Z-disk, in myofibrils. Moreover, Olson et al. (1977) showed that calcium-activated factor (CAF), a calcium-dependent protease endogenous to bovine muscle fibers, was responsible for the degradation of troponon T and the removal of Z-disks of myofibrils from bovine muscle. (CASF and CAF are abbreviations used for the same protease discovered by Busch et al., 1972.) This further strengthens the view that troponin and α-actinin are modified during postmortem muscle storage; consequently, the actin-myosin interaction, as measured by ATPase activity, could be modified because troponin and α-actinin are modified during postmortem muscle storage.

An increase in EGTA-modified activity is indicative of the loss of the calcium-sensitive component in myofibrils (Ebashi and Ebashi, 1964). EGTA-modified ATPase activity of myofibrils isolated from the red portion changed little during postmortem storage and had significantly ($P < 0.01$) less activity than did myofibrils isolated from the white portion (Fig. 2).

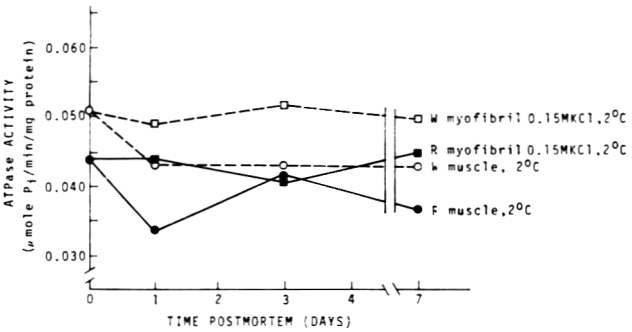

Fig. 3—Ca^{2+}-modified ATPase activity (high ionic strength) of myofibrils isolated from red and white portions of at-death porcine semitendinosus and stored in 0.15M KCl at 2°C, and of myofibrils isolated from red and white porcine semitendinosus portions stored at 2°C for 1, 3 and 7 days postmortem. Assay conditions: 0.5—0.7 mg protein/ml, 1 mM $CaCl_2$, 1 mM ATP, 0.5M KCl, 0.04M Tris-acetate, pH 7.0, incubated at 25°C for 5 min. (R) = red; (W) = white.

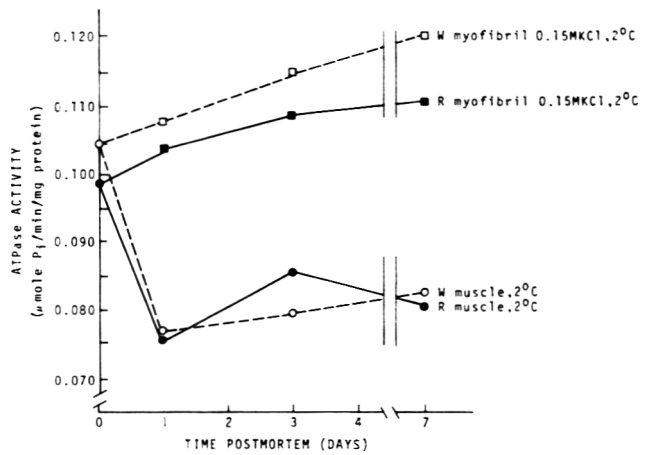

Fig. 4—EDTA-modified ATPase activity (high ionic strength) myofibrils isolated from red and white portions of at-death porcine semitendinosus and stored in 0.15M KCl at 2°C, and of myofibrils isolated from red and white porcine semitendinosus portions stored at 2°C for 1, 3 and 7 days portmortem. Assay conditions: 0.5—0.7 mg protein/ml, 1 mM EDTA, 1 mM ATP, 0.5M KCl, 0.04M Tris-acetate, pH 7.0, incubated at 25°C for 5 min. (R) = red; (W) = white.

At 7 days postmortem, the EGTA-modified ATPase activity of white myofibrils had increased significantly ($P < 0.01$). The marked increase in enzyme activity of myofibrils isolated from white muscle could possibly be due to the effect of the presence of a sarcoplasmic factor, CAF, because little change occurred in myofibrils isolated form the at-death white portion in which the sarcoplasmic proteins had been removed before storage in 0.15M KCl at 2°C.

Ca^{2+}-modified ATPase activity assayed at high ionic strengths, which corresponds to myosin ATPase, decreased at 1 day postmortem in both red and white myofibrils and remained essentially unchanged afterward in white myofibrils (Fig. 3). At-death myofibrils stored in 0.15M KCl solution, on the other hand, had little change in enzymic activity. No difference in enzyme activity due to fiber type was indicated by analysis of variance. Thus, these results indicate that the ATPase of myosin in myofibrils from postmortem muscle was modified, but that little or no modification occurred in myosin of myofibrils isolated from at-death muscle and stored at 2°C in 0.15M KCl (sarcoplasmic protein free).

ATPase activity at high ionic strength, modified by EDTA, measures K^+-modified myosin ATPase activity. A marked parallel decrease (about 25%) of EDTA-modified ATPase occurred in myofibrils from both types of postmortem muscle at 1 day, but at-death myofibrils stored in 0.15M KCl increased slightly (about 10%) during storage for 7 days (Fig. 4). This decline and increase in ATPase activity indicates some alteration in myosin of postmortem muscle and KCl stored myofibrils, respectively. No reasonable explanation can be given for these changes. Goll and Robson (1967) reported an increase and then a decrease of EDTA-modified ATPase of bovine myofibrils. There was no significant difference in ATPase due to fiber type, but there was a significant difference due to postmortem days of storage for both KCl stored myofibrils and myofibrils from postmortem muscle.

Simulated conditions of postmortem storage were carried out by isolating myofibrils from at-death red and white portions and then storing these myofibrils in buffered solutions (pH 5.5 and 7.0) at 2° and 25°C. The combination of low pH and high temperature would somewhat resemble the conditions that muscle encounters during rapid postmortem glycolysis (Briskey, 1964). Ca^{2+}-modified ATPase activity of myofibrils from both portions (Fig. 5) decreased significantly ($P < 0.01$) and precipitously at pH 5.5 and 25°C. Under the other simulated conditions of storage, activities were slightly erratic.

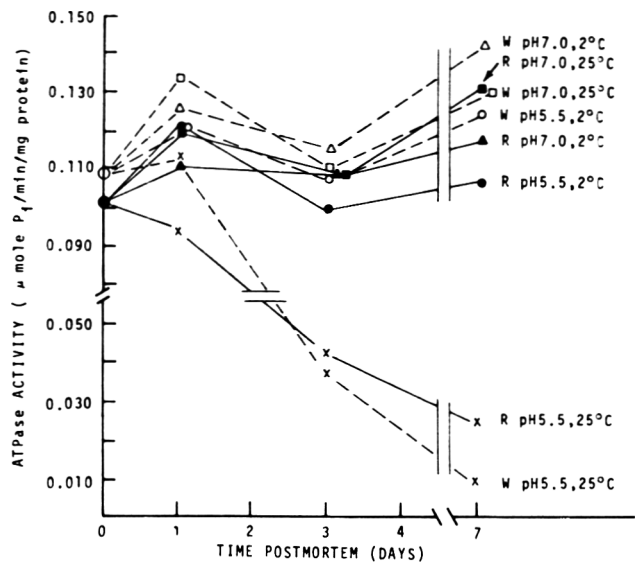

Fig. 5—Ca^{2+}-modified ATPase activity (low ionic strength) of myofibrils isolated from red and white portions of at-death porcine semitendinosus and stored in 0.15M KCl at pH 5.5, 2° and 25°C; and pH 7.0, 2° and 25°C. Assay conditions: 0.5—0.7 mg protein/ml, 1 mM $CaCl_2$, 1 mM ATP, 0.05M KCl, 0.05M Tris-acetate, pH 7.0, incubated at 25°C for 5 min. (R) = red; (W) = white.

That is, there was an increase, a decrease, and then an increase in activity when they were stored at pH 5.5, 2°C, or pH 7.0, 2° and 25°C. Samaha et al. (1970) reported that myosin prepared from white muscle at room temperature lost about 85% of its Ca^{2+}-modified ATPase activity when exposed to pH 4.35 for 5 min, whereas myosin prepared from red muscle lost about 50% of its activity. Mg^{2+}-modified ATPase activity of myofibrils isolated from both portions, although not shown, was very similar to those of Ca^{2+}-modified ATPase activities. Okitani et al. (1967) reported that the Mg^{2+}-modified ATPase activity of stored myosin B solution at pH 5.6 and 25°C in-

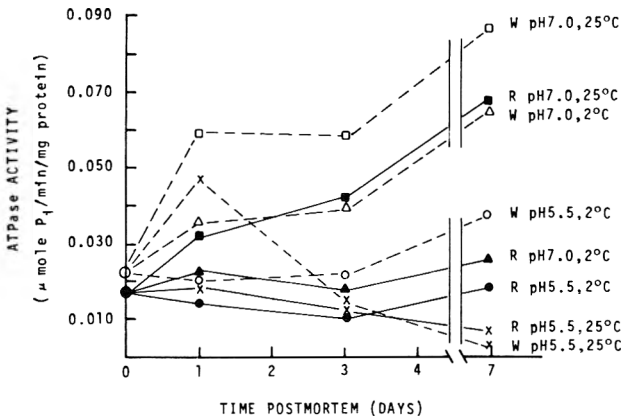

Fig. 6—EGTA-modified ATPase activity (low ionic strength) of myofibrils isolated from red and white portion of at-death porcine semitendinosus stored in 0.15M KCl at pH 5.5, 2° and 25°C; and pH 7.0, 2° and 25°C. Assay conditions: 0.5–0.7 mg protein/ml, 0.1 mM EGTA, 1 mM $MgCl_2$, 1 mM ATP, 0.05M KCl, 0.05M Tris-acetate, pH 7.0, incubated at 25°C for 10 min. (R) = red; (W) = white.

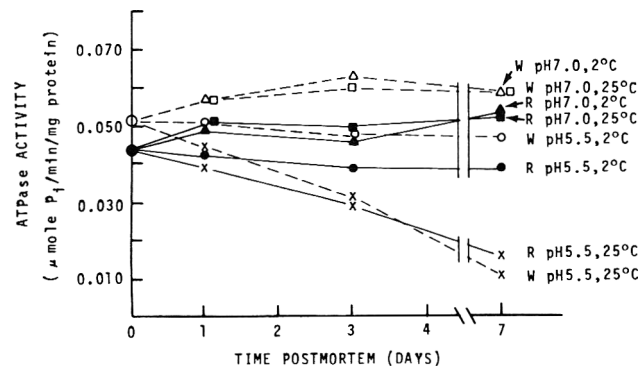

Fig. 7—Ca^{2+}-modified ATPase activity (high ionic strength) of myofibrils isolated from red and white portion of at-death porcine semitendinosus stored in 0.15M KCl at pH 5.5, 2° and 25°C; and pH 7.0, 2° and 25°C. Assay conditions: 0.5–0.7 mg protein/ml, 1 mM $CaCl_2$, 1 mM ATP, 0.5M KCl, 0.04M Tris-acetate, pH 7.0, incubated at 25°C for 5 min. (R) = red; (W) = white.

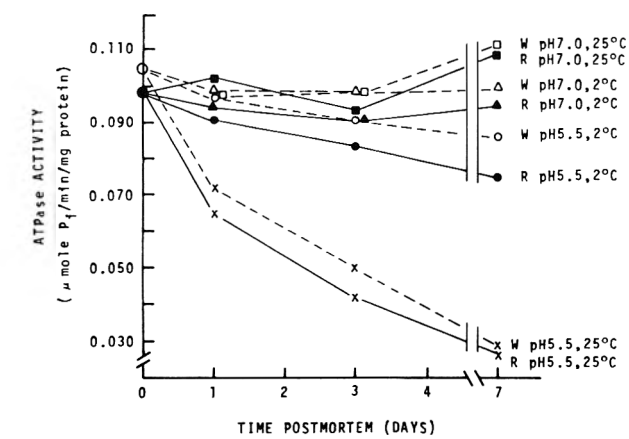

Fig. 8—EDTA-modified ATPase activity (high ionic strength) of myofibrils isolated from red and white portion of porcine semitendinosus stored in 0.15M KCl at pH 5.5, 2° and 25°C; and pH 7.0, 2° and 25°C. Assay conditions: 0.5–0.7 mg protein/ml, 1 mM EDTA, 1 mM ATP, 0.5M KCl, 0.04 M Tris-acetate, pH 7.0, incubated at 25°C for 5 min. (R) = red; (W) = white.

creased rapidly, then decreased. They also found, similar to our results, that the Mg^{2+}-modified ATPase activity of stored myosin B at pH 7.0 and 25°C increased steadily during 24 hr of storage.

EGTA-modified ATPase activity of white myofibrils increased about 100% 1 day postmortem for both pH groups at 25°C, but the activity of white myofibrils at pH 5.5 dropped sharply and the activity of those at pH 7.0 continued to increase (Fig. 6). Activity of red myofibrils, however, remained essentially unchanged with the exception of red myofibrils at pH 7.0, 25°C, which increased. The increases in ATPase activity indicate a loss of Ca^{2+} sensitizing ability of troponin. Arakawa et al. (1970b) reported that troponin from rabbit muscle had a slight loss in its Ca^{2+}-sensitizing ability when stored at pH 5.5 and 25°C for 16 hr. Our results suggest that the troponin-Ca^{2+}-sensitizing ability in white myofibrils is severely diminished for both pH-temperature groups whereas troponin in red myofibrils may be only slightly affected under the storage condition of pH 7.0 and 25°C.

Ca^{2+}-modified ATPase activity, assayed at high ionic strength to detect changes in myosin, decreased significantly ($P < 0.01$) for both red and white myofibrils stored at pH 5.5, 25°C; however, little change occurred in activity under other conditions of simulated storage (Fig. 7). The loss of Ca^{2+}-modified ATPase activity at high ionic strength in myofibrils stored at pH 5.5 and 25°C indicates that myosin molecules were denatured under these conditions, regardless of fiber type. Another measure of myosin integrity is the EDTA-modified ATPase activity. These activities, graphically illustrated in Figure 8, were very similar to those obtained for Ca^{2+}-modified ATPase activity, again indicating that pH 5.5 and 25°C severely reduced ATPase activity. This combination of low pH and high temperature would somewhat resemble the conditions that pale, soft exudative muscle encounters during rapid postmortem glycolysis (Briskey, 1964). Because myofibrillar proteins are important in water-holding capacity, loss of myosin integrity could be an explanation for the reduced water-holding capacity of rapidly glycolyzing muscle.

Three general observations can be made concerning the changes in ATPase activity of myofibrils from postmortem stored muscle and KCl stored myofibrils in combinations at 2°C and 25°C, pH 5.5 and 7.0.

First, the changes in myofibrillar ATPase activity of postmortem muscle were more profound in the myofibrils from the white portion than they were from the red portion. Although the myofibrillar ATPase activity of the white muscle portion was about 10–20% higher than the enzymic activity of myofibrils from the red muscle portion, this difference was not as great as those reported for white muscle myosin or actomyosin ATPase activity (Seidel et al., 1964; Barany et al., 1965; Sreter et al., 1966). An obvious explanation is that the red portion of porcine semitendinosus has about 50% red fibers, and the remainder are of white or intermediate fiber type. Other explanations for this difference may be species, preparative procedures and (or) the enzymic assay conditions.

Second, more marked changes were noted in the myofibrils prepared from postmortem muscle than from those stored in KCl from at-death muscle. Consequently, it seems that some factor in the sarcoplasm was exerting some modifying effect on myofibrillar proteins during storage. That is, myofibrils isolated from at-death muscle and stored (those myofibrils free of sarcoplasmic proteins) under similar conditions did not show a change in ATPase activity as did myofibrils isolated from postmortem muscle. This factor in the sarcoplasm could be calcium-activated factor (CAF), a calcium-dependent protease located in the sarcoplasm and endogenous to the muscle fiber and responsible for degradation of myofibrillar proteins during postmortem storage of muscle (Olson et al., 1977).

Third, myofibrillar ATPase activity indicated that high temperature-low pH combination was much more detrimental to

myofibrillar protein integrity than any other combinations of temperature and pH used in this study. Furthermore, fiber type had little influence on the behavior of myofibril ATPase activity and Ca^{2+} sensitivity.

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BÄRBEL HÄGERDAL[1]
Biochemistry 1, Chemical Center
P.O.B. 740, S-220 07 Lund 7, Sweden

STABILITY OF MYOGLOBIN TO ACETONE TREATMENT CHARACTERIZED BY DIFFERENTIAL SCANNING CALORIMETRY AND RESOLUBILITY IN WATER

ABSTRACT

The effect of organic solvents on globular proteins during extraction processes has been studied in a model system of myoglobin and acetone. Acetone was added to water solutions of myoglobin and acetone-water mixtures were added to solid samples of myoglobin. The acetone concentration range was 10–99% (v/v). The degree of irreversible transition caused by acetone in the myoglobin preparations was characterized both by differential scanning calorimetry and by water solubility. At corresponding acetone concentrations the myoglobin preparations resulting from the two different ways of adding acetone showed no significant difference in degree of irreversible transition. A constant fraction of any myoglobin preparation was resoluble in water. At medium acetone concentrations (50–70%) myoglobin underwent complete irreversible transition, whereas at low (30% and below) and high acetone concentrations (90% and above), only a fraction of the myoglobin sample underwent irreversible transition. In the low concentration range this was ascribed to the decrease in polarity of the acetone-water medium, and in the high concentration range to the decreased availability of water, expressed as a decrease in water activity. The myoglobin samples treated with high concentrations of acetone could be divided into two groups with different susceptibility to a following heat treatment. A model for predicting the degree of irreversible transition of protein concentrates and isolates that have undergone organic solvent extraction is presented.

INTRODUCTION

WHEN PREPARING PROTEIN concentrates or isolates for human consumption from new protein sources, lipids are often extracted by organic solvents from the raw material in order to obtain a storable product (Dambergs, 1969; Ohlsson, 1973). Organic solvents are also used to decolorize leaf and blood proteins to improve their acceptability (Pirie, 1957; Tybor et al., 1973). As the native protein conformation is mainly stabilized by weak interactions (hydrogen bonds, hydrophobic and electrostatic interactions; Kauzmann, 1959), the treatment with an organic solvent leads to protein denaturation (Tanford, 1969). The denaturation is irreversible when pH is close to the isoelectric point of the protein or when the protein concentration is high, which is the case during the production of protein concentrates and isolates. In turn the irreversible protein denaturation may result in poor technical properties such as water solubility, wetting ability and color (Dubrow et al., 1973).

Conformational changes of proteins due to organic solvents have been studied in dilute solutions at low or high pH (Weber and Tanford, 1959; Tanford et al., 1960; Herskowitz et al., 1970) to elucidate the importance of hydrophobic interactions for the stabilization of the tertiary structure of globular proteins (Kauzmann, 1959). In these studies organic solvent was added to water solutions of the proteins. In other investigations (Smith et al., 1951; Fukushima, 1969) solid samples of crude protein preparations have been exposed to organic solvent-water mixtures in order to study the influence of extraction processes on protein denaturation in the production of protein concentrates and isolates. The latter type of experiments, however, do not permit conclusions on a molecular level due to the complexity of the protein preparations examined and to the lack of proper analytical methods for solid samples.

In a previous study (Hägerdal and Martens, 1976) it was found that the apparent transition heat as determined by differential scanning calorimetry (DSC) could be correlated to the amount of "native-like" structure of proteins in solid samples and concentrated solutions. Therefore, in the present investigation DSC and water solubility analyses have been used to characterize the degree of irreversible transition of myoglobin preparations exposed to acetone of various concentrations. Acetone was added both to water solutions of myoglobin (cf Weber and Tanford, 1959; Tanford et al., 1960) and after prior mixing with water to solid samples of myoglobin (cf Smith et al., 1951; Fukushima, 1969) so the effect of these two different procedures of acetone treatment could be compared.

MATERIAL & METHODS

Myoglobin preparations produced by acetone treatements

Myoglobin from whale skeletal muscle (salt-free, lyophilized; Sigma Chemical Company) was treated with water and acetone [CHG (chromatographically homogeneous), < 1% water, BDH (British Drug House), Chemical Ltd.] using three different procedures. Thus, myoglobin preparations produced from an acetone concentration range of 10–99% (v/v) were obtained: (a) Myoglobin (50 mg, < 3% water) was dispersed in 10 ml of six different acetone-water mixtures with an acetone content of 10–95% (v/v); (b) Myoglobin (50 mg) wad dissolved in 9–0.5 ml water. Then 1–9.5 ml acetone was added instantly (within 5 sec) under vigorous stirring to give six dispersions of various acetone concentrations. The final acetone content of these dispersions was the same as in procedure (a); (c) Myoglobin (50 mg) was either equilibrated to 11 or 97% relative humidity (Hägerdal and Löfquist, 1973; Robinson and Stokes, 1959) (i.e. the protein absorbed about 5 or 30% water) or dried over P_2O_5 under vacuum (i.e. the residual water content of the protein was less then 2%) before the addition of 10 ml acetone. The final acetone content of these dispersions was around 10%. In all three procedures the dispersions were stirred vigorously for 15 min immediately after mixing to allow complete molecular transitions. In order to make the sample as reproducible as possible, all experiments were performed at 25°C in cylindrical screw-cap vessels, 2.5 cm in diamater and 5 cm high. No pH adjustments were made to eliminate the influence from counter ions and the final pH varied from 5.7–7.7. Insoluble material was sedimented at 14,500 x G for 15 min. In samples where the protein was partly dissolved, both soluble and insoluble material was collected to be analyzed. The insoluble material was washed twice with the corresponding aceton-water mixture; no material was lost during the washing. Both the soluble and the insoluble material was lyophilized. The amount of myoglobin remaining insoluble after the various acetone treatments was determined gravimetrically. The various preparations will be referred to by a designation so that $M30a_i$ designates the insoluble preparations prepared at 30% acetone by procedure (a). M99-5 designates the preparation obtained when myoglobin was equilibrated to a relative humidity equivalent to 5% water content and pure acetone was added to a final concentration of about 99%. Myoglobin treated with neither water nor acetone was used as a reference and is referred to as untreated myoglobin (cf Table 2).

[1] Present address: Dept. of Biochemistry & Biophysics, School of Medicine, Univ. of Pennsylvania, Philadelphia, PA 19104

DSC characterization of myoglobin preparations

The apparent transition heat and the transition temperature were determined by a Perkin-Elmer DSC-2 for the various myoglobin preparations after adding water to 75% of total weight (Hägerdal and Martens, 1976). In addition, thermograms were obtained for preparations M99-5 and M90b at several water contents in the range of 5–95%. The heating rate was 10°C · min^{-1} and instrument sensitivities of 1-0.1 mcal · sec^{-1} were used. The scanning range was 25–100°C. The apparent transition heat was also determined separately for the water soluble and water insoluble fractions of preparation M90b.

Water solubility of myoglobin preparations

To measure the water solubility of the lyophilized myoglobin preparations, suspensions containing either 2 or 4 mg of the preparations per ml were prepared. The suspensions were shaken gently for 24 hr at 25°C and centrifuged at 40,000 × G for 30 min. Aliquots of the resulting supernatants were incubated for 24 hr in 1M NaOH before determination of their myoglobin concentration by the absorbance at 280 nm ($\epsilon_{280}^{0.1\%}$ = 186). The water solubility was expressed as % of the suspended myoglobin preparation and could be determined within ± 5%. In some experiments suspensions containing 60 mg myoglobin preparation per ml were examined.

RESULTS

Variation of apparent transition heat with water content

The apparent transition heat values for preparations M99-5 were slightly lower than those reported by Hägerdal and Martens (1976) for untreated myoglobin over the range of water contents investigated (Fig. 1). The values were mainly constant between a water content of 30 and 80%, which also was observed for untreated myoglobin. The apparent transition heat values for preparation M90b, however, were significantly lower than for preparation M99-5 and they increased gradually with increasing water content. After preheating both preparations at 75% water content to 90°C, where the heat transition was complete, no further heat transition of these preparations was observed upon analysis at 95% water content. Thus 75% is a suitable water content when using DSC analysis to determine the amount of a preparation that has undergone irreversible transition due to acetone treatment.

When preparation M90b was heated at 25% water content to a temperature where the heat transition was complete (90°C), an apparent transition heat of 0.9 mcal/mg was recorded. If water was then added to 75%, the apparent transition heat was determined to be 1.1 mcal/mg which gives the sum of 2.0 mcal/mg for the two steps. This is in good agreement with the 1.8 mcal/mg found when the preparation was analyzed directly at 75% water content (Fig. 1). The apparent transition heat for the discrete steps of water content therefore seems to be additive for preparation M90b.

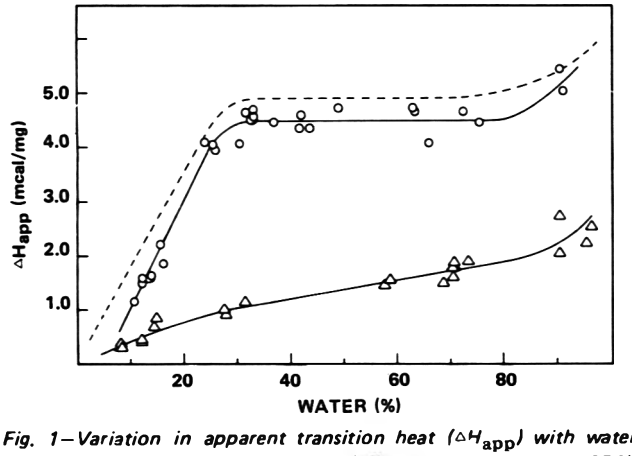

Fig. 1—Variation in apparent transition heat (ΔH_{app}) with water content. (---) untreated myoglobin (Hägerdal and Martens, 1976); (○) preparation M99-5; (△) preparation M90b. Heating rate 10°/min. The symbols represent individual measurements.

Solubility of myoglobin in various acetone-water media

When water and acetone were mixed prior to the addition to solid myoglobin [procedure (a)] most of the protein was dissolved below acetone concentrations of 30% (Table 1). At 50% of acetone and above the myoglobin was entirely insoluble. When acetone was added to a water solution of myoglobin [procedure (b)] the protein also precipitated completely at acetone concentrations above 50% (Table 1). At 50% of acetone a dispersion was obtained, which did not sediment completely at 14.500 × G. In this case the protein concentration of the supernatant varied widely for the various preparations. At 30% of acetone and below most of the myoglobin remained soluble. When myoglobin was dried over P_2O_5 or humidified to relative humidities of 11% or 97% before dispersion in acetone [procedure (c)], the protein was completely insoluble.

Characterization of myoglobin preparations obtained by acetone treatment

Since myoglobin was partially soluble in media of low acetone concentrations, two different acetone-treated myoglobin preparations, one soluble and one insoluble, were obtained from these media. The degree of irreversible transition of the preparations was determined separately by DSC and water solubility (Table 2).

An acetone treatment changes the amount of water available to the myoglobin sample and this was different for the various acetone treatments (Table 2). Procedures (b) and (c) caused a lowering of the amount of water available to the protein sample, while the reverse happened in procedure (a), where water and acetone were mixed prior to the addition to solid myoglobin. In spite of these differences between procedures (a) and (b), the water solubility and apparent transition heat for myoglobin preparations were similar when produced at corresponding acetone concentrations.

Water solubility values have been listed in Table 2 only for suspensions holding 2 mg myoglobin preparation per ml, because the water solubility determined as % of suspended myoglobin preparation did not vary significantly for suspensions holding 2, 4 or 60 mg per ml, so that a constant fraction of the preparations was soluble in water.

Apparent transition heat and water solubility. It is seen in Table 2 that the preparations obtained as insoluble material at low acetone concentrations (30% and below; $M10a_i$, $M30a_i$, $M10b_i$, $M30b_i$) had a water solubility of 5% and an apparent transition heat of around 0.5 mcal/mg. For preparations obtained at medium acetone concentrations (50–70%; M50a, M70a, $M50b_i$ and M70b) the water solubility was at a minimum and no apparent transition heat was observed. The prepa-

Table 1—Solubility of myoglobin in acetone–water media[a]

Acetone conc of the medium	Soluble myoglobin (%)	
	Acetone-water mixtures added to solid myoglobin (a)	Acetone added to water solutions of myoglobin (b)
10	96-97	98-99
30	71-78	92-93
50	0	0-86[b]
70	0	0
90	0	0
95	0	0

[a] Insoluble material of myoglobin–water–acetone dispersions holding 0.5% myoglobin was sedimented at 14,500 × G for 15 min and determined gravimetrically.
[b] This is not a real solution but a sol.

rations obtained at high acetone concentration (90 and 95%; M90a, M95a, M90b and M95b) showed a significant increase in water solubility to 69–90%, and an apparent transition heat of 1.8–3.2 mcal/mg. When the acetone concentration was increased further to about 99%, the preparations obtained (M99-30, M99-5, M99-2) showed a significant increase in apparent transition heat, while the water solubility increased only for preparation M99-2.

The myoglobin preparations obtained as soluble material at low acetone concentrations (30% and below; $M10a_s$, $M30a_s$, $M10b_s$, $M30b_s$) showed no significant variations in either water solubility or apparent transition heat (Table 2). The water solubility was around 82% and the apparent transition heat around 4.5 mcal/mg. The myoglobin preparation obtained with procedure (b) at 50% of acetone was derived from a sol rather than from a real solution. This preparation had a low water solubility (5%) and no peak of heat transition was observed. These results are similar to what was found for preparations M50a and $M50b_i$. The appearance of two different preparations at 50% acetone when applying procedure (b), and only one preparation when applying procedure (a), is probably caused by differences in the aggregation of myoglobin, which, in turn, might be due to differences in the initial water content of myoglobin in these two procedures.

It appears from Table 2 that there is a relation between water solubility and apparent transition heat for the myoglobin preparations obtained at high acetone concentrations. A graphic representation of these parameters (Fig. 2) reveals that the values for the preparations obtained by procedures (a) and (b) fit the same linear relation. The values of the preparations produced by procedure (c) seem to fit another linear relation earlier found for myoglobin samples heat treated at various water contents (Hägerdal and Martens, 1976). This line is therefore given in the figure. The relation between water solubility and apparent transition heat for the myoglobin preparations soluble in acetone-water media do not fit any of the linear relations, but for comparison these values have also been represented in the figure.

The linear relation for the heat-treat myoglobin samples (Hägerdal and Martens, 1976) which also fits the preparations obtained by procedure (c) has an intercept of 4.9 mcal/mg at 100% solubility. A regression analysis of the data of water solubility and apparent transition heat for the preparations produced by procedures (a) and (b) which did not differ significantly gave the equation:

$$\Delta H_{app} = 0.0344 \times (\text{Water solubility}) + 0.00092$$

Thus, the intercept at 100% solubility is 3.4 mcal/mg, which is significantly lower than the value for untreated myoglobin. The correlation between the apparent transition heat and water solubility was 0.96, and the standard error of estimate was 0.17.

The apparent transition heat of the water soluble fraction of preparation M90b in a 3% water solution, i.e. at 97% water content was 5.2 mcal/mg. When the water insoluble fraction was examined no peak of heat transition was found. Therefore, the same fraction of these preparations that account for the water solubility are also able to undergo thermal transition. These findings, together with the observation that a constant percentage of the myoglobin suspensions was soluble in water at concentrations up to a least 60 mg/ml imply that only a fraction of a myoglobin sample undergoes irreversible transition upon treatment with high concentrations of acetone.

Transition temperatures. As seen in Table 2 there is no significant difference in transition temperature for the various myoglobin preparations obtained by procedures (a) and (b). The mean value is 77.7°C, which is in good agreement with the transition temperature of 78°C for untreated myoglobin at 75% water content (Hägerdal and Martens, 1976). Thus, the acetone treatment did not cause a decrease in thermal stability of these preparations. The preparations obtained by procedure (c), showed slightly lower transition temperatures, with a mean value of 76.2°C.

Table 2—Characterization of myoglobin preparations obtained at various acetone concentrations by procedures (a), (b) and (c)[a]

Preparation procedures	Designation	Final acetone conc % (v/v)	Change in water content during preparation		Water solubility (%)		ΔH_{app} (mcal/mg)		T_m (°C)	
			Initial % (w/w)	Final % (w/w)	(i)	(s)	(i)	(s)	(i)	(s)
(a) Acetone-water	M10a	10	<3	91	–	82	0.5	4.4	77.9	77.2
mixtures added	M30a	30	<3	74	5	90	0.4	4.4	80.0	77.2
to solid	M50a	50	<3	56	1		0		–	
myoglobin	M70a	70	<3	35	1		0		–	
	M90a	90	<3	12	58		1.9		77.8	
	M95a	95	<3	6	88		3.2		77.6	
(b) Acetone added	M10b	10	99	91	–	76	0.5	4.6	78.5	77.6
to water	M30b	30	99	74	5	80	0.6	4.6	75.8	77.4
solutions of	M50b	50	99	56	4	5	0	0	–	–
myoglobin	M70b	70	98	35	5		0		–	
	M90b	90	95	12	60		1.8		77.3	
	M95b	95	91	6	80		2.6		78.0	
(c) Acetone added	M99-30	≈99	30(97% RH)	≈1	80		4.0		75.8	
to solid myoglobin	M99-5	≈99	5(11% RH)	≈1	88		4.5		76.3	
equilibrated to different humidities	M99-2	≈99	<2(P_2O_5)	≈1	96		4.8		76.4	

[a] Water solubility was determined from suspensions holding 2 mg myoglobin/ml water. Apparent transition heat (ΔH_{app}) and transition temperature (T_m) were determined on samples holding 75% water content. (i) and (s) designate preparations originating from material insoluble and soluble in the acetone–water media, respectively. Each preparation was made in triplicate and the measurements were made in duplicate. The variations in water solubility, apparent transition heat and transition temperature were: ± 5%, ± 0.25 mcal/mg and ± 0.5°C.

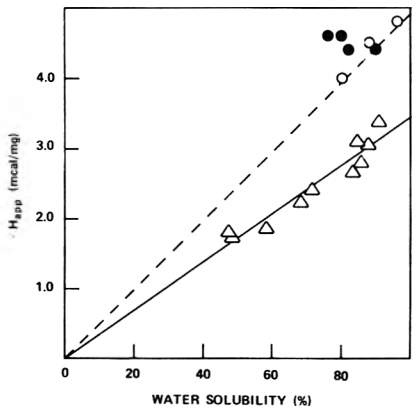

Fig. 2—Apparent transition heat (ΔH_{app}) as a function of water solubility. (○) preparations M99-2, M99-5 and M99-30. (△) preparations M90a, M95a, M90b and M95b. (●) preparations $M10a_S$, $M30a_S$, $M10b_S$ and $M30b_S$. The symbols represent mean values of duplicate measurements with a variation of ±0.25 mcal/mg in apparent transition heat and ±5% in water solubility. (----) The linear relation between apparent transition heat and water solubility for myoglobin heat treated at water contents below 30% obtained from Hägerdal and Martens, (1976).

DISCUSSION

LOW CONCENTRATIONS of organic solvents (< 30%) cause an unfolding of the protein structure in dilute solutions at pH different from pI. When the concentration of organic solvent is increased, the amount of secondary structure will increase compared to the native protein molecule (Weber and Tanford, 1959; Tandord et al., 1960; Herskowitz et al., 1970; Herskowitz and Solli, 1975). Nozaki and Tanford (1971) have suggested that this is due to the strong preference of the backbone peptide unit for a hydrophilic environment, which causes the formation of intramolecular hydrogen bonds and thus, might explain the increase in secondary structure. If, however, protein solutions are exposed to increasing concentrations of an organic solvent at or near pI, there is an increase in protein aggregation (Tanford, 1969). This was also found here when myoglobin was exposed to increasing concentrations of acetone. It also appeared from the DSC and water solubility analyses that only a fraction of the myoglobin preparations obtained at both low and high acetone concentrations underwent irreversible transition. This restricted irreversible transition at low and high acetone concentrations should be of different origin because of the differences in these acetone-water media.

When the acetone concentration is increased there is a decrease in polarity of the medium and at the same time a decrease in availability of water to the protein. The polarity of an organic solvent medium might be described by its surface tension (Bull and Breese, 1974). The surface tension for acetone-water mixtures (Morgan and Scarlett, 1917) is represented graphically in Figure 3. The availability of water for the protein sample can be expressed as the water activity of the acetone-water medium. Data on the partial pressure of water above acetone-water mixtures (Taylor, 1900) have been recalculated and are also represented in Figure 3. At increasing concentrations of acetone (<40%) the increase in partial irreversible transition of the myoglobin preparations seems to be due to the steep decrease in polarity of the medium. When the acetone concentrations increases above 90% the decrease in irreversible transition on the other hand seems to be due to the decrease in water activity, which is equivalent to a limited availablility of water to the protein. Thus, at high acetone concentrations the partial irreversible transition should be due

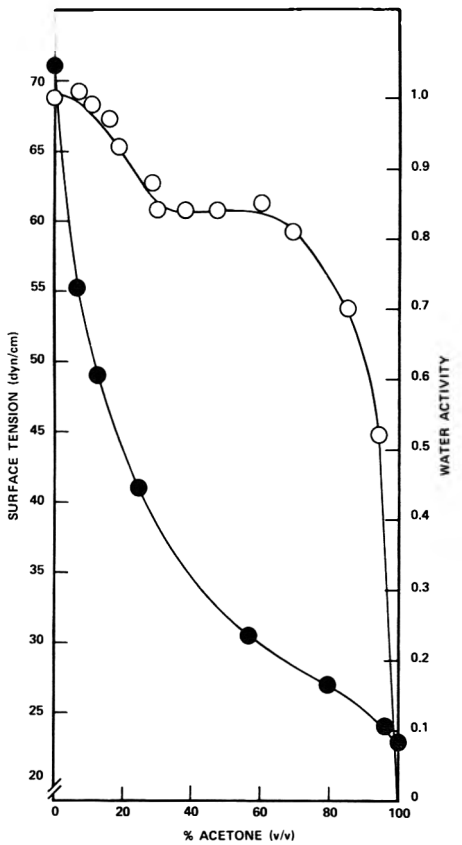

Fig. 3—Surface tension (●—●) and water activity (○—○) at various acetone concentrations. The surface tension data were taken from Morgan and Scarlett (1917), and the partial pressure values of water vapor at various acetone concentrations (Taylor, 1900) were recalculated to give the water activity.

to the lack of water medium promoting these transitions (Lewin, 1974). According to this hypothesis the complete irreversible transition of myoglobin preparations obtained at medium acetone concentrations (50–70%) should be due to the fact that the polarity is low enough to cause an unfolding of the native structure and the water activity is high enough to promote such structural transitions.

The suggestion that the water activity determines the degree of irreversible transition of the myoglobin sample at high concentrations of acetone is also in accordance with the results of a previous investigation (Hägerdal and Martens, 1976). Then it was found that the degree of irreversible thermal transition of myoglobin was proportional to the water content of the myoglobin sample at water contents below 30%, i.e. at water activities below 0.9 (Bull and Breese, 1968).

The myoglobin preparations obtained at high acetone concentrations could be divided into two groups as suggested by the different linear relations between water solubility and apparent transition heat (Fig. 2). The group obtained at 99% acetone fitted the linear relation with an intercept at 100% solubility of 4.9 mcal/mg, which is the same value as was found for untreated myoglobin when analyzed at 75% water content (Hägerdal and Martens, 1976). The group obtained at 90 and 95% acetone, on the other hand, gave a linear relation with the significantly lower intercept of 3.4 mcal/mg. Since the water-soluble fraction of preparation M90b accounted for the whole DSC signal, this fraction seems to have undergone conformational transitions, which resulted in lowering the apparent transition heat from 4.9 to 3.4 mcal/mg. It was actually found that a water solution holding 3% of the water-

soluble fraction had a lowered apparent transition heat of 5.2 mcal/mg, compared to 5.8 mcal/mg for a corresponding solution of untreated myoglobin (Fig. 1). If, however, this lowered apparent transition heat for the water-soluble fraction of preparation M90b should account for the whole difference between 3.4 and 4.9 mcal/mg, a value of 4.0 mcal/mg rather than 5.2 mcal/mg would have been expected.

Alternatively, the difference between preparations M99-5 and M90b may also be due to the water-insoluble fraction of preparations M90b. The apparent transition heat recorded in concentrated protein solutions (75% water content) is composed of endothermic heat transition and exothermic aggregation (Privalov et al., 1971), so the insoluble fraction of preparation M90b might cause an increased aggregation during the thermal analysis. In control experiments, where the water-soluble and insoluble fractions of preparation M90b were mixed, the apparent transition heat for the water-soluble fraction was not decreased.

When the variation in apparent transition heat with water content was compared for the myoglobin preparations M99-5 and M90b belonging to these two different groups (Fig. 1), it was found that preparation M90b underwent only half possible thermal transition at 25% water content, while preparation M99-5 underwent complete thermal transition at this water content. The thermal transition was, however, complete for these two myoglobin preparations at 75% water content, which was used in the DSC analyses, underlying the linear relations between apparent transition heat and water solubility. Thus, preparation M90b, belonging to the group with apparent transition heat of 3.4 mcal/mg at 100% solubility, required a much higher water content to undergo complete thermal transition, than preparation M99-5 belonging to the other group.

The present findings that the degree of irreversible transition of myoglobin reaches a maximum at medium acetone concentrations are in accordance with earlier data on crude protein preparations. Smith et al., (1951) found that organic solvent-water solutions holding 40–60% alcohols or acetone were most effective in denaturing soybean meal. The denaturing effect was determined as dispersible nitrogen after organic solvent treatment. Similar effects were noticed for soybean proteins treated with several organic solvents (Fukushima, 1969). In this case the degree of denaturation was determined by the susceptibility of the organic solvent treated proteins to proteolytic enzymes. This implies that the representation of the effect of acetone on myoglobin given in Figure 3 might serve as a general model for extraction processes involving organic solvents and globular proteins. Therefore, knowing the surface tension and the water activity of an organic solvent-water system make it possible to choose conditions so that the degree of irreversible transition that a protein concentrate or isolate undergoes during an extraction process can be predicted.

The use of the DSC analysis technique earlier developed for determination of the degree of irreversible thermal transition of proteins (Hägerdal and Martens, 1976) in combination with analyses of water solubility revealed that only a fraction of the myoglobin preparations obtained at high acetone concentrations had undergone irreversible transition. It further allowed a division of these preparations into two groups characterized by different susceptibilities to subsequent heat treatment.

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BÄRBEL HÄGERDAL[1] and BO LÖFQVIST
Biochemistry 1, Chemical Center
P.O.B. 740, S–220 07 Lund 7, Sweden

WETTABILITY AND SURFACE PRESSURE OF MYOGLOBIN TREATED WITH ACETONE

ABSTRACT

Myoglobin was exposed to acetone (50–99%) in three different ways. The wettability of the resulting preparations was determined by a screening method as well as by contact angle measurements, while the surface pressure was characterized by the decrease in surface tension of water. The wettability of the preparations could be ascribed to the surface properties of the fraction of the preparations which had undergone irreversible transition. Several other factors including roughness of the preparation, microstructure and amount of native-like molecules also had a significant influence on the apparent wettability. The surface pressure could be correlated to the amount of native-like molecules in a preparation, but was also influenced by the wettability of the preparation.

INTRODUCTION

MYOGLOBIN has been used as a model protein in an attempt to refer changes in technical properties of food proteins to basic chemical and physical features of the protein molecules. Thus, the relation between the degree of irreversible transition, the microstructure and color of myoglobin preparations produced by various acetone treatments was characterized (Hägerdal and Löfqvist, 1977; Hägerdal, 1978). During these studies it was observed that some of the preparations were poorly wettable and that this property was not directly related to their degree of irreversible transition. A myoglobin preparation could be poorly wettable even though it contained mainly native like molecules. A poor wettability has also been observed for proteins, which have been purified from lipids by extraction with organic solvents (Dubrow et al., 1973; Rasekh, 1974). In the present investigation the wettability of the myoglobin preparations has been characterized by a screening method as well as by the contact angle of water measured on a sessile droplet on the surface of the preparations pressed to tablets. The correlation between wettability and surface pressure, characterized as the decrease in surface tension of water on which the dry preparations were applied, has also been studied.

MATERIALS & METHODS

Myoglobin preparations

Preparations of myoglobin (whale skeletal muscle; salt-free, lyophilized; Sigma Chemical Company) were obtained by three different procedures (Hägerdal, 1978) involving treatment with water and acetone (CHG = chromatographically homogeneous, <1% water, BDH = British Drug Houses, Chemical Ltd.): (a) acetone and water premixed in different proportions and then added to dry myoglobin (<3% water); (b) acetone added to aqueous solutions of myoglobin; and (c) acetone added to dried or humidified myoglobin (containing 2–30% water). The myoglobin preparations were obtained in the acetone concentration range of 50–99% (v/v), the final myoglobin concentration in the dispersions was 0.5% (w/v) and the final volume was 10 ml. All the preparations were produced by rapid addition of acetone at 25°C. The resulting dispersions were then kept under heavy agitation for 15 min, lyophilized and ground in a mortar. A detailed description of the three different preparation procedures was presented by Hägerdal (1978). The various preparations will in the following be referred to by designations so that preparation M70b refers to the myoglobin preparation obtained at 70% acetone by procedure (b).

The wettability, surface pressure and degree of irreversible transition were investigated on lyophilized preparations. Preparation M90b was also separated into its water-soluble and water-insoluble fraction and the two resulting fractions were characterized separately after lyophilization.

Wettability

The wettability of the myoglobin preparations was studied by two different methods.

As a broad screening test a pinch (<0.1 mg) of the ground myoglobin preparations was gently applied to the surface of various acetone-water mixtures holding 0–90% acetone. The wettability was then characterized by the acetone concentration or the corresponding surface tension of the mixture in which the preparation sedimented within 5 min. Control experiments, in which the density of the acetone containing solutions was compensated to 1.0g/cm³ by the addition of sucrose (Analar, BDH, Chemical Ltd.), gave the same results as experiments, where no sucrose was used. Thus, the sedimentation of the myoglobin preparations could be entirely ascribed to the polarity of the acetone-water mixtures.

The wettability of some of the preparations was further characterized by the second method, namely measurement of the contact angle of a sessile droplet of double distilled water (10–20 μl) on the surface of tablets of the preparations. The measurements were made in a Ramé Hart goniometer, earlier as used for the characterization of the wettability of paper (Ekwall et al., 1958). The tablets, containing around 50 mg of the preparations, were made in a mould used for IR tablets. Pressures of 20 or 4000 kg/cm² were applied in vacuum (0.5 mm Hg) for 10 min.

Surface pressure

When characterizing the surface activity of proteins at an air-water interface, interfacial protein films are produced either by adsorbtion from a water solution holding a known amount of protein or by spreading protein solutions of known concentration at the air-water interface (Mitchell et al., 1970). None of these methods was applicable to the present study, where only small amounts of each preparation were available and where the water solubility varied widely from preparation to preparation (Hägerdal, 1978). Therefore, the surface activity of the myoglobin preparations was characterized by the ability of the dry preparations to lower the surface tension of water when applied at an air-water interface.

The surface activity will be expressed as the surface pressure, defined as the difference between the surface tension of water and the surface tension of water on which the dry preparations have been applied. A du Noüy tensiometer (A. Krüss, Optischmechanische Werkstätten, Hamburg) was used for the measurements.

The ground preparations (5 mg) were applied on the surface of 140 ml double distilled water in a beaker with a surface area of 20 cm², while the platin ring of the tensiometer was submerged in the subphase. The water was not stirred during the application of protein. Separate measurements were made after 5 and 30 min to avoid any interferring disturbances of the interfacial protein film. The variation in surface pressure with amount of material applied was studied for untreated myoglobin and preparations M83a and M83b.

Residual native-like structure

The amount of residual native-like structure in the preparations was determined by measuring the apparent transition heat (ΔH_{app}) at a water content of 75% by differential scanning calorimetry (DSC) using the method of Hägerdal and Martens (1976).

[1] Present address: Dept. of Biochemistry and Biophysics, School of Medicine, Univ. of Pennsylvania, Philadelphia, PA 19104.

Table 1—Wettability, surface pressure and apparent transition heat (ΔH_{app}; after addition of 75% water) for acetone-treated myoglobin preparations[a]

Preparation procedure	Prepn. medium Acetone conc (%)	Wetting medium: Acetone conc. (%)	Wetting medium: Surface tension (dyn/cm)	Contact angle of water (°): 20 kg/cm²	Contact angle of water (°): 4000 kg/cm²	Surface pressure (dyn/cm) measured after: 5 min	Surface pressure (dyn/cm) measured after: 30 min	ΔH_{app} (mcal/mg)
(a)	50	25	40.0	128	98	1.7	1.9	0
	70	30	38.0	132	73	0	0	0
	77	40	34.5	124	–	0	0	0.4
	83	50	32.0	114	73	5.9	7.8	0.9
	90	60	30.0	138	65	13.8	14.4	1.9
	95	70	28.5	74	64	19.9	16.0	3.2
(b)	50	0	72.4	0[b]	70	6.5	–	0
	70	10	51.0	131	96	0	0	0
	77	15	47.0	121	97	0	0	0.4
	83	30	38.0	134	–	7.8	6.6	0.8
	90	40	34.5	136	75	15.7	12.2	1.8
	95	50	32.0	102	74	19.3	16.6	2.6
(c)	≈99 (30% water)	0	72.4	0[b]	–	18.9	15.9	4.0
	≈99 (2% water)	0	72.4	0[b]	–	19.1	17.4	4.8
Untreated myoglobin		0	72.4	0[b]	71	17.1	13.4	4.8

[a] Maximum error in wettability determined by the screening method ± 2 dyn/cm; determined by the contact angle ± 5°; in surface pressure ± 2 dyn/cm; in apparent transition heat ± 0.25 mcal/mg.
[b] The water droplet goes down into the tablet and the contact angle is per definition taken as zero.

RESULTS

Wettability of myoglobin preparations

All the preparations produced in the 70–95% range of acetone concentrations could be wetted only in contact with solutions containing acetone, and therefore of lower polarity than water (Table 1). As the polarity of the acetone-water mixtures do not decrease linearly with increasing concentrations of acetone (Morgan and Scarlett, 1917) the corresponding surface tension values have also been presented in Table 1. The polarity of the wetting medium decreases with the polarity of the preparation medium, but all preparations were wetted by a medium of higher polarity than the medium from which they were prepared. It also appears that the preparations obtained when acetone-water mixtures were added to dry myoglobin had a poorer wettability than those obtained at corresponding acetone concentrations when acetone was added to aqueous solutions of myoglobin.

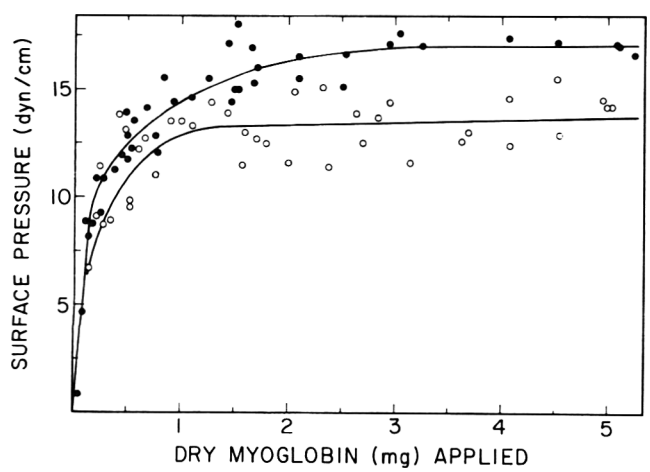

Fig. 1—Surface pressure of untreated myoglobin measured 5 (•) and 30 (○) min after application of increasing amounts of dry myoglobin at the air-water interface.

The contact angle values measured on tablets pressed at 20 kg/cm² (Table 1) were in agreement with the wettability data obtained by the screening test. Accordingly, preparation M50b which could be wetted by water gave no measurable contact angle, while preparations wetted only by water-acetone mixtures gave contact angles considerable higher than 90°.

In order to evaluate the influence of the roughness of the tablet surfaces on the contact angles (Wenzel, 1936), tablets of the preparations were also made at a pressure of 4000 kg/cm² (Table 1). The contact angle values of the preparations wetted by water, i.e., untreated myoglobin and M50b, increased to 70° while the contact angle values for the poorly wettable preparations decreased. Preparation M90b gave the same low contact angle, 75°, as untreated myoglobin, and thus becomes wetted by water when roughness is reduced.

The two preparations obtained when acetone was added to dried or humidified myoglobin (≈ 99% acetone) were both wetted by water and gave accordingly no measurable contact angle for low-pressure tablets. When investigated by the screening test for wettability over the whole range of acetone concentrations, it was found that these preparations were immediately dissolved in acetone-water mixtures holding up to 30% acetone.

Surface pressure of untreated myoglobin

The surface pressure of untreated myoglobin was analyzed and used as a reference for the acetone-treated myoglobin preparations (Fig. 1). When measured 5 min after application of myoglobin the surface pressure increased with increasing amounts of sample and reached a plateau of about 17 dyn/cm at around 3 mg. The relatively high amount of protein needed to attain a constant value only expressed that diffusion of the myoglobin molecules to the air-water interface becomes a rate limiting factor under the prevailing measuring conditions. Consequently, when the surface pressure was measured 30 min after application of myoglobin, a constant value was reached

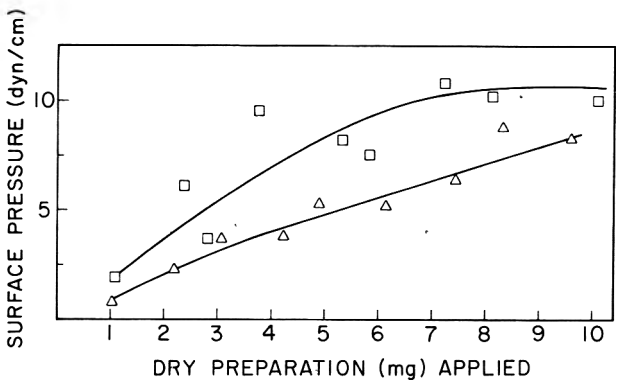

Fig. 2—Surface pressure of one preparation M83a (△) and one preparation M83b (□) measured 5 min after application of increasing amounts of dry preparation at the air-water interface.

Table 2—Wettability, surface pressure and apparent transition heat (ΔH_{app}; after addition of 75% water) of water-soluble and water-insoluble fractions of preparations M90b and M50b, immediately lyophilized[a]

Preparation	Wetting medium Acetone conc, (%)	Surface pressure (dyn/cm) after 5 min	Surface pressure (dyn/cm) after 30 min	ΔH_{app} (mcal/mg)
M90b Water-soluble fraction	0	17.4	13.3	3.4
M90b Water-insoluble fraction	40	12.6	11.2	0
Water-insoluble fraction retreated with 90% acetone	40	0	–	0
M50b (immediately lyophilized) the whole preparation	0	17.1	14.0	1.2
M50b (immediately lyophilized) water-insoluble fraction	0	5.7	–	0

[a] Maximum errors see Table 1.

already at 1 mg. In this case, however, the surface pressure only amounted to 13 dyn/cm. The protein film is, therefore, most likely subjected to surface denaturation or aging (Bull, 1972).

These methodological results on untreated myoglobin pointed out that 5 mg was a suitable amount for the investigation of the acetone-treated preparations. Measurements were made 5 as well as 30 min after application of myoglobin.

Surface pressure and residual native-like structure of the nonwettable myoglobin preparations

The surface pressure was zero for preparations obtained in 70 and 77% acetone and then increased progressively with the concentration of acetone in the preparation medium (Table 1). The surface pressure does therefore not correlate with the wettability of these preparations. On the other hand, the residual native-like structure, characterized by the apparent transition heat (ΔH_{app}), of the corresponding myoglobin preparations increased with increasing acetone concentration (Table 1). This was ascribed to the limited availability of water in these preparation media, which restricted the occurrence of irreversible molecular transition. Thus, it appears that the surface pressure of the poorly wettable myoglobin preparations is correlated to their content of residual native-like structure, i.e., their content of water-soluble myoglobin.

When the surface pressure was measured after 5 min, the preparations obtained when acetone-water mixtures were added to dry myoglobin, except M95a, gave a lower surface pressure than those obtained when acetone was added to aqueous solutions of myoglobin. This is somewhat contradictory to their correspondence in apparent transition heat (Table 1). In order to investigate these differences in further detail, preparations M83a and M83b were chosen and their surface pressure was measured 5 min after applying increasing amounts of sample. Figure 2 clearly shows that, in spite of some variation, the surface pressure of preparation M83b is higher than that of preparation M83a. In addition to the variation in surface pressure within the preparations seen in Figure 2, there was also a variation between different preparations obtained by the respective preparation procedures. Therefore, the surface pressure was measured for several preparations obtained by each one of the two preparation procedures and the results were analyzed statistically. Also this analysis showed that preparation M83b gave a significantly higher surface pressure than preparation M83a. The result might be interpreted as if the molecules in native-like conformation from preparation M83a are set free to the air-water interface at a slower rate from M83b.

Preparation M95a did not show any deviation in surface pressure (Table 1), which could be understood by the fact that this preparation has a high solubility in water.

Preparations M95a, M83b, M90b and M95b give lower surface pressure values after 30 min then after 5 min (Table 1). This seems to be due to the same aging phenomenon that was observed for untreated myoglobin (Fig. 1). Preparations M83a and M90a, on the other hand, showed a slight increase in surface pressure after 30 min, which might further support the suggestion that the diffusion of native-like myoglobin molecules from these preparations to the air-water interface is delayed.

Surface properties of the water-soluble and water-insoluble fractions of preparation M90b

The positive correlation of the surface pressure to the amount of native-like structure in the myoglobin preparations and the absence of correlation to their wettability were further investigated on the water-soluble and water-insoluble fractions of preparation M90b (Table 2). When the water-soluble fraction was extracted from the original preparation, the remaining fraction still showed as low a wettability as the intact preparation (Table 1). The water-soluble fraction had the same properties as untreated myoglobin. The water-insoluble fraction also gave a considerable surface pressure. This surface pressure could not be lowered by further extraction with water, but was completely eliminated by a second treatment with 90% acetone. It is therefore most likely that a few remaining native-like molecules, which could not be removed by water extraction, were responsible for the surface pressure of the insoluble fraction. Thus, the wettability of the intact myoglobin preparation could be ascribed to the water-insoluble fraction, while the ability to lower the surface tension of water was exercised by the water-soluble fraction.

Surface pressure and residual native-like structure of the wettable preparation M50b

Preparation M50b, which had a good wettability in water, was found to have a surface pressure of 6.5 dyn/cm (Table 1). It was not possible to register any apparent transition heat for this preparation, nor could the surface pressure be eliminated by a second treatment with 50% acetone. At this acetone concentration the irreversible transition was not limited by the availablity of water and the properties of the insoluble M50b therefore differed from those of the insoluble fractions of the poorly wettable preparations. This different type of irreversible transition as well as the above described relation between surface pressure and residual native-like structure was also demonstrated for another M50b preparation, which was ob-

tained when the preparation was lyophilized before the irreversible transition had gone to completeness (unpublished results). This preparation showed a measurable apparent transition heat and a surface pressure similar to that of untreated myoglobin (Table 2). It could also be separated into a water-soluble and water-insoluble fraction, and the surface pressure of the insoluble fraction was similar to that of M50b obtained by the normal preparation procedure.

DISCUSSION

THE WETTABILITY of synthetic polymers and polyaminoacids, characterized by the critical surface tension, has been suggested to be determined by the nature and packing of the surface atoms (Zisman, 1964; Fort, 1964; Baier and Zisman, 1970). Furthermore, it has been shown that the side chains of nonpolar aminoacids like tyrosine and tryptophane become increasingly exposed to the medium when a protein is subjected to a solvent of low polarity (Herskowitz et al., 1970). This suggested direct relation between wettability and polarity of the molecular surfaces does, however, not explain the observed wettability of the acetone-treated myoglobin preparations. A better understanding of the observed apparent wettabilities for the different preparations is achieved when factors like amount of water-soluble molecules, roughness and microstructure are considered together with the polarity of the molecular surfaces.

From the separate investigation of the water soluble and water insoluble fractions of preparation M90b it could be established that the apparently poor wettability of the intact preparation was determined by the polarity of the water-insoluble fraction, i.e., the fraction that had undergone irreversible transition and thereby had become fixed during the acetone treatment. The apparent wettability of the unfractionated preparations was, however, also determined by the amount of native-like molecules and the solubility in water of these molecules, as was demonstrated by the low contact angle value obtained for the high pressure tablets of preparation M90b. The native-like myoglobin molecules in this case act as surfactant molecules both at the air-water and the tablet-water interface. Similarly, the preparations obtained by adding acetone to humidified or dried myoglobin (≈99% acetone) appear wettable even though they have been prepared from a low polarity medium. These preparations contain a high amount of water-soluble native-like molecules.

The roughness of a surface contributes to its apparent wettability (Wenzel, 1936) so that a low polarity surface shows a better wettability while a high polarity surface shows a poorer wettability, when roughness decreases. Thus, by comparing the contact angles values for low and high pressure tablets it could be established that roughness was one factor contributing to the apparent wettability of the acetone-treated myoglobin preparations. Since preparations M50a, M70b and M77b, contain no or only a very small fraction of native-like molecules, the improved wettability of the high pressure tablets seems to be entirely related to the reduced roughness of the irreversibly denatured low polarity sample surface. The opposite relation was observed for preparation M50b, which has a surface of higher polarity and therefore shows an improved wettability when the roughness of the sample surface is increased.

The contact angles for the high-pressure tablets of preparations M50a, M70b and M77b were almost identical, indicating that the surfaces of these three preparations had the same polarity. A variation in the contact angles was, however, found for the low-pressure tablets and, thus, the degree of roughness seems to vary for these different preparations. This variation could not be related to the presence of microstructural beads (Hägerdal and Löfquist, 1977). Instead it seemed to be due to irregularities one or two orders of a magnitude above the diameter of the bead-like particles.

The surface pressure of a protein preparation at an air-water interface is a function of the ability of the protein molecules to arrange and unfold at this interface (Mitchell et al., 1970; James and Augenstein, 1966). It is, therefore, quite comprehensible that the surface pressure of the acetone-treated myoglobin preparations was positively correlated to the amount of native-like molecules in the preparation. These molecules have retained their ability to undergo such conformational transitions, as unfolding at an interface, while the molecules, constituting the fraction that has undergone irreversible transition, have lost this ability. This relation was further supported by the findings that the surface pressure of preparation M90b could be related to the water-soluble molecules.

In the method applied, the surface pressure was also determined by the diffusion rate of native-like molecules from the dry preparation to the air-water interface. Thus, the preparations obtained by adding acetone-water mixtures to dry myoglobin gave a lower surface pressure when measured 5 min after applying the material than those obtained by adding acetone to aqueous solutions of myoglobin. The reverse result was obtained when measurements were made after 30 min. The preparations obtained by adding acetone to aqueous solutions of myoglobin had a microstructure composed of mainly bead-like particles with a diameter of about 150–500 nm, while the microstructure of the preparations obtained by adding acetone-water mixtures to dry myoglobin had no such regular features (Hägerdal and Löfqvist, 1977). A microstructure composed of bead-like particles might therefore increase the diffusion rate of native-like molecules by means of an increased surface area. This might also be the reason why these preparations have a better wettability than those without regular features.

The surface pressure measurements also indicate that this method is more sensitive for determining the residual amount of native-like structure in a preparation than is differential scanning calorimetry (DSC). This was demonstrated by the preparations obtained at 50% acetone, which gave a surface pressure without showing a detectable peak of heat transition. On the other hand, a minor DSC signal is registered for the poorly wettable preparations M77a and M77b, which did not give any surface pressure. In this case it is most likely the poor wettability and thereby the low diffusion rate of the native-like molecules from the preparation to the air-water interface that determines the surface pressure.

The apparent surface properties of acetone-treated myoglobin preparations having undergone various degrees of irreversible transition were found to be determined by a delicate balance of different parameters including amount of native-like structure, polarity of the surface, microstructure and roughness. These parameters could be related to different factors of the acetone treatment. This model study on myoglobin and acetone therefore points out the possibility to obtain protein concentrates and isolates with desired surface properties by a proper choice of the organic solvent concentration as well as the way of mixing the components in the extraction process.

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FLORENCE S. BURNETTE and GEORGE J. FLICK JR.
Dept. of Food Science & Technology, Seafood Processing Research & Extension Unit
Virginia Polytechnic Institute & State University, P.O. Box 369, Hampton, VA 23669

ACTIVITY AND RESISTANCE TO THERMAL INACTIVATION OF PEROXIDASE IN THE BLUE CRAB (Callinectes sapidus)

ABSTRACT

Peroxidase is one of the most heat resistant enzymes and may cause undesirable quality changes in thermally processed foods. Peroxidase activity and its resistance to thermal inactivation in fresh and pasteurized lump, claw and flake meat of both male and female blue crabs was determined spectrophotometrically. Male crabs usually exhibited a greater initial activity (ΔOD_{460}/min/mg N) than did females of equal size. Heat inactivation curves indicated two straight line decreasing segments which varied by rate of descent. D values obtained for the enzyme based on the second straight line segment were D_{80} = 47 min, D_{110} = 18.2 min and D_{150} = 11.2 min. A "z" value of 92°F was also obtained for the enzyme.

INTRODUCTION

PEROXIDASE is a member of a large group of enzymes called the oxidoreductases and is considered to have an empirical relationship to off-flavors and off-colors in food products (Joslyn and Bedford, 1940; Joslyn, 1949; Joslyn, 1966; Bedford and Joslyn, 1939; Weaver and Hautala, 1970). Only limited research has been conducted with peroxidase in animal tissue and seafood products, particularly in the blue crab, *Callinectes sapidus*. Loss of flavor and the development of off-flavors due to oxidative and enzymatic changes in crab meat has been a problem for many years. The use of antioxidants has not previously been proven very effective with seafood products (Sweet, 1973). Also, additives are usually ineffective in preventing enzyme-induced discoloration or off-flavors in foods (Furia, 1968). Therefore, a study of peroxidase was undertaken to obtain a better understanding of the chemical and physical properties of the enzyme in crab meat and to establish an optimum time-temperature relationship for pasteurization to help maintain quality of crab meat during storage. Presently, crab meat is pasteurized at an internal temperature of 185°F in the geometric center of the can for 1 min. The product can be stored up to 6 months under refrigerated conditions (32–36°F). However, with an extended storage period, off-flavors may develop. By inactivating peroxidase in crab meat and preventing or retarding its regeneration the shelflife of the product could possibly be extended while maintaining good quality; or quality during the shelflife could be improved.

MATERIALS & METHODS

Test organism

Live blue crabs (*Callinectes sapidus*) used in this study were supplied by Graham and Rollins, Inc., Hampton, VA. Pasteurized lump (backfin), flake and claw meat samples processed at P.K. Hunt and Son, Hampton, VA, were purchased from a seafood market also located in Hampton, VA.

Sample extraction

Ten grams of meat were removed from a crab, rapidly weighed, placed into a blender for 30 sec at high speed. The homogenate was filtered through a Whatman No. 42 filter paper. Since only a crude extract was needed for the qualitative studies, this crude extraction was considered adequate.

Enzyme assay

Peroxidase activity in all samples was determined following the procedure described by Burnette and Flick (1977). The substrate consisted of 0.1 ml of H_2O_2 (30%) in 100 ml of 0.01M potassium phosphate buffer (pH 6.0). A 1% (w/v) solution of o-dianisidine in methyl alcohol was used as the hydrogen donor during the enzymatic reactions since the oxidized dye is chromogenic. Consequently, the rate of the reaction could be spectorphotometrically determined by measuring the rate of oxidized dye formation at maximum wavelength adsorption.

The enzyme assays were performed at 25°C in a Perkin-Elmer double beam Coleman model 124 spectrophotometer equipped with a Perkin-Elmer model 56 recorder (Coleman Instruments Division, Maywood, Ill). The total volume of reaction mixture in each cuvette was 3 ml (2.8 ml of 0.01M phosphate buffer containing H_2O_2, 0.1 ml of o-dianisidine solution and 0.1 ml of extract). The reactions were initiated by the addition of 0.1 ml of the crude extract. The rates of the reactions were measured as the initial change in absorbance (1 cm light path) at a fixed wavelength of 460 nm per unit of time and expressed as ΔOD_{460}/min/mg N.

Influence of pH

The influence of pH on enzymatic activity was determined by changing the pH of the phosphate buffer by the addition of 1.0N HCl or 1.0M NaOH and measuring ΔOD_{460}/min/mg N. Increments of 1.0 pH unit were tested over the pH range from 3–10.

Chemical analysis

The percent nitrogen of raw and pasteurized blue crab extracts was determined using the macro-Kjeldahl method (AOAC, 1975). Peroxidase activity was based on ΔOD_{460}/min/mg N.

Gel-electrophoresis

Polyacrylamide disc gel electrophoresis was performed by combining the procedures of Nagle and Haard (1975) and Lee et al. (1976). The whole gel consisted of two portions: stacking gel (upper gel) and separating gel (lower gel). The solutions for both gels were all made to a final volume of 100 ml. For a run of 13 gels, the separating gel was made by mixing 3.0 ml of a solution [A30g acrylamide (Eastman X-5521) plus 0.8g bis-acrylamide], 3.0 ml of solution B [24 ml 1.0N HCl, 18.18g THAM (Tris (hydroxymethyl) aminomethane) (pH 8.9) and 0.4 ml TEMED (N,N,N',N'-tetramethylenediamine] and 6.0 ml of solution C (1.0 mg riboflavin).

Following polymerization of the separating gel, the stacking gels of 1 cm in length were formed on top of the separating gels. The stacking gels were prepared by mixing 2.0 ml of solution D (5.0g acrylamide plus 1.25 g bis-acrylamide), 1.0 ml solution E (12.8 ml 1.0M H_3PO_4, 2.85g THAM and 0.1 ml TEMED) and 1.0 ml of solution F (2.0 mg riboflavin). After complete polymerization of the gels, they were placed in an electrophoresis unit and immersed in a bath buffer (pH 8.3) containing 0.025M THAM and 0.192M glycine. A predetermined amount of the prepared sample (0.1 ml) was applied to the top of each gel. Five milliliters of bromphenol blue (0.05%) were placed in the upper buffer to serve as a tracking dye. Electrophoresis was carried out using a current of 1.0 mA per gel until the bromphenol blue marker dye passed through the stacking gel (about 1 hr). The current was then changed to 2.0 mA per gel until the marker dye was approximately 1.0 cm from the bottom of the gel (about 3 hr). After electrophoresis, the gels were removed from the tubes and stained overnight in test tubes at room temperature with an iodide stain (0.02M potassium iodide, 0.125M phosphate citrate buffer and 5×10^{-4}M H_2O_2 (Murphy, 1970). The gels were rinsed and placed in a destainer (Isco Model 422 rapid destainer tank) filled with 7% acetic acid and destained until distinct blue-black bands could be seen. After complete destaining, the gels were photographed and drawn.

Thermal enzyme inactivation study

Two-ml samples of the crude crab homogenate were placed in 10-inch long, 1/4-inch diameter glass tubes and subjected to different heating temperatures (80, 110, 150 and 200°F) for varying time periods (1, 2, 8, 20 and 30 min) to determine percent enzyme activity remaining after heating in a hot oil bath (Haake Model FS-2). Following the desired heating time, the tubes were removed and transferred to an ice-water (32°F) bath in order to cool the homogenate. The homogenate was then centrifuged at 20,384 × G for 1.0 min and supernatant fluid tested for enzyme activity.

Heat penetration curve

One pound of commercially hand-picked crab meat was placed in each of 6 cans (301 × 401) which contained one copper-constantan thermocouple inserted into the geometric center of the can (cold point). The thermocouples were attached to a temperature recorder (Honeywell 24-point recorder) which monitored the temperature of each thermocouple every min. The cans were filled with the crab meat leaving 1/8 inch headspace and sealed with a commercial can seamer. The canned crab meat was then placed in a hot water bath at 195°F (Napco Model 220) until an internal can temperature of 185°F was obtained. The cans were removed and allowed to cool in an ice-water bath (32°F) until internal can temperature was 60°F.

RESULTS & DISCUSSION

PEROXIDASE ACTIVITY in three market styles of crab meat was found to be active at the high and low ranges of the pH scale (Fig. 1). The high relative percent activity at both ends of the pH range could be due to the denaturation of some proteins exposing heme groups (Eriksson and Vallentin, 1973; Lu and Whitaker, 1974) and to the release of NH_3 which will increase the rate of peroxidation of o-dianisidine (Fridovich, 1963). Weinryb (1966) indicated that the active site of peroxidase (using horseradish peroxidase) involved an apoprotein as well as a heme group.

Peroxidase activity was measured at pH 6.0 which according to Figure 1 was not in the range of maximum activity. This was considered important since at maximum activity the reaction would be too fast to give reliable data for initial activity. The actual pH of raw crab meat determined immediately following extraction fell in a range from pH 6.0 to pH 7.0, therefore, the enzyme assays were performed at the pH of the extract itself.

Polyacrylamide starch gel electrophoresis performed with male and female blue crab extracts indicated nine isozyme bands for the male and eight for the female crab (Fig. 2). The male crab used to prepare the gel shown in Figure 2 weighed 115g, the female 116g. The visualization of isoperoxidases in polyacrylamide gels in strongly influenced by substrate concentration used in the "staining procedure" (Novacky and Hampton, 1968). A relatively high substrate concentration may actually mask some of the sites of enzyme activity because of substrate or product inhibition. Since varying enzyme concentrations will also yield greater or fewer bands, there is a need to obtain an optimum crab extract concentration for peroxidase activity determination.

Polyacrylamide starch gels were also used to determine whether the weight of the crab was relative to the number of bands produced and to the size and intensity of these bands (Fig. 3). Female crabs which weighed 155, 121 and 96g were tested. Eight bands appeared on the gels containing an extract from the 155g and 121g females, however, only seven bands appeared on the gel containing an extract from the 96g female. The mobility of the bands produced from the three female crab extracts varied, as did the intensity of the bands. The larger the crab, the more isozymes detected. Also, the greater the concentration of enzyme in the extract, the darker the bands produced in the gel. The spectrum of peroxidase isozymes revealed on starch gel and acrylamide gel electrophoresis has been shown to be relatively constant for a given species under specific conditions and is apparently related to age, species, variety, growth regulating substances and disease (Novacky and Hampton, 1968). Previous studies showed that in several cases isoperoxidases that were not detected in young healthy plant tissues were later detected in tissues upon aging or after infection. Such changes have been attributed to biosynthesis of new proteins (Novacky and Hampton, 1968). The "new" isoperoxidases may simply represent an increase in activity of isoperoxidases normally present at low activity or low concentrations in younger tissues.

Additional isozyme bands appeared on the gels prepared from extracts of the 155, 121 and 96g female blue crabs by extending the time of electrophoresis by 1 hr. Isozymes are separated on the basis of size, charge and molecular weight,

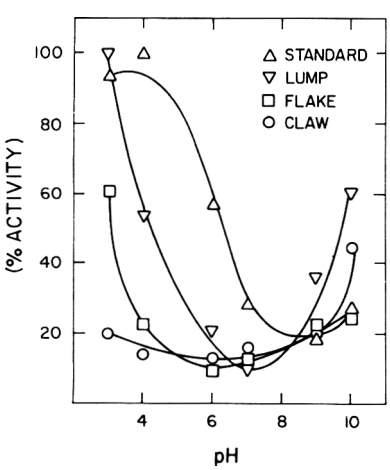

Fig. 1—Percent peroxidase activity in a purified horseradish peroxidase standard and in extracts of lump, flake and claw meat from the blue crab at different pH intervals. The data are based on the percent activity of the purified standard at pH 4.0 equaling 100%.

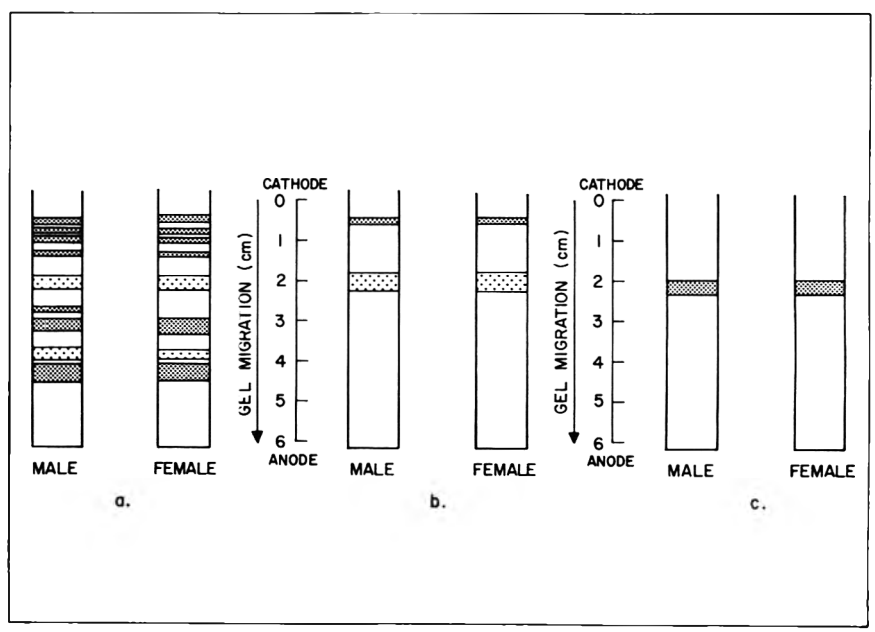

Fig. 2—Diagrams of gel patterns for peroxidase in extracts of male and female blue crabs: (a) Raw extracts; (b) Extracts cooked at 200°F for 4 min; (c) Extracts cooked at 200°F for 30 min.

therefore. any two or more isozymes which are quite similar in physical and chemical properties may appear as one band in the usual 4-hr electrophoresis period.

A comparison of the initial peroxidase activity in male and female blue crabs is given in Table 1. Male crabs usually exhibited a greater initial activity (ΔOD_{460}/min/mg N) than did females of equal size. Also, the larger the crab within a given sex, the more activity was detected. A direct relationship exists between the size and age of male crabs, therefore, one can postulate that the older the crab, the more peroxidase activity will be present. This relationship between size and age can only be applied to immature females or between mature and immature females. It is not possible to correlate age with size among mature females. This relationship between size and age is in agreement with Novacky and Hampton (1968).

Information as to relative activity in different sexes of the blue crab is not advantageous to the seafood processor at this time since no discrimination is made as to sex prior to picking or pasteurizing the crabs. These are basically informative data that could be of possible future significance.

A comparison of the six gels shown in Figures 2 reveals that as the time of heating is increased at a specific temperature (200°F) there is a loss of isozyme bands and the remaining bands become less intense. Maier et al. (1955) have shown that various isozymes of peroxidase have different heat constants. Heat will cause the denaturation of proteins and the longer the heat treatment at a particular temperature, the greater the loss in activity. This agrees with research data published by Zoueil and Esselen (1959).

Data on the effect of time and temperature on the initial concentration of peroxidase where log C equals percent activity in blue crabs is given in Figure 4. There was a decrease in peroxidase activity for the first minute of heating, than an increase in activity for the next 7–9 min for all temperatures included in this study, followed by another decrease in activity. Yamamoto et al. (1962) suggested that during heat inactivation of peroxidase in sweet corn, two independent first-order inactivation reactions took place. The initial inactivation line represented inactivation of the heat-labile enzyme and the second represented inactivation of the heat-stable enzyme.

Table 1—Peroxidase activity in male and female blue crabs

Sex	Crab size[a] (in.)	Crab wt (g)	ΔOD_{460}/min/mg N
Male	5-1/2	145	11.2
Female	6	144	7.6
Male	5	125	6.8
Female	5	136	5.2

[a] Crab measured from point-to-point of the carapace

Another reason suggested by Yamamoto et al. (1962) for the two reactions was that a protective enzyme-substrate complex was formed which affected the heat stability of a portion of the enzyme. The initial rapid inactivation represented the disappearance of the unprotected peroxidase. From these data, the following D values (time required for a 90% reduction in enzyme activity) were determined using the second decreasing line segment: D_{80} = 47 min, D_{110} = 18.2 min and D_{150} = 11.2 min.

The heat inactivation reactions for peroxidase in the blue crab appear to be time dependent rather than temperature dependent. The increase in activity after approximately 1 min could be due to the release of prooxidants in the crabs which will increase the rate of oxidation of o-dianisidine (Fridovich, 1963).

Figure 5 presents the relationship between temperature and log D values. A "z" value of 92°F has been determined for peroxidase in crab meat by using the D values calculated for the second decreasing straight line segment of the enzyme at different heating temperatures. By using this "z" value plus data from the heat penetration curve (Fig. 6) for different types of crab meat in cans of specified size, a time and temperature can be determined for pasteurizing crab meat whereby all peroxidase can be inactivated.

Peroxidase will regenerate in pasteurized crab meat. The histogram in Figure 7 shows that after 2 wk of storage the pasteurized backfin, flake and claw meat showed very similar

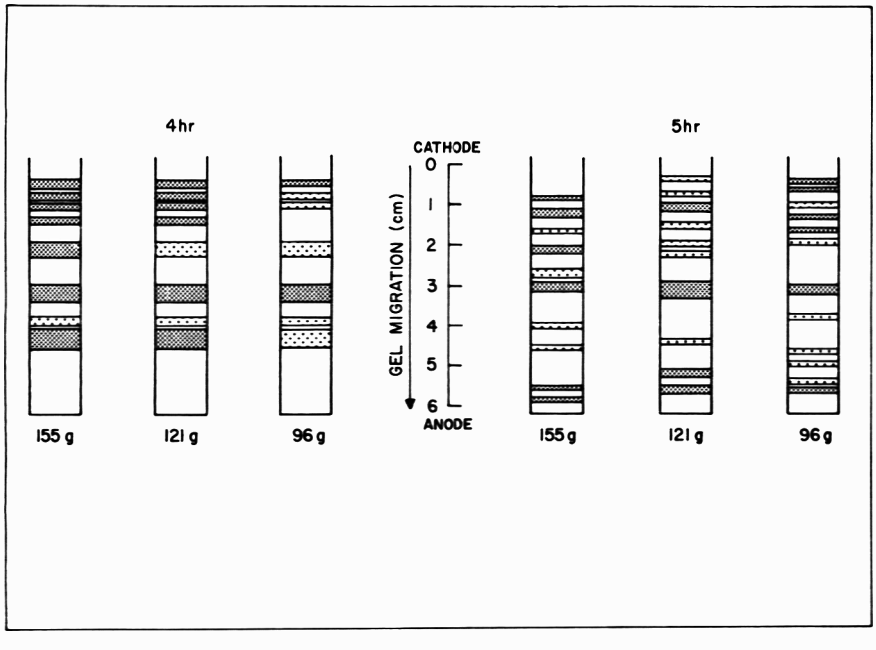

Fig. 3—Diagrams of gel patterns for peroxidase in fresh extracts of female blue crabs of various weights (155, 121 and 96g) following 4 and 5 hr of electrophoresis.

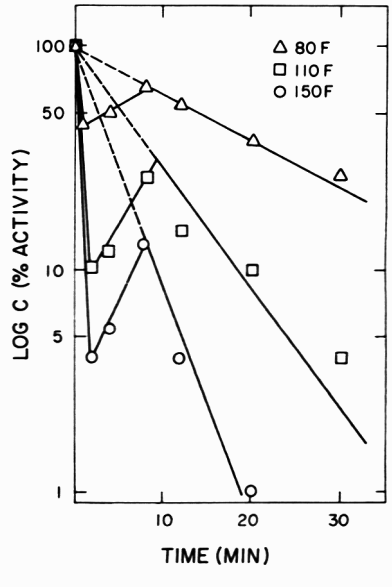

Fig. 4—Percent peroxidase activity in raw backfin extracts following heat treatments at 80, 110 and 150°F for 2, 4, 8, 12, 20 and 30 min.

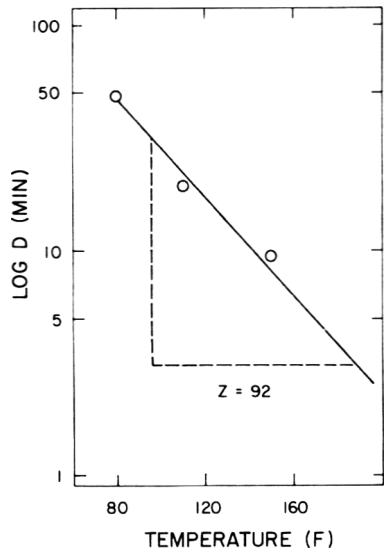

Fig. 5—Relationship between log D values and temperature for raw blue crab peroxidase.

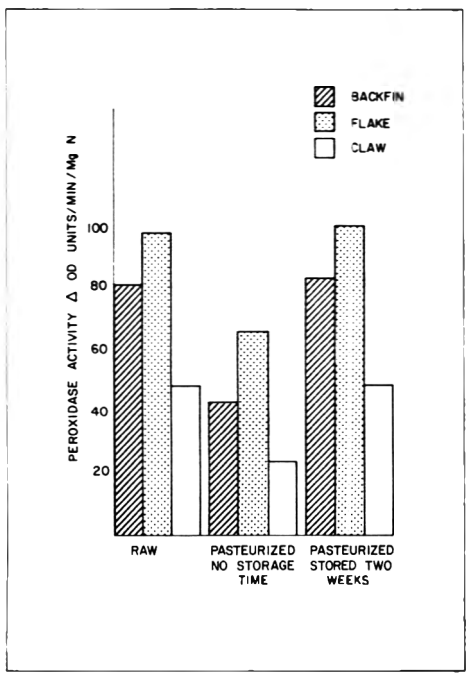

Fig. 7—Comparison of peroxidase activity in raw, pasteurized and stored pasteurized crab meat extracts.

peroxidase activity when compared to the raw backfin, flake and claw meat. In all cases, the flake extract exhibited the most activity and the claw extract the least.

Peroxidase in blue crabs is heat stable and not inactivated by thermal processing at 250°F for 12 min which is the heat treatment used prior to pasteurization to aid in the removal of crab meat from the shell and subsequent pasteurization to an internal temperature of 185°F for 1 min. An almost 100% regeneration of the enzyme in crab meat that was processed following commercial times and temperatures proves that present processing methods are inadequate to effect a significant reduction in activity from the natural level. It is possible, therefore, that peroxidase may cause undesirable changes

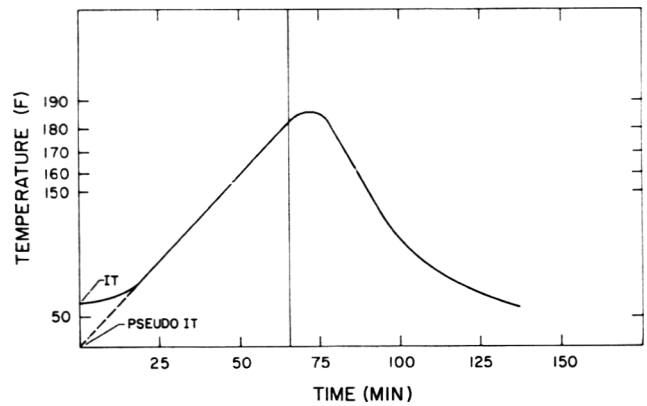

Fig. 6—A heat and cooling penetration curve for canned blue crab meat (backfin) obtained from the geometric center of a 401 X 301 can during water bath heating at 195°F and cooling at 32°F. (It represents initial temperature).

during refrigerated (32–36°F) or frozen (32°F) storage. By extending the times and temperatures required to inactivate peroxidase may cause denaturation of the proteins in crab meat and destroy the texture of the product. Due to this problem, it may not be possible to satisfactorily inactivate peroxidase in blue crab meat and still retain acceptable quality.

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YU BANG LEE, DAVID A. RICKANSRUD, ELROY C. HAGBERG AND RICHARD H. FORSYTHE
Campbell Institute for Food Research, Campbell Place, Camden, NJ 08101

POSTMORTEM BIOCHEMICAL CHANGES AND MUCSLE PROPERTIES IN SURF CLAM (Spisula solidissima)

ABSTRACT

Postmortem biochemical changes and their relationship to the eating quality of surf clam muscle were studied. Shear value and cook loss continuously increased with the length of storage time up to 9 days postmortem and the rate of increase was much greater after 4 days of storage. A rapid breakdown of high energy phosphate compounds and muscle glycogen with a concomitant fall in pH after 4 days postmortem suggested that rigor onset occurred at 4 days and was completed between 6 and 9 days postmortem. The biochemical changes in surf clam muscles were much slower than those reported for the muscle systems from other species and were in a close coincidence with the change of shear values and cook loss. Another significant finding was that pyruvate instead of lactate was accumulated as glycolysis proceeded.

INTRODUCTION

POSTMORTEM BIOCHEMICAL CHANGES in muscle have a profound effect on the eating quality of meat from mammals, fish, poultry and crustaceans and extensive reviews and research data are available on the development of rigor mortis and associated biochemical changes in the muscle of these animals (Bendall, 1960; De Fremery, 1966; Newbold, 1966; Tarr, 1966; Sidhu et al., 1974). However, comparable studies on clam muscle have been few and primarily limited to the breakdown of nucleotides (Arai and Saito, 1961) and the bacteriological quality (Hoff et al., 1967).

Clams are the most affordable shellfish which can be dug up along any unpolluted sand or mud flats. Surf clam (*Spisula solidissima*, also called sea clam) found along the Atlantic coast are increasingly being processed for food use. The present commercial processing involves steam shucking and extensive washing to remove sand and foreign materials. The final product is quite tough and chewy. A better understanding of postmortem biochemical changes and their relationship to eating quality might help in establishing the best processing procedure.

The objective of the present study was to investigate the postmortem changes of high energy phosphate compounds and glycolytic intermediates as related to the onset of rigor mortis and tenderness in surf clam.

EXPERIMENTAL

Animals and muscle samples

Live surf clams, *Spisula solidissima*, dredged off the coast of Virginia were transported by air to the Campbell Institute for Food Research Laboratory, Camden, and held in a 4°C cooler. All clams were used within 2 days after catching and their average shell length was 13 cm. One hundred twenty clams were hand shucked and foot and adductor muscles were immediately removed and rinsed in cold 3% NaCl solution. About 60-g samples of adductor and foot muscles were separately placed in polyethylene bags and stored in the cooler at 4°C. At different storage periods of 0, 4 and 8 hr, 1, 2, 4, 6 and 9 days, four bags of foot muscle and three bags of adductor muscle were taken for the analyses of metabolic intermediates and for the meaurements of shear value and cook loss.

The whole experiment was repeated with 120 additional clams and the data presented in this paper are combined results of two trials.

Shear value and cook loss measurements

Foot and adductor muscles were cooked separately in a boiling water bath for 20 min, cooled and drained. Extra surface moisture was blotted with tissue paper and percent cook loss was determined. The cooked samples were then ground through a coarse plate of hand-operated food chopper and shear values were measured on 20g of this ground sample using a Kramer shear press.

Analysis of metabolic intermediates

Foot and adductor muscles were rapidly frozen in liquid nitrogen, pulverized according to the procedure described by Borchert and Briskey (1965) and stored on dry ice until analyses were completed.

Two grams of pulverized muscle were placed in a cold polyethylene centrifuge tube and extracted with 4.4 ml of 0.6N perchloric acid. After centrifugation at 12,000 × G for 10 min, the perchloric acid extract was neutralized with 5M K_2CO_3 using methyl orange as indicator, while stirring. After precipitation of potassium perchlorate, the clear supernatant was used for the subsequent enzymatic analyses.

Glucose-6-phosphate, ATP and phosphoarginine were determined sequentially by the enzymatic procedure described by Lemprecht and Trautschold (1963), pyruvate, ADP and AMP by the method of Adam (1963) and lactate by the method of Hohorst (1963).

For glycogen determination, it was first isolated by treating pulverized samples with a potassium hydroxide-ethanol procedure described by Dalrymple and Hamm (1973). The isolated glycogen was solubilized in distilled water and determined using phenol-sulfuric acid (Dubois et al., 1956).

Measurement of pH

pH was measured on powdered muscle samples homogenized in 10 volumes of 5 mM sodium iodoacetate as described by Marsh (1952).

RESULTS & DISCUSSION

Tenderness of clam meat

Changes in shear value and cook loss of foot and adductor muscles during storage are shown in Figure 1. Three different stages were observed in the rate of change in shear values and cook loss: (1) fast increase until 8 hr postmortem; (2) slow increase from 8 hr to 4 days postmortem; and (3) fast increase after 4 days of storage up to 9 days. The initial fast increase in shear and cook loss could be attributed to the excitation and temporary shortening caused by initial handling (shucking, excision, washing). The following slow increase suggested that the muscle system was stabilized and undergoing slow metabolic processes. The final stage of fast increase indicated that muscle probably reached the stage of rigor, resulting in contracted and shortened state. These changes in shear value and cook loss during storage are very closely related to the biochemical changes as discussed later.

Of significance was the finding that shear values were still increasing even at 9 days of storage and no conditioning (resolution of rigor) took place during this time period. Such a development was much slower than bovine and poultry muscles which showed maximum shear values at 12 hr and 2–4 hr, respectively, followed by a decrease in shear values (Goll et al., 1964; De Fremery, 1963). It was also slower than scallop muscle which developed maximum stiffening at about 4 days of storage at 5°C, followed by softening (Dyer and Hiltz, 1974).

Adductor muscle had higher shear value but lower cook loss than foot muscle. Further study is needed to define the length of storage time required to reach maximum shear value.

Postmortem changes in high energy phosphate compounds

Postmortem changes of phosphoarginine, ATP, ADP and AMP are shown in Figure 2. Phosphoarginine (PA) instead of creatine phosphate was found in clam muscle. The initial values for PA were comparable to creatine phosphate values reported for vertebrates (Lee et al., 1971; Newbold, 1966), but were much lower than phosphoarginine values for rock lobster muscle (Sidhu et al., 1974). Initial ATP values in surf clam were comparable to those reported for pig (Lee et al., 1971) and fish (Tarr, 1966); slightly higher than those reported for king crab, scallop, abalone and squill (Arai and Saito, 1961; Porter, 1968); lower than those for rock lobster (Sidhu et al., 1974), squid and prawn (Arai and Saito, 1961).

Breakdown of phosphoarginine was very rapid for the initial 4 hr after shucking, then at a slower rate thereafter. It required 6 days for phosphoarginine to drop to a negligible concentration, which is much longer than 2–6 hr reported for pork, beef, poultry and fish (Lee et al., 1971; Newbold, 1966; De Fremery, 1966; Partman, 1965), and also longer than 72 hr reported for rock lobster muscle (Sidhu et al., 1974).

Breakdown of ATP was slow until 4 days and 2 days of storage for foot and adductor muscle, respectively, followed by much faster breakdown thereafter. As might be expected in view of the function of PA in furnishing high energy bonds to resynthesize ADP into ATP, the high levels of ATP were maintained until the concentrations of PA had fallen significantly.

Since the loss of muscle extensibility or stiffening of muscle is closely related to the breakdown of ATP (Newbold, 1966), a close coincidence between the rapid breakdown of ATP and rapid increase of shear values in surf clam muscle was expected. Although rigor measurements using a rigorometer were not conducted in the present study, the data on the breakdown of ATP and PA strongly indicated that rigor onset occurred at about 4 days postmortem and was completed between 6 and 9 days postmortem. Newbold (1966) stated that many published data showed a close coincidence between the completion of the physical changes and the virtually complete disappearance of creatine phosphate. In surf clam muscle, the virtually complete disappearance of PA occurred at 6 days postmortem. The time course of rigor development in surf clam muscle appeared to be much slower than that reported for other animals.

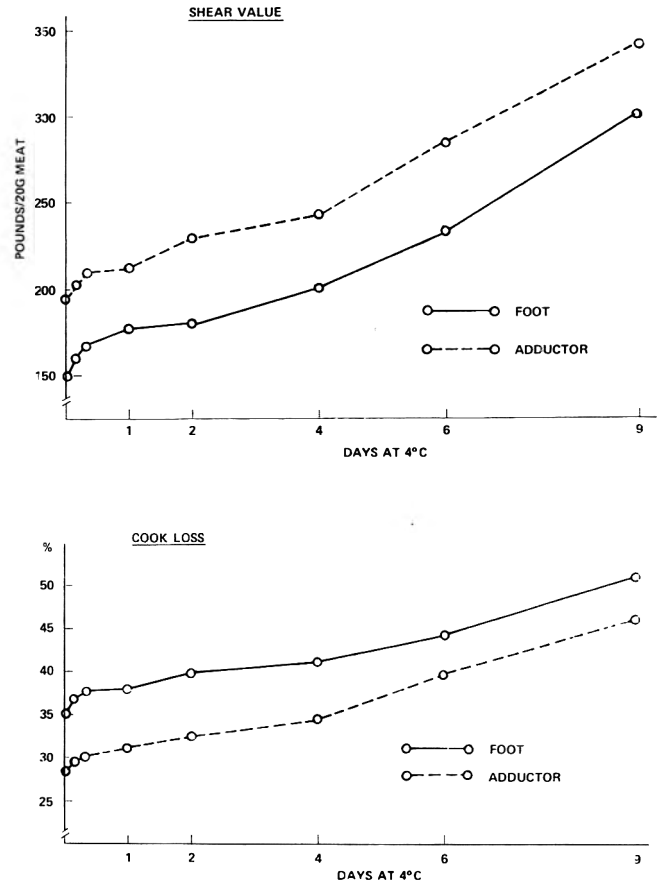

Fig. 1—Shear value and cook loss of surf clam muscle during storage at 4°C.

The level of ADP gradually increased until 6 and 4 days of storage for foot and adductor muscle, respectively, and then decreased thereafter. The maximum peak of ADP concentration occurred at the midpoint of fast breakdown of ATP. The accumulation of AMP reached the highest level at 6 days post-

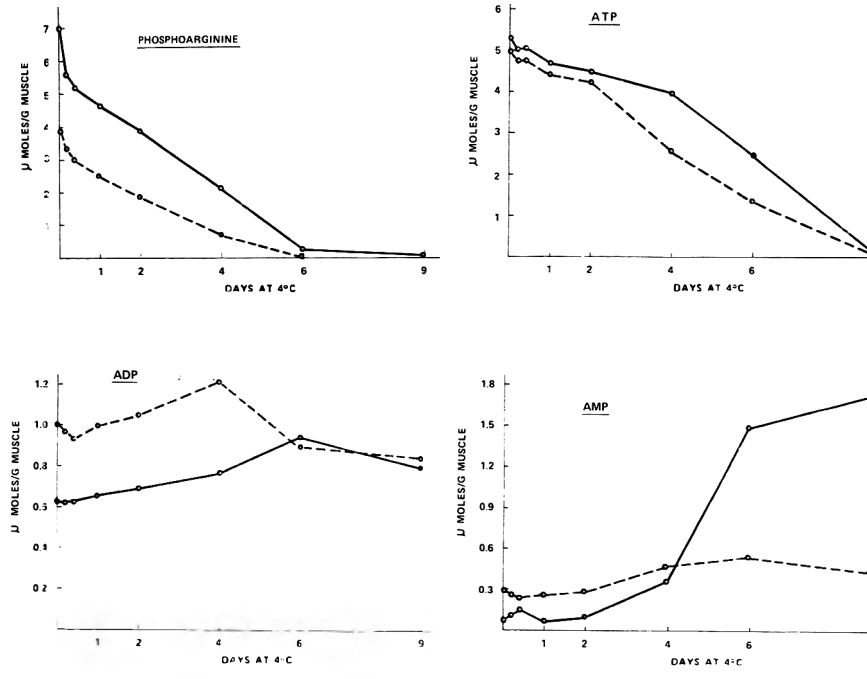

Fig. 2—Postmortem changes of high energy phosphate compounds: o——o foot muscle; o---o adductor muscle.

mortem for both the foot and adductor muscles, although the increase of concentration was much greater for the foot muscle. The decreased AMP concentration in the adductor muscle and the reduced rate of AMP increase in the foot muscle after 6 days of storage suggested that AMP was further degraded possibly to IMP and then inosine as reported by Arai and Saito (1961) and the rate of breakdown was much greater in the adductor muscle.

Postmortem glycolysis

Postmortem changes in the level of glycogen, glucose-6-phosphate and pyruvate, and pH change are shown in Figure 3. Lactate was found to be absent in surf clam muscle.

The initial average glycogen content of 35 mg/g muscle was much higher than those reported for porcine muscle (Lee et al., 1971; Dalrymple and Hamm, 1973), bovine muscle (Dalrymple and Hamm, 1973) and rock lobster muscle (Sidhu et al., 1974).

The level of glycogen content remained almost unchanged until 4 days of storage, followed by a fast breakdown thereafter. A fairly high glycogen content (about 50% of the initial level) still remained even at 9 days postmortem. Adductor muscle contained higher glycogen than foot muscle. As glycolysis proceeded, pyruvate concentration increased rapidly after 4 days postmortem with no detectable lactate. This indicated that surf clam muscle might possess a different metabolic pathway from other muscle systems which normally produce lactate as an end product of glycolysis. To verify the lack of lactate, the lactate dehydrogenase (LDH) activity was measured. The activity was about 1 unit per min per gram wet tissue which is remarkably low as compared to 200–700 units in rat muscle (Baldwin et al., 1973) and 1400 units in chicken breast muscle (Lee et al., 1976). A very low LDH activity could be either due to the lack of enzyme itself or due to the possible presence of enzyme in an inactive form, which must be verified in a future study.

The existence of alternate pathways of glycogen breakdown which led to the production of compounds other than lactate were also reported in cod muscle (Burt, 1966) and in rock lobster muscle (Sidhu et al., 1974). A further study is needed to determine possible alternate glycolysis pathways in surf clam muscle.

The breakdown of glycogen and accumulation of pyruvate were well reflected in postmortem pH change. There was an initial rapid fall until 8 hr postmortem, followed by a very slow change until 4 days, and then, a rapid decrease to 5.6–5.7 at 9 days of storage. As in the case of breakdown rate of high energy phosphate compounds, glycolysis rate in surf clam muscle is remarkably slower than that reported for other animals (Newbold, 1966; Lee et al., 1971; Sidhu et al., 1974). The data also indicated that adductor muscle showed a slightly faster glycolysis rate than foot muscle.

It was concluded from the foregoing data that the physical and biochemical changes in surf clam muscles were much slower than those reported for other muscle systems and did not occur to an appreciable extent until 4 days of storage at 4°C. The faster breakdown of ATP and glycogen after 4 days postmortem suggested that rigor onset occurred at 4 days and was completed between 6 and 9 days postmortem. These biochemical changes were in a close coincidence with the changes of shear values which increased greatly after 4 days of storage.

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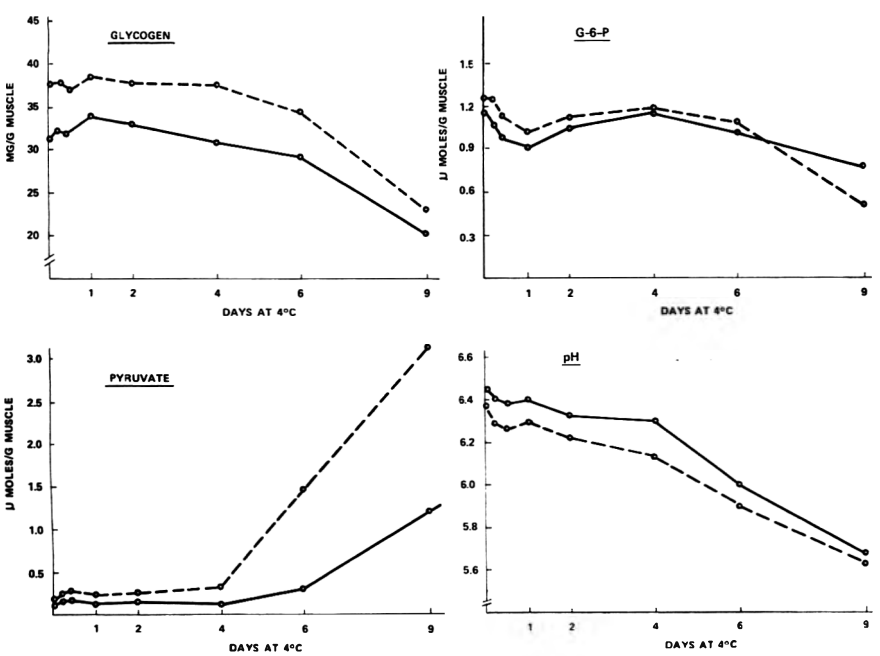

Fig. 3—Postmortem changes of glycolytic intermediates and pH: ○——○ foot muscle; ○- - -○ adductor muscle.

P. J. KE, B. A. LINKE and R. G. ACKMAN
Dept. of Fisheries and the Environment, Fisheries and Marine Service
Halifax Lab., 1707 Lower Water St., Halifax, Nova Scotia B3J 2R3, Canada

ACCELERATION OF LIPID OXIDATION IN FROZEN MACKEREL FILLET BY PRETREATMENT WITH MICROWAVE HEATING

ABSTRACT

Fresh mackerel fillets with skin on were pretreated, before frozen storage at −15°C by microwave heating at 2450 MHz for 5, 10 and 20 sec. During 6 months, the lipids of the skin and meat of the fillets were separately examined for quality variations by chemical methods such as TBA, POV and FFA. Pro-oxidant effects on lipids in mackerel fillets pretreated for 20 sec were shown by an increase of TBA molar value of 200% and 100% in the skin and meats, respectively, after 6 wk of frozen storage at −15°C. In fillets stored for 6 months, the rate of increase in POV in the pretreated sample was twice that in the control. According to the RIM quality evaluation system, the life in frozen storage of mackerel fillets pretreated with microwave heating decreased proportionately with the time of pretreatment. The loss of storage life was as much as 60% (from 16 to 7 wk) compared with the control samples. It is suggested that the promotion of lipid oxidation in the fish pretreated with microwave energy should be considered as a particularly important factor affecting the quality during frozen preservation.

INTRODUCTION

THROUGH IMPROVEMENTS in thermal processes applicable in fish industry, (Belyaev et al., 1975; Bezanson, 1976) microwave treatment is not only able to give fast and efficient heating but can also be used to retard losses of moisture and volatiles (Armbruster and Haefele, 1975). Although many hurdles remain to be passed, application of microwave heating to food processing is inevitable in the areas of freeze drying or thawing (Bezanson, 1976; Crepey, 1969; Tsuyki and Shuto, 1970), detoxification (Armstrong and Stanley, 1975), and biochemical deactivation (Henderson et al., 1975; Yeargers et al., 1975).

Microwave heating in processing various fish products has been studied in different research and application fields (Bezanson, 1976; Baldwin et al., 1971; Belyeav et al., 1975; Crepey, 1969; Tsuyki and Shuto, 1970), but amelioration of enzymatic and catalytic autoxidation of fish lipids has received little attention. However, fat oxidation and post-treatment flavor in meats were certainly affected by microwave heating (Ziprin and Carlin, 1976). Since most fish fats contain more than 50% unsaturated fatty acids, of which about half are polyunsaturated (Ackman, 1976), lipid oxidation is always considered to be one of the major factors contributing to difficulties in the preservation of fish quality during frozen storage, especially in the fatty fish (Ke et al., 1976a, 1977).

One of the more striking possibilities in the utilization of microwave heating in the fishing industry has been that of modifying the complex reactions which promote autoxidation of unsaturated fats in fish. In the induction period of oxidation of fish lipids, the formation rate of free radicals is ultimately related directly to fish quality in terms of oxidative rancidity (Ke and Ackman, 1976) and the effect of microwave radiation during this sensitive period has not been investigated.

In this paper we report studies on the susceptibility of pro-oxidant catalytic effects in the autoxidation of skin and meat fats from Atlantic mackerel fillet during frozen storage at −15°C following pretreatment by microwave heating in 2450 MHz. Based on the RIM system (Ke et al., 1975), the frozen storage life of these samples pretreated with microwave energy from 5 to 20 sec have been compared.

EXPERIMENTAL

Preparation of fish

Atlantic mackerel (*Scomber scombrus*), obtained in October of 1974 from trap nets outside Halifax harbor, were used for this study. The round fish were carefully iced in 100-lb boxes after landing in the early morning and were received at the Laboratory less than 6 hr out of water. The fish (avg length 36 cm, avg weight 470g) were washed under clean conditions and filleted. The proximate composition of the skin-on fillet was: protein, 18%, water, 65%. The fat content in these mackerel fillets (hand-skinned) was determined separately for skin as 49% with iodine value (I.V.) 109, and for meat 17% with I.V. 118. The fillets were iced briefly before microwave treatment.

Pretreatment by microwave heating prior to frozen storage

A microwave oven (Litton Industries Ltd., U.S.A., model 850) operating at 2450 MHz (2.5 KW) was used for pretreating the mackerel fillets. Four fillets placed in two layers, all with skin side down, were preheated in the microwave oven for 5, 10 and 20 sec. A thermometer (dyed alcohol) was inserted between the upper and lower layers of fillets for measuring temperature changes. Before heating, readings in the range 4–5°C were recorded for fillets. The temperatures were elevated about 1–2 and 3–4 degrees respectively in the fillets heated by microwave for 5–10 and 20 sec. These pretreated mackerel fillets were then wrapped in a polyethylene film and frozen at −15°C in packages of four fillets. Control fish did not receive any microwave treatment.

Procedures for quality assessment

At suitable intervals of frozen storage, all fillets from one package were examined. The skin part (including the thin fat layer under the skin) was removed from the meat part while the latter was in a partially thawed condition. These meat and skin samples, pooled separately from four fillets, were used for analytical determinations.

The thiobarbituric acid (TBA) test was applied directly to these skin and meat samples as described in a previous report (Ke et al., 1977). Lipids were extracted from the balance of the two samples by the method of Bligh and Dyer (1959), and were used for the determination of peroxide value (POV) according to the official method of the American Oil Chemists' Society (AOCS, 1974), and the content of free fatty acid (FFA) by an improved method using a multiple solvent system and meta-cresol purple as the indicator (Ke et al., 1976b).

RESULTS & DISCUSSION

THE RATES OF LIPID OXIDATION in the skin and meat samples of mackerel fillets stored frozen at −15°C after being pretreated with microwave heating for 10 and 20 sec were followed for 6 months. The results in terms of TBA and POV changes respectively have been plotted in Figures 1 and 2. The unusually rapid oxidation in all skin samples in comparison with the meats with and without microwave pretreatment, has been recorded previously, and are similar to results for unprocessed fish described in our previous study (Ke et al., 1977). As shown in Figure 1, the TBA molar values for skin lipid from fillets pretreated with microwave for 20 sec, have increased 200% and 100% after storage times of 6 and 14 wk, respectively, in comparison with the sample with no pretreatment. In the meat samples for fillets from these frozen storage periods, the increases of 100% and 50%, about half of the changes in the skin were measured.

The similar trends due to catalytic effects of microwave pretreatment on oxidation in terms of POV changes in both skin and meat samples of mackerel fillet frozen at −15°C are shown in Figure 2. The increase in POV due to microwave

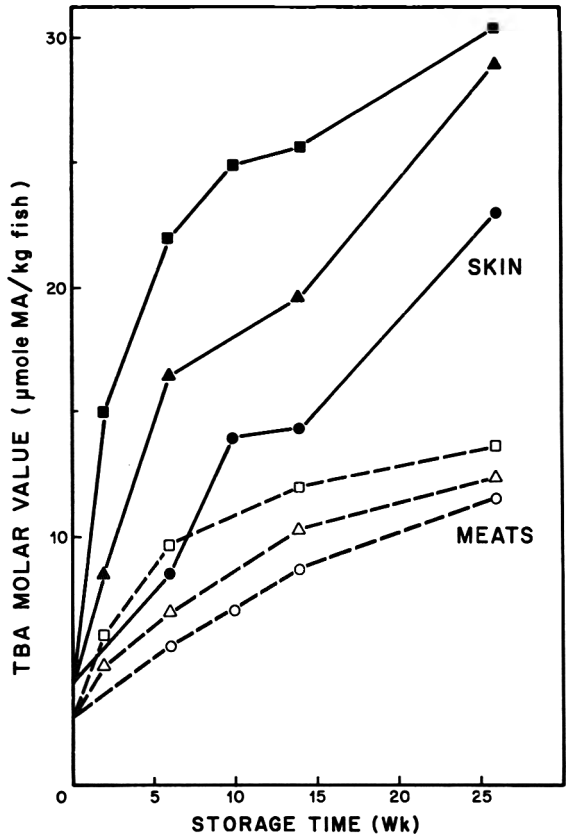

Fig. 1—Variation of TBA molar value in skin (solid line) and meats (broken line) of mackerel fillet frozen at −15° C after microwave pretreatment for 0 (○●), 10 (△▲) and 20 sec (□■), respectively.

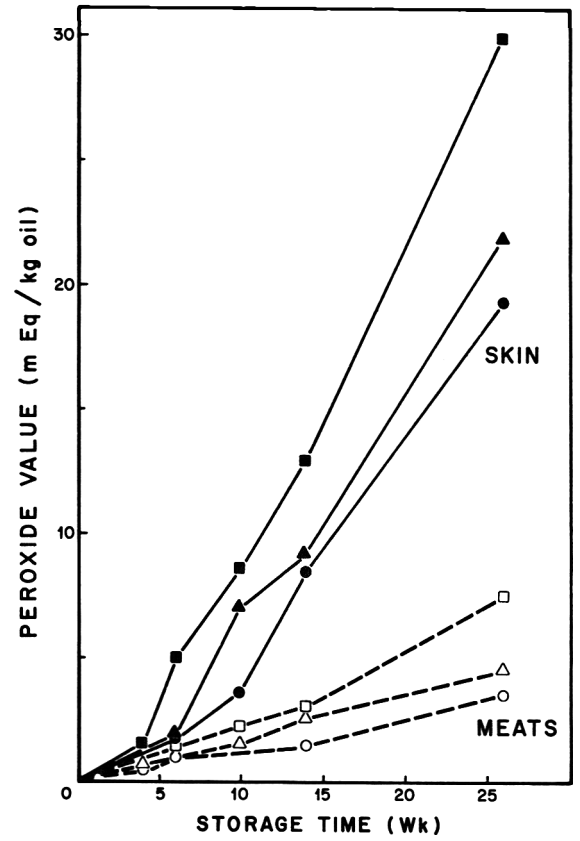

Fig. 2—The change of peroxide value in the skin (solid line) and meat samples of mackerel fillet frozen at −15° C after microwave pretreatment for 0 (○●), 10 (△▲) and 20 sec (□■), respectively.

pretreatment for 20 sec amounted to an increase more than 60% of the POV of controls, both for the skin and meat samples, after 6 months of frozen storage at −15°C.

Differences in the FFA content of lipids in the skin and meat of frozen mackerel fillet pretreated by microwave at 2450 MHz for 10 and 20 sec are compared in Figure 3. The subcutaneous (skin) fats were more slowly hydrolyzed than the fats in the meat samples, as found in our previous investigations (Ke and Ackman, 1976; Ke et al., 1976a). It is also obvious that the formation of FFA by lipid hydrolysis in both the skin and meat of the frozen mackerel fillet was increased significantly by microwave pretreatment for various radiation periods. The total FFA in all samples of lipid from mackerel fillet frozen for 6 months was less than 3%. The combination of increased FFA with the increased rate of oxidation strongly suggests that in the first instance the energy from the microwave treatment disrupts membranes and/or subcellular structures, thus liberating the normal enzymes and their substrates.

The frozen storage life of mackerel fillets pretreated by microwave energy for 5, 10 and 20 sec can be evaluated according to the Rancidity Indexes for Mackerel (RIM) (Ke et al., 1975) as "acceptable," when its POV are no more than 12 in the skin and 2 in meats. By comparing the sample pretreated with microwave for 20 sec with the controls, the shelf life have been shortened as much as 60%, from 16 to 7 wk. It should also be noted that the decrease of the shelf life was approximately proportional to the heating time of microwave pretreatment. Thus it has been demonstrated that the pretreatment of fish by microwave heating at 2450 MHz is an important factor affecting the initial quality and subsequaently catalyzing the development of oxidative rancidity during the posttreatment frozen storage at −15°C.

The initiation of lipid oxidation by catalytic factors is the

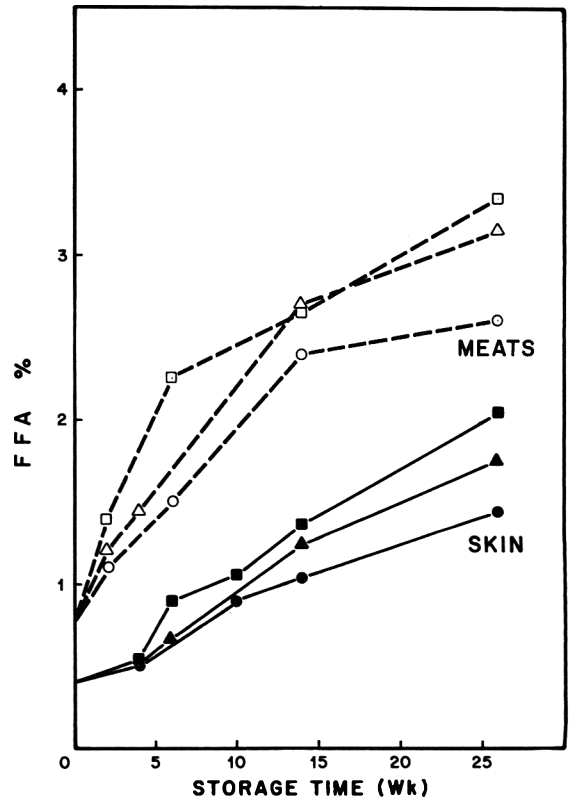

Fig. 3—The change of free fatty acid in the skin (solid line) and meats (broken line) samples of mackerel fillet frozen at −15° C after microwave pretreatment for 0 (○●), 10 (△▲) and 20 sec (□■), respectively.

most important problem for quality preservation in products based on fatty fish (Ke and Ackman, 1976). Once a radical chain reaction has been initiated, the breakdown of products is responsible for oxidative rancidity and the myriad of other reactions which reduce the shelf life and nutritive value of various frozen fish (Ke et al., 1976a). The possible role of catalysis in the initiation of lipid oxidation by microwave heating cannot be fully understood and discussed with the limited research of the present investigation. However, some promotion seems logical from our results, because the microwave energy at 2450 MHz used for pretreatment of the fish in this investigation is great enough to activate oxygen on either a direct attack basis, or to promote a reaction to produce singlet oxygen using tissue pigments as sensitizers (Rawls and van Santen, 1970). Clearly, research into the use of microwave pretreatment in the processing of fatty fish should be particularly centered on the enhancement of oxidative rancidity, in post-treatment storage as well as on any change of flavor (Angelini and Merritt, 1975), nutritive values (Ziprin et al., 1976), or enzymes (Henderson, 1975).

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NANCY LARSON-POWERS[1] and ROSE MARIE PANGBORN
Food Science & Technology, University of California, Davis, CA 95616

PAIRED COMPARISON AND TIME-INTENSITY MEASUREMENTS OF THE SENSORY PROPERTIES OF BEVERAGES AND GELATINS CONTAINING SUCROSE OR SYNTHETIC SWEETENERS

ABSTRACT

By paired comparison methods, concentrations of 0.75% and 0.86% calcium cyclamate and of 0.17% and 0.19% aspartame were equivalent in sweetness to 10% sucrose in distilled water at 3° and 22°C, respectively. Inherent bitterness of the compounds prevented precise assessment of relative sweetness for sodium saccharin in distilled water, and for the saccharin and cyclamate in flavored drinks. By application of linear regression to the paired comparison data, 0.07% aspartame was calculated as equal in sweetness to 10% sucrose in lemon, strawberry and orange drinks. Because the underlying bitterness of saccharin interfered with assessment of its sweetness, a time-intensity technique was applied. Using a chart recorder to monitor time, time-intensity (T-I) measurements were made of the intensity and the duration of sweetness, bitterness, sourness and flavor in distilled water, and the same characteristics, plus flavor in three flavored drinks, and two flavored gelatins, sweetened with sucrose, cyclamate, or saccharin. T-I curves for the sensory properties of aspartame closely resembled those for sucrose in all media. Cyclamate and saccharin imparted a marked, persistent bitterness to all carriers. In gelatin, samples containing 18% sucrose were firmer initially and took longer to manipulate to a liquid in the mouth than did gelatins containing 0.105% aspartame, 0.55% cyclamate, or 0.05% saccharin.

INTRODUCTION

THE DEVELOPMENT and testing of synthetic sweeteners involves a lengthly sequential procedure. First, it must be established that the new ingredient is safe for human consumption, that it is stable under normal conditions of handling and storage, and that it is not detrimental to the physical or functional attributes of the products in which it is to be used. Of prime importance are the sensory properties – appearance, aroma, texture, taste and aftertaste. Traditionally, a single sensory parameter is measured – sweetness equivalence to sucrose, usually by paired comparison methods. In the interest of expediency and efficiency, most investigations are conducted using model systems (compounds dispersed in distilled water), with the hope that similar relationships would hold in more complex food and beverage systems. We felt that measurements of perceived intensity, sensory quality, and stimulus duration in several systems would be useful supplements to the conventional, unidimensional "relative sweetness" measurement. The present investigation was undertaken to compare the relative taste properties of aspartame, sodium saccharin and calcium cyclamate with those of sucrose in distilled water, in lemon, orange and strawberry drinks, and in orange- and strawberry-flavored gelatins, using both paired comparison and a new approach to time-intensity (T-I), wherein time is continuously monitored by a strip chart recorder (Larson, 1975).

MATERIALS & METHODS

Ingredients

The sweeteners used were: sucrose (99% pure, C & H Sugar Co.), sodium saccharin (Sherwin Williams, lot x5768), calcium cyclohexylsulfamate (Searle Biochemics, lot x5735), and aspartame (L-aspartyl-L-phenylalanine methyl ester, Searle Biochemics, lot 5270-7). Drinks and gelatins were prepared from powdered bases supplied by General Foods Corp., Tarrytown, NY (Table 1).

Sample preparation

The water solutions were prepared (w/v) using double-distilled water and sweetener, covered, and allowed to stand at 22°C for 16 hr prior to tests conducted at room temperature, and at 3°C for 16 hr prior to tests conducted with solutions at refrigerator temperature. The chilled samples were poured into the test beakers 1 hr before the test session, covered, and replaced in the refrigerator to allow the temperature to equilibrate to 3°C.

The three flavored drinks were prepared from a stock solution of the powdered base and double-distilled water. The stock was added to the solid sweeteners, agitated to dissolve them, diluted to volume at 22°C, and stored at 3°C for approximately 12 hr.

Stock solutions of the two gelatins were prepared by mixing the base with 3/4 of the total volume of boiling, distilled water and stirring for 2 min by hand. After mixing for 10 min with a magnetic stirrer, the gelatin was cooled to room temperature and brought to volume. Aliquots of the sweetener, which had been dissolved in distilled water (w/v) to a specific volume, dependent upon the desired concentration, were combined with the gelatin solution, brought to volume, and poured into enamel pans to a height of 2 cm. Any surface foam was removed, and the pans were covered with a plastic wrap and stored at 3°C ± 1°C for 18 hr. All gelatins were tested within 24 hr of preparation, at which time they were served as 2 cm cubes at 7° ± 1°C.

Sensory procedures

The paired comparison, constant stimulus, forced-choice method (Amerine et al., 1965) was used to compare seven concentrations of each of the three synthetic sweeteners with solutions of 10% sucrose, at 3° and at 22°C. In the three flavored drinks, five concentrations of each synthetic sweetener were compared with samples containing 10% sucrose. Judges selected the sweeter sample within each pair, with a total of three replications obtained per judge per paired concentration. The percentage of the responses selecting the 10% sucrose sample as sweeter

Table 1—Composition of powdered bases used for drinks and gelatins

	Drinks (w/w%)		
	Strawberry	Orange	Lemon
Citric acid	52.36	48.71	69.89
Monocalcium phosphate	43.64	42.35	22.07
Flavoring	0.74	6.61	2.90
FD&C color	1.49	0.61	0.01
Vitamin C	1.60	1.55	0.81
Vitamin A	0.17	0.17	0.09
Clouding agent	–	–	4.23
	Gelatins (w/w%)		
	Strawberry		**Orange**
Gelatin	70.9		70.7
Adipic/fumaric acid	20.3		20.1
Sodium citrate	6.7		6.6
Flavoring	1.7		2.4
Color	0.4		0.2

[1] Present address: 1015 Campbell, Prosser, WA 99350

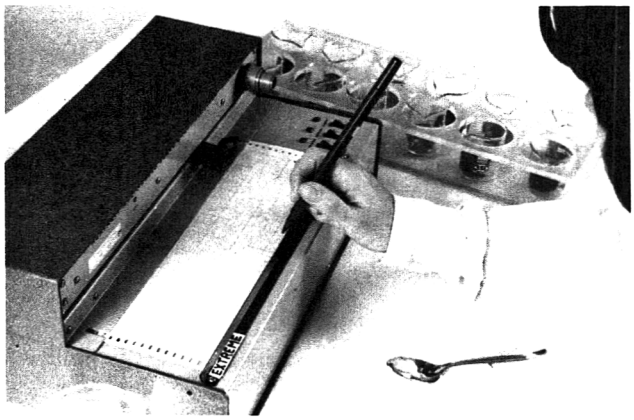

Fig. 1—Time intensity method. Judge places sample in mouth, activates strip-chart and records perceived intensity on moving chart until extinction point.

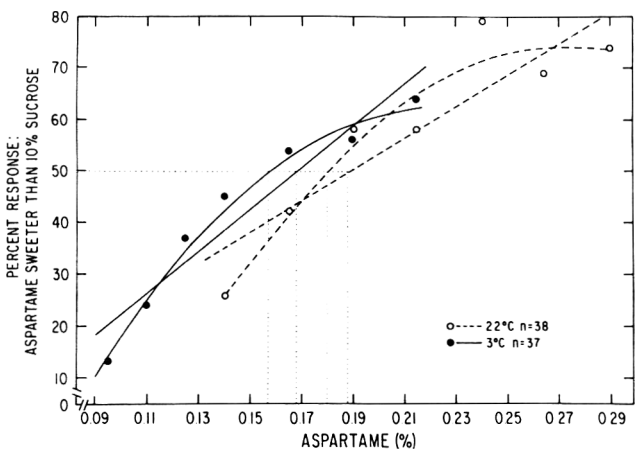

Fig. 2—Sweetness of aspartame relative to 10% sucrose in distilled water at 3° and 22°C, showing both linear and parabolic lines fitted to the data.

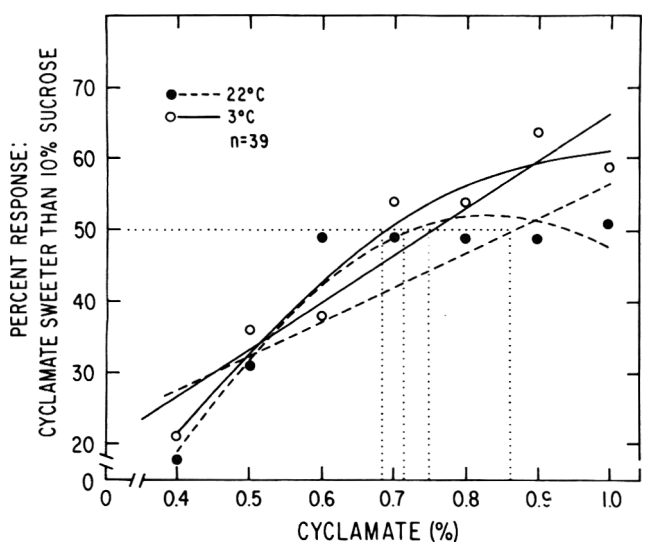

Fig. 3—Sweetness of calcium cyclamate relative to 10% sucrose in distilled water at 3° and 22°C, showing both linear and parabolic lines fitted to the data.

was plotted against the concentration of the synthetic sweetener, and a regression line fitted to the data. Equisweetness was defined as that concentration of synthetic sweetener at which 50% of the responses indicated the synthetic was sweeter than sucrose, as read from the linear regression line. For the water solutions, only, parabolic lines were fitted to the data by computer, also. Gelatins were not evaluated by paired comparison due to difficulties encountered with the liquids.

Judges consisted of nine females and four males, students and employees, selected on the basis of interest in participation and consistency of response to perceived sweetness and bitterness in paired presentation of test solutions. Within all test series, samples of approximately 35 ml of solution were served in randomized order in 50-ml beakers coded randomly with numbers from 1–999. Distilled water was provided for oral rinsing and judges were instructed not to swallow samples. Test sessions were conducted three to four times per week, between 10:00 am and noon, in individually partitioned booths maintained at 21° ± 2°C.

For the T-I measurements, time was monitored by a strip chart recorder (Heathkit Multi-speed Servo Chart Recorder, Model 1R-18M). The paper was set to advance at a rate of 0.05 in/sec when liquids were being tested, and at 0.1 in/sec for testing gelatin. Perceived intensity was recorded manually with a felt-tip pen on the moving chart, using a 100-division, unstructured scale labeled "none" to "extreme" on the stationary paper-cutter bar (Fig. 1). The bar served both as a guideline for marking intensity, and to cover the previous response, forcing judges to concentrate on the immediate intensity. Samples were presented in 50-ml beakers identified with small, gummed labels marked with the sequence and with the single sensation being measured, e.g., "1-sweet," or "2-bitter." The gummed label was transferred from the beaker onto the chart paper to identify the curve, and to focus the judge's attention on the single sensation under consideration. The judge placed the entire sample into the mouth, retaining the 10 ml of liquid for 20 sec, or the 2-cm cube of gelatin for 10 sec before expectoration, while recording the intensity on the moving chart. For sweetness measurements in distilled water (Fig. 2, 3, and 4), judges first recorded initial intensity on the stationary chart. Then, a second sample of the same stimulus was placed in the mouth while simultaneously initiating the advancement of the chart paper. Beginning from the initial intensity point, the judge marked perceived intensity on the moving chart, expectorating at the designated times, and continuing to mark the moving chart until the sensation was no longer perceived, i.e., to the extinction point (Fig. 1).

In addition to recording taste and flavor intensities, hardness and rate of breakdown of the gelatins were assessed by the T-I method. First the judge placed the gelatin cube between the molar teeth, bit down and expectorated, recording the initial hardness or firmness on the stationary chart. Next, the second sample of the same gelatin was placed between the molars, bitten and manipulated between the tongue and palate to a complete liquid, recording the perceived rate of breakdown on the moving chart. For both liquids and gelatin, a total of eight samples were tested per session, with distilled water provided for oral rinsing.

For evaluation of the water solutions and the three drinks by the T-I method, nine judges, with experience on the previous paired tests, participated. For evaluation of gelatins, a new group of subjects was screened and trained, with ten being selected on the basis of reproducibility of judgment, two of whom had participated in the previous taste studies.

To tabulate the T-I data, points on the graphs were joined to form a smooth curve, with mean intensities calculated for the entire panel at 4-sec intervals for the liquids, and at 2-sec intervals for the gelatins. These mean intensities were then replotted against time for each sensation for each sweetener. The total area under the curve was estimated using third-order exponential smoothing, based on an input of points from a digitizing table to a calculator (Hewlett-Packard, 9810). Analysis of variance was applied to the initial intensity values read from the individual charts, and to the values representing areas under the curves.

RESULTS & DISCUSSION

Paired comparison method

Sensory responses to the sweetness of aspartame, cyclamate and saccharin compared to 10% sucrose in distilled water are plotted in Figures 2, 3, and 4, respectively. For aspartame, it was necessary to test a different concentration series at each solution temperature to bracket the sweetness match (Fig. 2).

Table 2—Concentration range, equisweet concentrations and correlation coefficients for solutions of synthetic sweeteners compared to 10% sucrose, paired comparison method

Medium of dispersion	Aspartame			Cyclamate			Saccharin++
	Conc range tested %	Equiv. sweetness[a] %	r	Conc. range tested %	Equiv. sweetness[a] %	r	Conc range tested %
Distilled water							
22°C	0.075–0.290	0.190	0.903**	0.15–1.0	0.86	0.818*	0.03–0.50
3°C	0.065–0.290	0.170	0.955***	0.04–1.0	0.75	0.937**	0.05–0.35
Drinks							
Strawberry	0.05–0.15	0.069	0.955*	0.25–0.85	++[b]	–	0.05–0.25
Orange	0.05–0.270	0.068	0.986**	0.20–1.25	++	–	0.02–0.30
Lemon	0.05–0.10	0.066	0.963**	0.45–1.15	++	–	0.05–0.15

*Significant at $p < 0.05$
** Significant at $p < 0.01$
*** Significant at $p < 0.001$
[a] Values determined from linear regression line.
[b] ++Equivalent sweetness concentration point was not determinable.

The aspartame data fit more closely to a parabolic (r = 0.965 at 22°C and r = 0.990 at 3°C, $p < 0.01$) than a linear regression (r = 0.903 and 0.955, respectively, $p < 0.01$ and 0.001). The cyclamate data (Fig. 3) also fit more closely to a parabolic (r = 0.962, $p < 0.01$) than a linear regression (r = 0.818, $p < 0.05$). Using the conventional measurement of reading values from a linear regression line, 10% sucrose was determined to be equivalent to 0.190% aspartame and 0.86% cyclamate in solutions tested at 22°C, and to 0.170% aspartame and 0.75% cyclamate at 3°C (Table 2). Lower aspartame values have been reported in the literature, e.g., a 10% sucrose match of 0.075% aspartame (Beck, 1974) and 0.050% aspartame (Guadagni et al., 1974). The latter investigators did not specify solution temperatures, but we presume samples were served at room temperature. Although sucrose and cyclamate have been tested by several investigators (Vincent et al., 1955; Schutz and Pilgrim, 1957; Kamen, 1959; Hellaur, 1966; Stone and Oliver, 1969; Yamaguchi et al., 1970a, b none compared cyclamate directly to 10% sucrose. The best estimate of the sweetness of calcium cyclamate from these literature values can be expressed as a relative sweetness of 30–80 compared to sucrose being equal to 1.

The linear regression lines in Figure 3 are highly questionable, particularly for solutions tested at 22°C, because of the obvious plateauing of responses at the 50% mark for concentrations ranging from 0.6–1.0% cyclamate. An inspection of the individual data showed a time-order bias for the cyclamate-sucrose comparison, only. The second sample within a paired set was significantly selected as sweeter 226 times in 364 trials, or 62% of the time ($p < 0.001$), compared to the chance selection of 50%. This overselection of the second sample, despite random presentation, strongly suggests mutual synergism of the two compounds, agreeing with Kamen (1959) and Yamaguchi et al. (1970b) who reported sweetness synergism of intermediate concentrations of sucrose and cyclamate. It should also be pointed out that several investigators have observed a bitter off-taste in cyclamate solutions, starting with concentrations of 1.3% (Helgren et al., 1955). Vincent et al. (1955) found a linear relationship between the percentage of their 76 judges reporting off-taste and the log of the concentration of sodium cyclamate. Twenty percent detected an off-taste at 0.42%, and 50% at 0.66% cyclamate. The off-taste was perceived at higher levels of relative sweetness for the sodium salt than for the calcium salt of cyclamate. Using 150 female testers, Tiwald (1971) reported significant differences between equisweet solutions of 0.17% sodium cyclamate and 7.5% sucrose, which he attributed to the bitter aftertaste of the former.

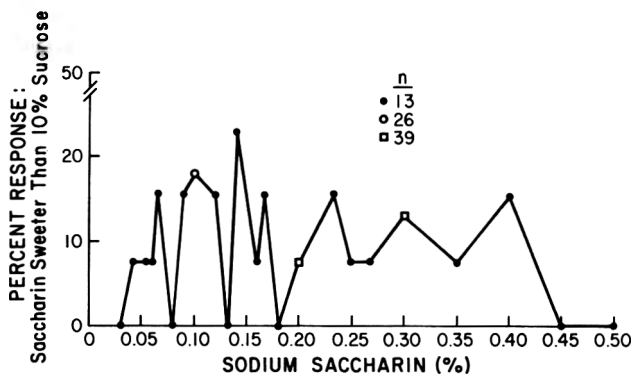

Fig. 4—Sweetness of sodium saccharin relative to 10% sucrose in distilled water at 22°C, demonstrating the impossibility of fitting a line to the data to determine the relative sweetness.

Figure 4 dramatically illustrates the impossibility of using the paired comparison technique to establish the concentration of saccharin equivalent to 10% sucrose. A 20-increment concentration series, ranging from 0.03–0.05% saccharin resulted in a tracking-type response pattern which never approximated the 50% response criteria. A second concentration series of three levels of saccharin, 0.10, 0.20 and 0.30%, fared no better, despite the increased number of replications. A reappraisal of the recent literature proved futile, as a 10% sucrose standard was not tested by paired comparison. The closest concentration values were 9.12% sucrose ≅ 0.03% saccharin (Schutz and Pilgrim, 1957) determined by a single stimulus, 9-point intensity scale, and 9.18% sucrose ≅ 0.08% saccharin (Yamaguchi et al., 1970a) established by paired comparison. Neither investigator reported difficulty in testing the relative sweetness of this compound. In his review of a large number of early articles on relative sweetness, Nieman (1958) reported a summary value of 30,000 for saccharin relative to 100 for 10% sucrose. As shown by Amerine et al. (1965), Täufel and Klemm (1925) reported a value of 18,900 for saccharin relative to 100 for 10% sucrose.

Vincent et al. (1955) calculated that sodium saccharin was 240–350 times sweeter than sucrose at a level of

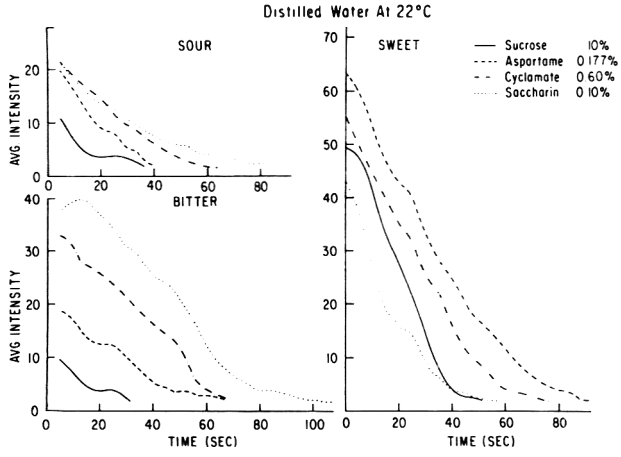

Fig. 5—Time-intensity curves for sourness, bitterness, and sweetness of four sweeteners in distilled water at 22°C.

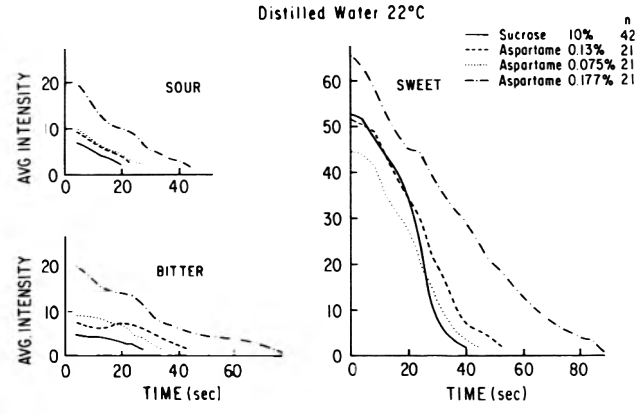

Fig. 6—Time-intensity curves for sourness, bitterness, and sweetness for 10% sucrose and three concentrations of aspartame in distilled water at 22°C.

0.007–0.0125%, but at higher concentrations the sweetness dropped rapidly due to increasing bitterness of the saccharin. This was substantiated by Moskowitz (1970), using magnitude estimation. Although we did not quantitate the bitterness intensity in the paired comparisons, we concur with Moskowitz (1970) that the bitterness of the saccharin concentrations increases at a faster rate than does the sweetness. These changes in sweetness-bitterness functions with increasing concentration preclude the establishment by paired comparison of a concentration at which 50% of the responses would indicate the saccharin solution was sweeter than 10% sucrose.

No difficulty was encountered in calculating sweetness equivalence by paired comparison for aspartame in the three flavored drinks. The aspartame values equivalent to 10% sucrose were so similar for the three drinks, that a single concentration of 0.07% aspartame was used for subsequent testing of all drinks.

Our experience with paired comparison testing of cyclamate, and particularly with saccharin, emphasized the need for a sensitive, indirect estimate of the multiple sensory properties of these compounds, hence the development of the T-I technique. For the T-I method the final concentrations selected for each sweetener are specified on the individual graphs (Fig. 5–9).

Time-Intensity method

Average T-I curves for the four sweeteners dispersed in distilled water and tested at 22°C are shown in Figure 5. Similar curves were obtained with solutions tested at 3°C, except that sourness of the three synthetic sweeteners was significantly lower ($p < 0.001$) at the lower temperature, as were sweetness and sourness for all compounds. Bitterness intensity was the same, possibly because solutions may have been closer to mouth temperature when they made contact with the bitter

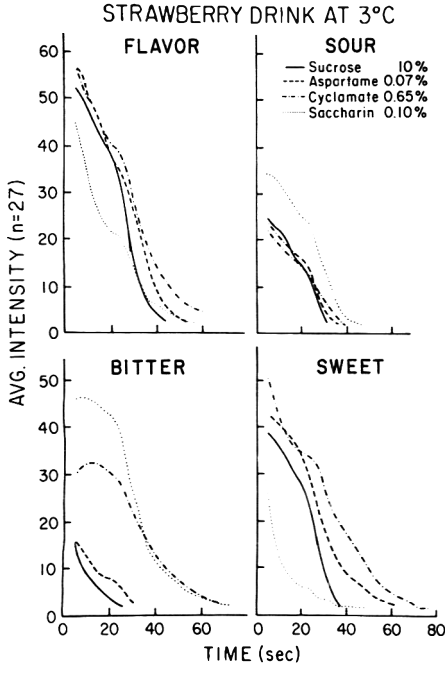

Fig. 7a—Time-intensity curves for flavor, bitterness, sourness and sweetness in strawberry drink at 3°C.

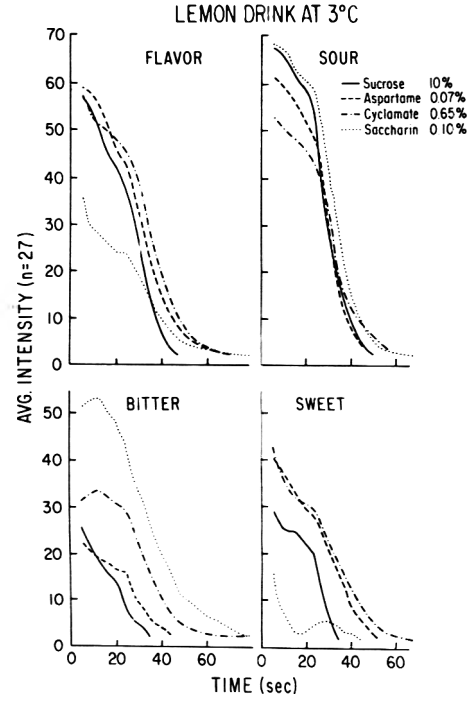

Fig. 7b—Time-intensity curves for flavor, bitterness, sourness and sweetness in lemon drink at 3°C.

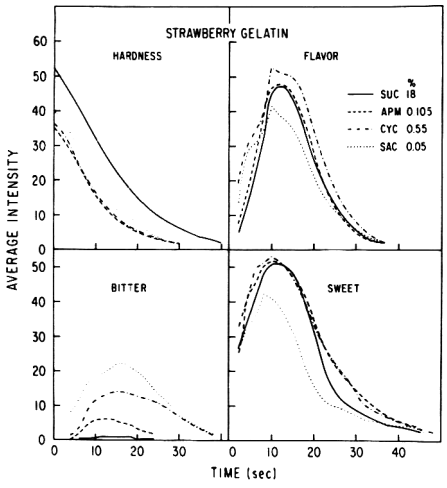

Fig. 8a—Time-intensity curves for strawberry gelatin with four sweeteners: (n = 30 for hardness and flavor; n = 24 for bitterness; n = 27 for sweetness).

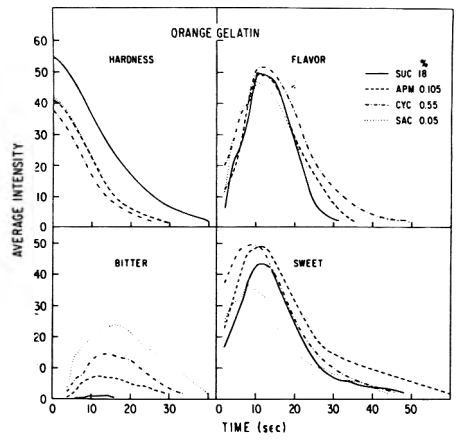

Fig. 8b—Time-intensity curves for orange gelatin with four sweeteners: (n = 30 for hardness and flavor; n = 24 for bitterness; n = 27 for sweetness).

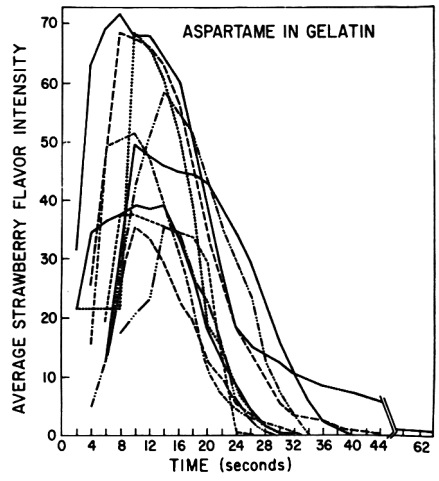

Fig. 9—Average T-I curves for strawberry flavor in gelatin for each of ten judges, demonstrating variation in initial intensity, maximum intensity and duration (n = 3).

receptors at the base of the tongue. At both temperatures, the greater sourness and bitterness of saccharin and of cyclamate, compared to sucrose was quite evident. Also, it is very obvious that 0.10% saccharin was considerably less sweet initially, with a shorter sweetness duration, compared to the other sweeteners. The aspartame samples were initially sweeter than the sucrose solutions, but at the lower temperature, the sweetness duration of both solutions was identical. The persistence of all taste sensations in the synthetic sweeteners tested at 22°C is striking, particularly the bitterness and sourness of saccharin. Table 3 presents values in cm^2 for the total areas under the curves, with the corresponding LSD values.

Since the concentration of 0.177% aspartame, calculated from the paired comparison experiment as equivalent to 10% sucrose proved to be sweeter, two lower concentrations were compared to sucrose using the T-I method (Fig. 6). The concentration of 0.075% was taken from a literature value (Beck, 1974), while the level of 0.13% was derived arithmetically from the data in Figure 5, i.e., 0.177% aspartame /64 initial intensity = 0.13% aspartame/49 initial intensity (the starting point for 10% sucrose). The latter aspartame concentration gave sweetness, sourness, and bitterness curves remarkably similar to those for 10% sucrose when tested experimentally (Fig. 6). This demonstrates the use of T-I data to interpolate and match sweetness intensities for compounds with no interfering secondary tastes.

T-I curves for the orange and strawberry drinks were almost identical, hence only the latter data are shown (Fig. 7a), contrasted with curves from the lemon drink (Fig. 7b). The main difference in the curves for strawberry (pH 3.0) and lemon (pH 2.75 was the greater perceived sourness of the latter. The data suggest that sourness supressed perceived sweetness intensity, which agrees with previous findings (Pangborn, 1963). Flavor intensity curves were similar in both drinks for sucrose, aspartame, and cyclamate, with significantly less initial flavor for drinks sweetened with saccharin. In both drinks, the saccharin samples were very bitter, cyclamate samples were moderately bitter, and the sucrose and aspartame samples had a slight, transient bitterness. Bitterness curves for samples containing saccharin and for those containing cyclamate suggest a slight increase in intensity up to the time of expectoration (20 sec), then a pronounced drop. This slight delay in perception of maximum bitterness, only, might be related to the delay in the stimuli reaching the circumvallate papillae at the base of the tongue, the receptors primarily responsive to bitter compounds.

Marked differences can be seen in sweetness intensity and in duration, with aspartame and cyclamate being the greatest

Table 3—Mean areas under the curve (cm^2) for solutions, drinks and gelatins sweetened with sucrose or three synthetic sweeteners, T-1 method[a]

		SUC	APM	CYC	SAC	LSD[b]
Distilled water						
Sour	3°C	5.5	10.7	14.6	17.0	7.1
	22°C	6.5	14.4	22.7	28.1	7.1
Bitter	3°C	7.3	15.7	43.1	57.3	13.3
	22°C	6.0	20.3	41.5	65.6	13.3
Sweet	3°C	37.1	61.9	44.0	22.6	13.1
	22°C	38.1	76.2	53.7	26.7	13.1
Drinks						
Flavor	Strawberry	43.4	53.5	61.7	35.3	8.2
	Orange	36.9	47.5	48.8	37.1	9.1
	Lemon	49.6	62.0	71.7	37.5	8.3
Bitter	Strawberry	7.1	10.4	40.9	51.7	9.5
	Orange	13.6	7.8	36.4	53.3	10.7
	Lemon	16.9	20.2	41.3	65.3	11.1
Sour	Strawberry	17.7	18.3	17.7	30.9	8.1
	Orange	20.0	20.4	26.1	33.2	6.7
	Lemon	64.9	59.4	56.0	75.1	10.1
Sweet	Strawberry	31.2	44.6	56.3	16.2	10.0
	Orange	30.2	43.3	51.3	20.4	10.9
	Lemon	23.4	39.6	48.6	11.0	10.1
Gelatins						
Hardness	Strawberry	53.4	25.5	26.0	29.7	6.3
	Orange	56.8	32.4	25.9	33.5	7.6
Bitter	Strawberry	0.8	6.0	20.5	30.3	7.5
	Orange	0.7	8.8	18.4	35.4	11.7
Flavor	Strawberry	48.6	52.7	63.2	47.1	11.5
	Orange	49.3	56.1	73.1	59.9	13.9
Sweet	Strawberry	68.9	77.9	82.0	55.3	17.5
	Orange	57.0	82.1	70.0	48.8	16.4

[a] SUC, APM, CYC and SAC are, respectively, sucrose, aspartame, cyclamate, and saccharin. For concentration, consult Fig. 6-8.
[b] Least significant difference among sweetener means at $p < 0.05$.

and saccharin being the least. Quantification of areas under the curve in cm^2 and the corresponding LSD values (Table 3), emphasize the magnitudes of greater bitterness and sourness, and of lesser sweetness and flavor of the saccharin samples.

The data indicate that lower concentrations of aspartame and of cyclamate should be used to match the initial sweetness intensity of 10% sucrose. Applying the ratio described previously, one could predict that the initial sweetness intensity of sucrose in these drinks would be equivalent to 0.06% aspartame, and to 0.45–0.47% cyclamate. Due to the unavailability of the same judges, we were unable to verify these predicted concentration matches.

Despite the almost identical curves obtained for T-I measurements of orange and of strawberry gelatin, both figures are included to illustrate the reproducibility of the panel using this method (Figs. 8a and 8b). As would be anticipated, gelatins sweetened with sucrose were firmer ($p < 0.001$) and broke down in the mouth more slowly than did the synthetically-sweetened samples. Maximum firmness was perceived at "0" time, whereas maximum flavor and sweetness were perceived after 10 sec of oral manipulation, and maximum bitterness after approximately 15 sec. Again, greater bitterness and less flavor and sweetness were assigned to samples containing saccharin. A good matching of curves was obtained for flavor and for sweetness of samples with 0.55% cyclamate, 0.105% aspartame, and 18% sucrose, also demonstrated in Table 3. An increase in the amount of saccharin would definitely have increased bitterness, but might not have increased sweetness, due to the interference of bitterness.

Figure 9 is included to illustrate the typical range of individual judge responses to gelatin samples, using the T-I method. Although all ten judges were requested to begin marking the moving chart at the same time (2 sec after placing the gelatin cube in the mouth), they differed markedly in their perception of initial flavor intensity, maximum flavor intensity, and extinction point. Most likely these differences are caused by variations in sensory sensitivity to the stimulus and to variations in the usage of the scalar points. Despite the large between-judge variation, observed in all sensory tests, there was excellent within-judge reproducibility of response. The latter was demonstrated by the general lack of significant F-ratios for the judge X replication interactions in the analysis of variance of these T-I data.

We feel that the T-I technique described herein for liquids and gelatins has considerable potential for testing sensory attributes of a variety of foods and beverages, after trained judges master the mechanics of marking intensity on a moving chart. The use of a recorder to monitor time is an improvement over the T-I methods described by Nielson (1957) and by Jellinek (1964) in which judges watch the second hand of a clock, by Todd et al. (1971) where judges used a stop-watch to time duration of bitterness of beer, and by McNulty and Moskowitz (1974) where 1-sec auditory signals indicated the time intervals.

In conclusion, it should be emphasized that paired comparison methods could be inappropriate with stimuli which exhibit masking or synergistic effects, or with stimuli which differ in more than one parameter.

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NANCY LARSON-POWERS[1] and ROSE MARIE PANGBORN
Food Science & Technology, University of California, Davis, CA 95616

DESCRIPTIVE ANALYSIS OF THE SENSORY PROPERTIES OF BEVERAGES AND GELATINS CONTAINING SUCROSE OR SYNTHETIC SWEETENERS[1]

ABSTRACT

Two descriptive sensory methods, anchored (deviation from a sucrose reference) and unanchored, were used to quantify differences in aroma, flavor and aftertaste in five media – strawberry, lemon and orange drinks, and strawberry and orange gelatins – varying in type of sweetener. With both methods, samples sweetened with sodium saccharin deviated the most from the sucrose standard, those sweetened with aspartame the least, and calcium cyclamate was intermediate. In general, drinks sweetened with sucrose or with aspartame could be characterized as "sweet-clean," and those sweetened with cyclamate or with saccharin as "sweet-chemical" and "bitter." Gelatins containing synthetic sweeteners generally were more astringent, bitter and sour, with less strawberry flavor, and were significantly less hard, springy and viscous than those sweetened with sucrose. In all media, more significant differences were observed among the sweeteners with the anchored method than with the unanchored procedure. Advantages and limitations of these two quantitative descriptive procedures are discussed.

INTRODUCTION

PAIRED COMPARISON and time-intensity methods were used to measure the relative taste intensities of flavored drinks and of gelatins sweetened with sucrose, aspartame, sodium saccharin, or calcium cyclamate (Larson-Powers and Pangborn, 1978). In addition to these quantitative measures, qualitative attributes of the aromas, tastes and aftertastes of these stimuli were established by two descriptive procedures: multiple-comparison, unanchored, and anchored to a sucrose reference (Larson, 1975). Preliminary testing of commercial milk chocolate, using the anchored descriptive analysis, gave greater reproducibility of response than did unanchored methods, such as the nonquantitative A.D. Little "Flavor Profile" (Caul, 1957) and the General Foods "Textural Profile" (Civille and Szczesniak, 1973) or Tragon's "Quantitative Descriptive Analysis" (Stone et al., 1974). The present paper evaluates the data from the anchored and unanchored descriptive techniques and contrasts them with information derived from the previous quantitative methods.

MATERIALS & METHODS

THE SWEETENERS, the powdered bases for the drinks and gelatins, and the procedures for their preparation have been described previously (Larson-Powers and Pangborn, 1978).

Sensory procedures

Five females and one male served as judges for the drinks, and four females and two males for the gelatins. Two additional females participated in the development of vocabulary and in sessions using unanchored description of all products. However, because these two subjects prepared and served samples and hence knew of the "blind" sample, their responses were not recorded in the data obtained from anchored descriptive analysis. Judges were students or employees, between the ages of 22–35 yr, selected on the basis of interest in participation in extended groups sessions, and ability to reproducibly describe flavor attributes of the test samples. These judges developed a vocabulary of terms to describe the aroma, flavor and aftertaste of the samples in daily group sessions of approximately 1-hr's duration. At the onset of each group meeting, judges listed all terms which applied to the samples, with recommendations for substances which could serve as physical references in subsequent sessions. All impressions were discussed by the group, definitions established, and references agreed upon. The panel leader developed preliminary score cards and brought reference materials to anchor aroma terminology at the following training sessions. After agreement was reached on appropriate descriptors and references (six to eight sessions), the score sheets were finalized for each stimuli – strawberry, orange and lemon drinks, and strawberry and orange gelatins. At all test sessions thereafter, judges evaluated samples individually at a partitioned round table with no group discussion. The aroma references were continually available from a rotating "lazy susan."

Samples were served at 3°C in 80-ml blue cobalt glasses immersed in ice water. The glasses were covered with aluminum lids containing two- or three-digit codes, and were served as a complete block in randomized order. At each session, four samples were presented, one from each sweetener – sucrose, aspartame, sodium saccharin, or calcium cyclamate. The concentrations of each sweetener are given in Figures 1–10. For beverages, 10-ml samples were served for aroma evaluation, and 60-ml samples for evaluation of flavor and aftertaste. For gelatin, six 2-cm cubes were served. For each of the five stimuli (three drinks and two gelatins), five sessions were held. This relatively small number of replicate sessions, which totalled 4 wk of daily testing/commodity, was necessitated by the availability of the student judges during various academic sessions. Within each stimulus, the first session was considered orientation, and the data were not included in the final tally. At the next three sessions, samples were evaluated in terms of deviation from a sucrose reference, i.e., using an anchored, descriptive analysis. At the fifth and final session, unanchored descriptive analysis was used, i.e., the four samples were presented simultaneously and judged on an absolute basis, on an unstructured scale consisting of a 100-mm horizontal line labeled "none" to "extreme" for each descriptor.

The anchored, descriptive analysis was a modification of a method reported by Daget (1974) for evaluating chocolate. The sensory data obtained was analyzed by Vuataz et al. (1974). In the present study, judges indicated the degree of difference in intensity of each characteristic from the reference (the sucrose-sweetened sample) by placing a mark on an unstructured, horizontal line, 120 mm in length, labeled "Less" and "More" at the ends, with the center labeled "Same as Reference." A hidden reference, the sucrose-sweetened sample, was included as a coded, test sample to check the internal variation of the judges' responses.

A fixed model analysis of variance was applied to the individual scores within each of the five stimuli, for aroma, for flavor and for aftertaste descriptors separately. Main effects tested were sweeteners, descriptors, judges, and replications, as well as all two- and three-way interactions. Least significant differences were calculated for all significant sweetener × descriptor interactions.

RESULTS & DISCUSSION

Drinks

Because of the great similarity in the responses to the strawberry, lemon and orange drinks, only results from the orange drink will be presented herein. The complete set of data are available in the thesis by the senior author (Larson, 1975).

Anchored descriptive analysis. Responses to the aroma, flavor and aftertaste of the sucrose sample compared with itself as a blind control are shown in Figure 1. The mean deviation did not exceed ±5 mm on the 120-mm intensity scale, attesting to the ability of the group to match the reference to itself. The magnitude of the standard deviations indicate good agreement on aftertaste, with greater variation for aroma and

[1] Present address: 1015 Campbell, Prosser, WA 99350

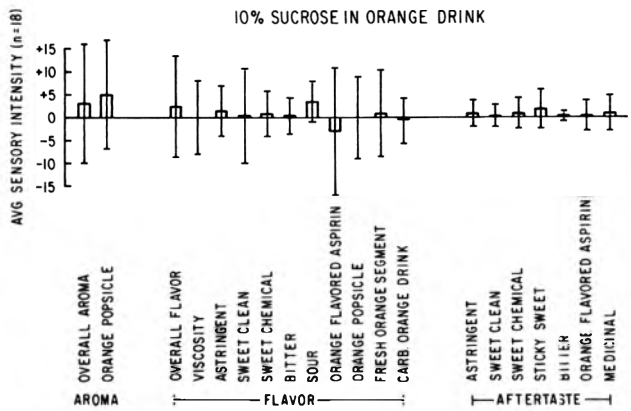

Fig. 1—Mean intensity differences and standard deviations for the sensory characteristics of orange drink with 10% sucrose, compared with itself as a reference.

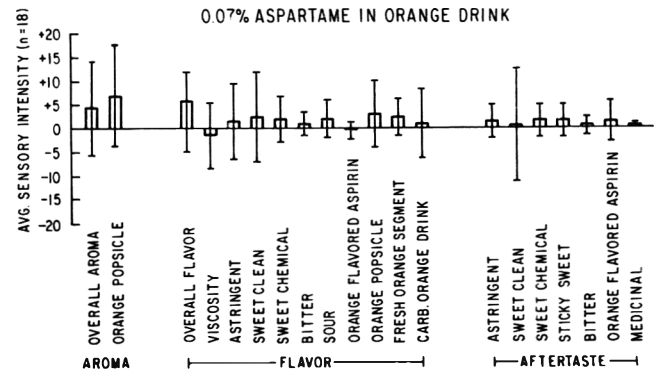

Fig. 2—Mean intensity differences and standard deviations for the sensory characteristics of orange drink with 0.07% aspartame compared with the 10% sucrose reference.

selected flavor terms. The large standard deviations for general descriptors like "Overall Aroma" and "Overall Flavor" indicate lack of concurrence among the judges, possibly because it was impossible to provide a reference sample for those descriptors. The best match and greatest group agreement was for bitter flavor and bitter aftertaste.

Figures 2, 3 and 4 depict the direction and magnitude of difference of the sensory characteristics of orange drink sweetened with aspartame, cyclamate or saccharin, contrasted with the sucrose-sweetened reference. Samples sweetened with saccharin deviated the most and those sweetened with aspartame deviated the least from the reference. "Overall flavor," the composite of all oral sensations, was significantly greater in the cyclamate and saccharin samples (Fig. 3 and 4). The term "sweet chemical," used to describe a synthetic-type of sweetness (in contrast with "sweet clean," which was associated with the sucrose sample), was significantly more pronounced in the cyclamate samples, both in flavor and in aftertaste. For the saccharin sample, however, "astringent" and "bitter" flavors were significantly more intense than for the sucrose reference. Large standard deviations were obtained, particularly for the drink containing saccharin (Fig. 3). This is attributed to the variation in perceived intensity of characteristics among, rather than within judges, as the F ratios for replication were not significant in the analysis of variance (df = 2/390, F = 2.10 for aroma, 1.81 for flavor, and 2.50 for aftertaste). Furthermore, the interactions of sweetener by replication, and of sweetener by descriptor were not significant. Large variations among judges also were reported by Daget (1974) who used a similar method to characterize the sensory properties of milk chocolate.

Unanchored descriptive analysis. Results from the unanchored descriptive analysis method permits intercomparison among all four sweeteners in orange drink (Fig. 5a and 5b). No significant differences in overall aroma were obtained. Only two of the 13 individual aroma descriptors differed significantly among the sweeteners – "fresh orange peel," and "orange-flavored aspirin," which were more intense in the sucrose sample. For flavor, overall intensities did not differ significantly but seven of the 16 individual flavor descriptors did (Fig. 5a). In general, drinks sweetened with sucrose or with aspartame could be characterized as "sweet-chemical," and "bitter." Sucrose and saccharin imparted more astringency than did the other two sweeteners. Saccharin was considered significantly more sour than the other sweeteners, possibly due

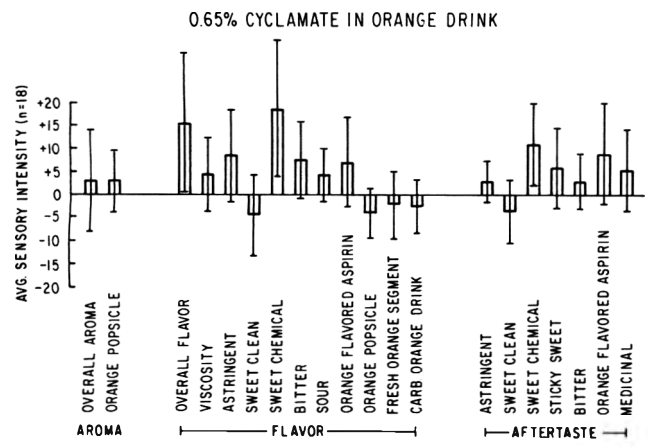

Fig. 3—Mean intensity differences and standard deviations for the sensory characteristics of orange drink with 0.65% cyclamate compared with the 10% sucrose reference.

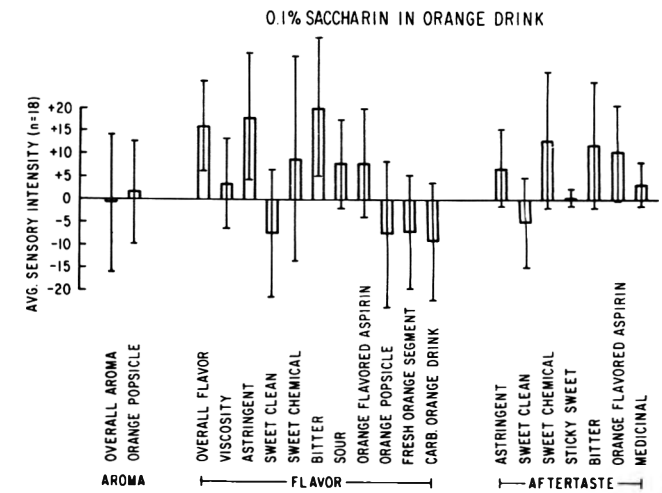

Fig. 4—Mean intensity differences and standard deviations for the sensory characteristics of orange drink with 0.1% saccharin compared with the 10% sucrose reference.

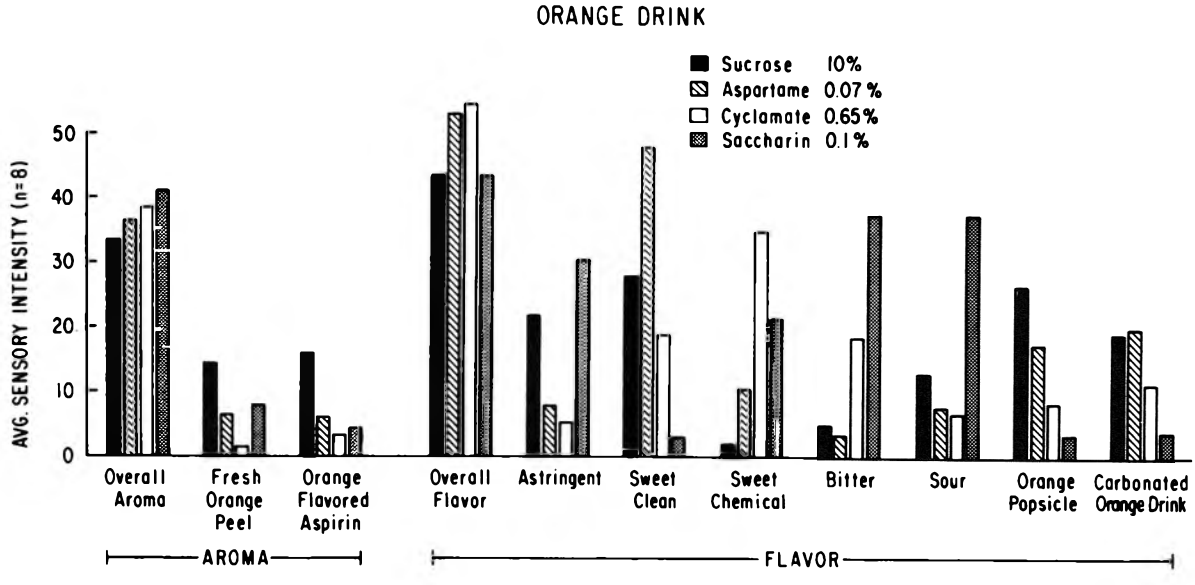

Fig. 5a—Mean intensities for aroma and flavor characteristics of orange drink for each of four sweeteners. (Unanchored descriptive analysis)

to the judges' association of sourness with bitterness, as observed also by other investigators (Meiselman and Dzendolet, 1967; Robinson, 1970). The comparative means and standard deviations (SD) for sourness and bitterness, respectively, were 12.8 ± 14.4 and 4.9 ± 11.8 for sucrose samples, and 37.5 ± 29.1 and 37.4 ± 39.3 for the saccharin samples. For the sucrose series, the foregoing SD fall within the same range as the SD for other descriptors. For the saccharin series, however, these SD are much higher than the SD for most other descriptors. In retrospect, we might have grouped the terms into a composite called "sour-bitter." Of the 11 terms used to describe aftertaste, seven differed in intensity among the four sweeteners (Fig. 5b). Again, samples with sucrose or aspartame were "sweet-clean," and those with cyclamate or saccharin were "sweet chemical." The saccharin sample continued to exhibit an astringent and bitter aftertaste, while the cyclamate sample had a cloying, "sticky-sweet" aftertaste. These latter sweeteners were considered medicinal, also.

Intercomparison of the means and of the analyses of variance for the two descriptive methods, showed that for all drinks, the anchored method (comparison with the sucrose reference) was more sensitive. More significant differences were observed among the sweeteners for more descriptors by the anchored, than by the unanchored procedure. Part of these differences may be attributable to the smaller number of judgments collected by the unanchored procedure, and by the sequence of presentation of methods, i.e., the three "anchored" sessions always preceded the "unanchored" session.

Gelatins

Similar results were obtained for the two gelatins – orange and strawberry; therefore, only the data from the latter are presented herein.

Anchored descriptive analysis. In gelatin, aroma could not be broken down into individual characteristics. Consequently, only "overall aroma intensity" was examined. Comparison of the sucrose sample against itself showed that only one term, "sour" deviated from the reference by more than 5 mm on the 120-mm scale (Fig. 6), demonstrating good internal consistency of the judges as a group. Small standard deviations were obtained for terms such as "bitter" and "metallic" but an exceptionally large standard deviation was obtained for the key flavor term, "strawberry." it is suspected that judges used

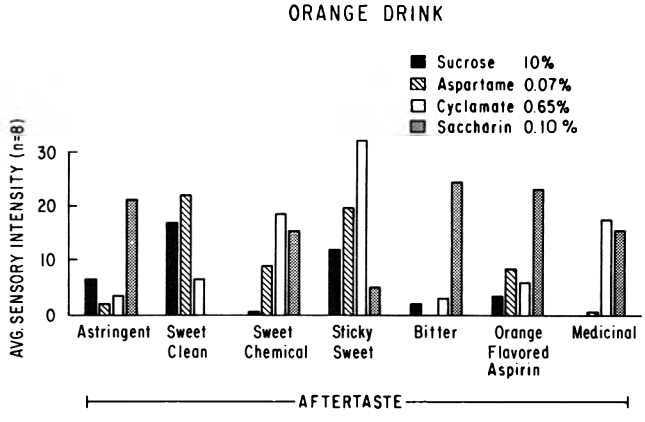

Fig. 5b—Mean intensities for the aftertastes of orange drink for each of four sweeteners. (Unanchored descriptive analysis)

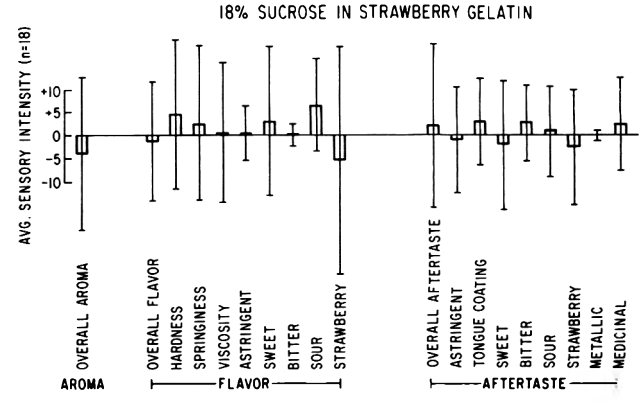

Fig. 6—Mean intensity differences and standard deviations for the sensory characteristics of strawberry gelatin with 18% sucrose, compared with itself as a reference.

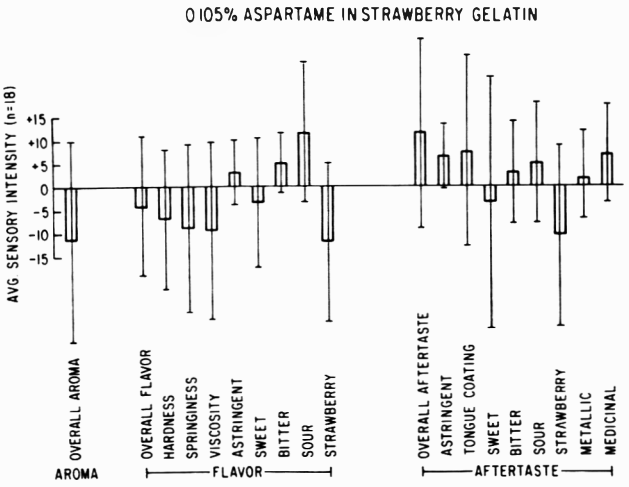

Fig. 7—Mean intensity differences and standard deviations for the sensory characteristics of strawberry gelatin with 0.105% aspartame compared with the 18% sucrose reference.

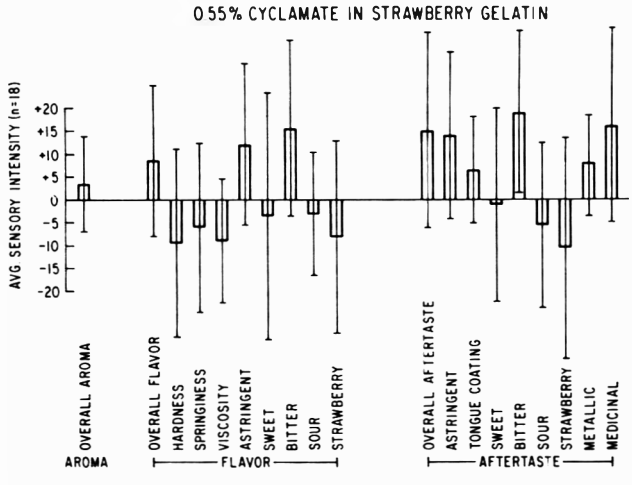

Fig. 8—Mean intensity differences and standard deviations for the sensory characteristics of strawberry gelatin with 0.55% cyclamate compared with the 18% sucrose reference.

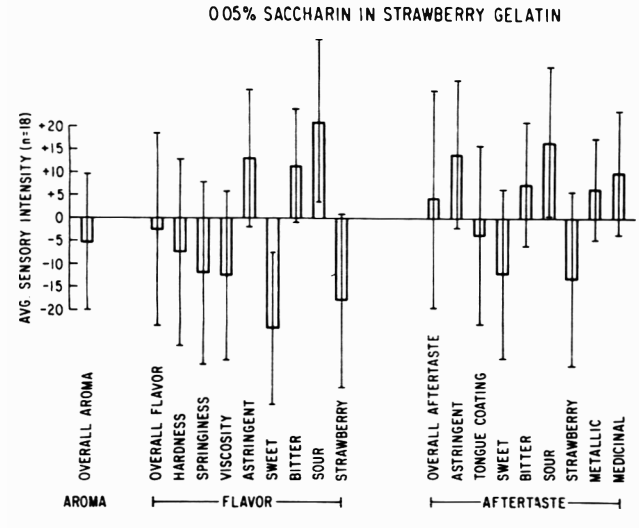

Fig. 9—Mean intensity differences and standard deviations for the sensory characteristics of strawberry gelatin with 0.05% saccharin compared with the 18% sucrose reference.

different criteria for evaluating the complex sensations they labeled as "strawberry."

The direction and magnitude of difference from the sucrose reference of strawberry gelatin sweetened with synthetic sweeteners are depicted in Figures 7, 8 and 9. In most characteristics, aspartame differed the least, and saccharin differed the most from the reference. Gelatins containing synthetic sweeteners were significantly less hard, springy and viscous than the sucrose reference. This is consistent with results obtained in time-intensity studies, where gelatin sweetened with sucrose was firmer than gelatin containing the synthetic sweeteners (Larson-Powers and Pangborn, 1978). As noted in Figures 7, 8 and 9, much less strawberry flavor and strawberry aftertaste was reported for synthetically-sweetened gelatins ($P < 0.05$). Gelatins with cyclamate and with saccharin had significantly more bitter flavor and bitter aftertaste than did the samples with sucrose. Considerable sourness was ascribed to the saccharin sample. Again, it should be mentioned that many tasters equate, or confuse sensations of sourness and bitterness.

Unanchored descriptive analysis. In Figure 10, mean intensity values are presented for gelatins evaluated simultaneously in a multiple-sample presentation. Significant differences ($P < 0.05$) were obtained among the four sweeteners for the descriptors "sweet," "bitter," and "strawberry" flavors. Again, samples with sucrose or with aspartame were sweeter, less bitter, and had more strawberry flavor than did samples containing cyclamate or saccharin. Bitter, sour, and medicinal aftertastes were more perceptible in these latter samples, also. A greater number of descriptors differed significantly among the four sweeteners using the anchored analysis than by the unanchored method, as indicated previously for the drinks.

CONCLUSIONS

DESCRIPTIVE ANALYSES allowed intercomparison of multiple sensory characteristics, rather than a single parameter as described previously for paired-directional tests and time-intensity testing (Larson-Powers and Pangborn, 1978). Anchoring the description to a reference, and expressing results in terms of the positive and negative deviation from the reference improved both the precision and the accuracy of the responses, compared to the unanchored descriptive method.

In all five test media – strawberry, lemon and orange drinks, and strawberry and orange gelatins – the anchored method resulted in a greater number of descriptors which were significantly different among the four sweeteners, indicating it was more sensitive than the unanchored method. Comparison of analyses of variances between anchored and unanchored test data showed much higher F ratios for sweeteners for the former, and higher F ratios for judges for the latter method. In other words, we found more differences among sweeteners in the anchored, and more judge variability in the unanchored method. Again, it should be noted that there were fewer judgments with the latter method.

Additional real and potential advantages of an anchored descriptive method would include: (a) Provision of an internal measure of judge reliability by comparison of the reference against itself as a blind sample; (b) Provision of a fixed criterion of comparison to minimize drifting of responses with time, or comparison against faulty memory standards; (c) In incomplete block designs where samples cannot be compared against each other, there is a potential increase in reliability because they can all be compared against the same standard; (d) In product matching or product formulation, the method provides a quick measure of attributes, and hence ingredients, which need to be increased or decreased relative to a fixed reference.

The disadvantage of the anchored method would include an indirect, rather than a direct knowledge of the degree to which

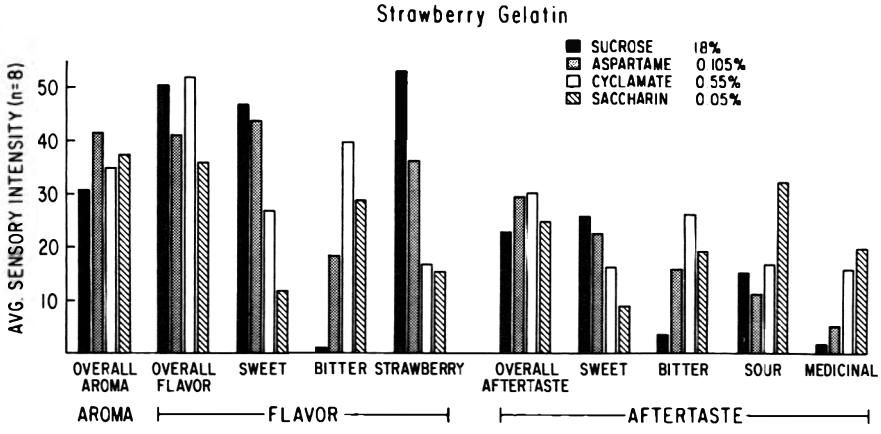

Fig. 10—Mean intensities for aroma, flavor and aftertaste characteristics of strawberry gelatin for each of four sweeteners. (Unanchored descriptive analysis)

samples compare with each other. In studies in which it is difficult to designate a reference, the anchored method would, of course, be of limited value.

Relative to the sweeteners, samples containing sucrose or aspartame had little bitterness and were termed "sweet clean," whereas those containing cyclamate or saccharin were very bitter and were labeled "sweet chemical." These observations on sweetness and bitterness are in agreement with conclusions obtained using the time-intensity technique (Larson-Powers and Pangborn, 1978).

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JUHANI OLKKU[1] and STEVEN W. FLETCHER III
Dept. of Food & Agricultural Engineering, University of Massachusetts, Amherst, MA 01002
CHOKYUN RHA
Dept. of Nutrition & Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139

STUDIES ON WHEAT STARCH AND WHEAT FLOUR MODEL PASTE SYSTEMS

ABSTRACT

A Brabender Visco/amylo/Graph model VA-V modified to accommodate higher torque was used to study gelatinization of wheat flour and wheat flour components in limited water concentration (solid concentration between 50.2 and 59.7%). Other solid ingredients in the mixture were sugar and molasses. Effect of ingredient variables (starch content of flour components, fraction of flour components or flour in the flour-sugar mixture, and moisture contents of the mixture) and cooking variables (rpm of the cooker, temperature above the pasting temperature, and duration of cooking after reaching the pasting temperature) on maximum consistency and consistency of the paste at the end of cooking and moisture loss during cooking were investigated. Second order polynomial equations expressing relationships between ingredient and processing parameters are developed from the experimental results. Water content, flour content of solids, and starch content of flour all affect the consistency of the flour-sugar-water paste. The pasting temperature of starch or flour in sugar solution is a function of sugar concentration.

INTRODUCTION

THE GELATINIZATION of starch in aqueous solutions at temperatures above 60°C has been recently reviewed (Olkku and Rha, 1977). Gelatinization takes place in two stages, which appear as a rise in the viscosity of the solution due mainly to hydration, and a release of solubilized starch exudate. The release of exudate can be delayed by other flour components such as proteins, pentosans, and lipids, either through complex formation and/or limiting the amount of water available for the starch hydration. Other ingredients in food, such as sugars and salt, also have a delaying effect on the onset of gelatinization. The mode of action of these ingredients on starch gelatinization may be similar to that of non-starch components of flour. It may be due in part to a decrease in the water available for the hydration of starch and in part to the interactions of these ingredients with the starch itself. This seems to suggest that there is a certain level of "reactivity of available water" which is required for gelatinization of starch. That is, in the presence of ingredients which decrease the availability of water for starch hydration, the temperature must be increased until this level of "the reactivity of available water" is reached. When this condition is met, the gelatinization of starch takes place.

Gelatinization of wheat flour and of wheat flour components in limited water concentration was investigated in this study. The solids concentration ranged between 50.2 and 59.7%. Other solids used in these experiments were sugar and molasses. Generally the term "gelatinization" is used to indicate the thickening of starch materials. However, in the strict chemico-physical sense, starch does not gel. Therefore in this study the term "pasting" is preferred.

MATERIALS & METHODS

Instrumentation

Gelatinization instrument. A Brabender Visco/amylo/Graph model VA-V (C.W. Brabender Instruments Inc., South Hackensack, NJ) was modified to electronic recording as described by Voisey and Nunes (1968). The modification was made using a Daytronic 300D transducer-amplifier with Type 91 strain gauge transducer input module, and a Riken Denshi SP 65V recorder (Queensboro Instruments, Ottawa, Ontario, Canada).

In order to simulate industrial conditions, the instrument was equipped with a CWB No. 031-20-029 thermoregulator which has a 2.5°C/min temperature increase and a maximum temperature of 150°C. The thermoregulator was equipped with a protective tube for high torque operations.

The torque encountered in this experiment exceeded the torque limit of the recording system of the normal Brabender Visco/amylo/Graph bowl and bin system. Therefore a CWB No. 03-20-44 open bowl with one central rod and the mixing-torque-sensing system given in Figure 1 were used. It was established that 1 cm-g torque recorded with the modified system was equivalent to 3.5 cm-g, or about 5 B.U., recorded with the standard amylograph system. The maximum torque limit of the instrument was 4000 cm-g or about 20,000 B.U., compared with 2000 B.U. for the normal instrument.

Two 10 microfarad capacitors were added parallel to the recorder input to reduce the torque fluctuation amplitude in the torque curves. A rubber O-ring was attached on the measurement shaft in the instrument for the same purpose.

Because the cooking mass tended to rise over the edges of the bowl wall, the thermoregulator stem was supplied with a small scraper wing that was kept in its place with rubber O-rings. A bowl wall scraper was installed to keep the upper bowl wall clean. These details are shown in Figure 2.

Additional Instruments

A JSM U-3 Scanning Electron Microscope was used to examine the structure of the products.

Moisture contents were determined at 155°C for 20 min and 120°C for 25 min for flour and starch respectively using a Brabender Moisture/Volatiles Tester (C.W. Brabender Instruments Inc., South Hackensack, NJ).

A blender, Catalog No. 34 K 82932 (Sears, Roebuck & Co.) was used for mixing the experimental recipes.

Ingredients

Wheat starch used was the thick boiling powder (Supergell Code 2052) containing 7.0% moisture, 0.2% protein, 0.2% ash and pH 6.5. Wheat solubles (code 9271) had 15.5% moisture and 21.3% protein on dry basis. Wheat flour was first clears with 13% moisture content. These three ingredients were obtained from Industrial Grain Products (P.O. Box 6089, Montreal 101, Quebec, Canada). Molasses (SuCrest Corp., 120 Wall St., New York, NY 10005) ahd 22.5% moisture and 77.5% total solids including 71.8% sugars, 2.5% ash and 3.1% organic non-sugars.

Variables and experimental design

Ingredient and cooking variables. The independent variables were selected for this study on the basis of the work of Knoch (1972), industrial experience in the field, and preliminary experiments (Olkku and Rha, 1975). The variables are shown in Table 1. The ratios of

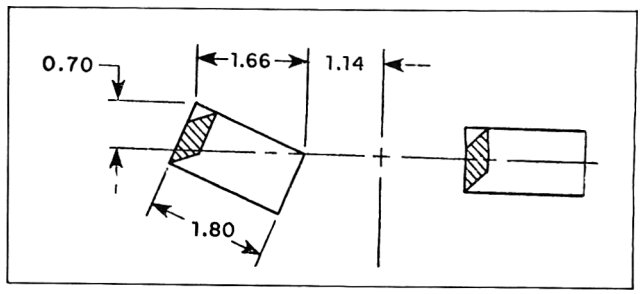


Fig. 1—Location of mixing rods on sensor base plate (full scale, in centimeters).

[1] Present address: Technical Research Center of Finland, Food Research Laboratory, 02150 Espoo 15, Finland

Table 1—Independent and dependent variables

Independent variables

Ingredient variables

x_1 = the fraction of starch in flour components on dry basis
x_2 = the percentage of flour components (or flour) in the flour-sugar mixture on dry basis
x_3 = the moisture contents of the mixture in percent on wet basis

Cooking variables

x_4 = the rpm of the cooker (an indication of the rate of shear)
x_5 = the temperature difference above the pasting temperature in °C (equivalent to the temperature at 250 cm-g torque)
x_6 = the duration of cooking in minutes after reaching the pasting temperature

Dependent variables

y_1 = the average consistency at the end of cooking in kgcm
y_2 = the consistency spread amplitude around y_1 in kgcm
y_3 = the average consistency at consistency maximum in kgcm
y_4 = the consistency spread amplitude around y_3 in kgcm
y_5 = the maximum consistency at the end of cooking in kgcm
y_6 = the maximum consistency at consistency maximum in kgcm
y_7 = the moisture loss during cooking (in percent per minute w.b.)

Table 2—Values of variables at experimental levels

	−a	−1	0	+1	+a
x_1	0.795	0.807	0.815	0.823	0.835
x_2	30.3	33.0	35.0	37.0	39.8
x_3	40.3	43.0	45.0	47.0	49.8
x_4	52	65	75	85	98
x_5	0.3	3.0	5.0	7.0	9.7
x_6	31	39	45	51	59

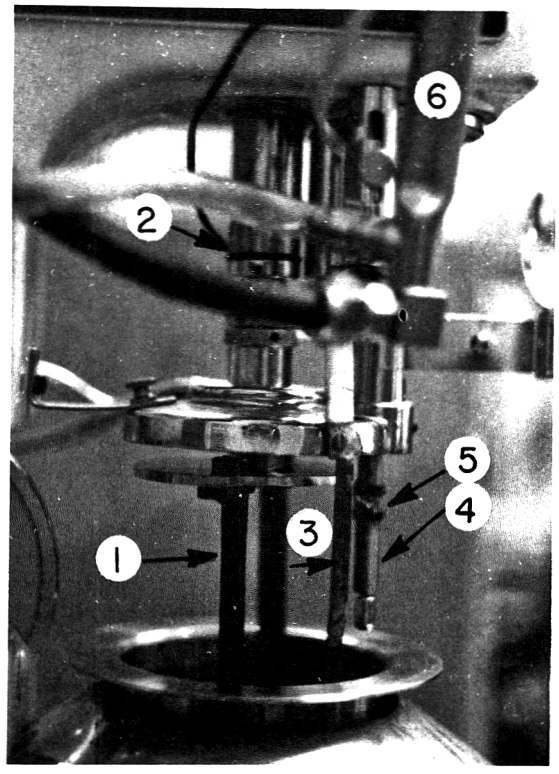


Fig. 2—Details of the bowl scraping and recording equipment: (1) Mixing-torque sensing head fixed on the instrument; (2) Rubber O-ring to reduce the amplitude of torque fluctuations; (3) Bowl wall scraper blade; (4) Thermoregulator with the protective tube; (5) Small scraper blade on the thermoregulator stem; and (6) Amylograph cooling probe which has been removed from the bowl, being unnecessary during the experiments, and giving rise to high torque.

wheat starch and wheat flour solubles were controlled in the model systems.

Experimental variables. The factorial experimental design uses coded variables, where the 0-level of each variable is in the center of the experimental variable ranges, and levels +1, −1, and +a and −a are used. The variable values at each level are given in Table 2. These levels have been chosen according to Knoch (1972) within the limits of instrumentation. The following coded variables have been used:

$$(1)\quad x_1 = \frac{X_1 - 0.815}{0.008} \qquad (4)\quad x_4 = \frac{X_4 - 75}{10}$$

$$(2)\quad x_2 = \frac{X_2 - 35}{2} \qquad (5)\quad x_5 = \frac{X_5 - 5}{2}$$

$$(3)\quad x_3 = \frac{X_3 - 45}{2} \qquad (6)\quad x_6 = \frac{X_6 - 45}{6}$$

The flour composition (flour ingredients) in the model systems had X_1 fraction of starch and 0.01 part of gluten, the remainder being wheat flour solubles. The sugar was 20% sucrose and 80% sugar from molasses, on dry basis.

Dependent variables. The variables are shown in Table 1 and in Figure 3.

Experimental design. A $1/2 \times 2^6$ near rotable central composite design (Myers, 1971) was executed with the levels given in Table 2. A star point block (runs on the midpoint, one variable either on (+a) or (−a) value; the others on 0 level) was run to discern significant differences between flour and the nearly gluten-free starch (0.2% protein) and soluble mixtures in the models. The value of (a) for this design is 2.3446.

Description of experiment

Experimental run. The flow diagram of a typical experimental run is given in Figure 4.

RESULTS & DISCUSSION

Analysis and results of the cooking stage

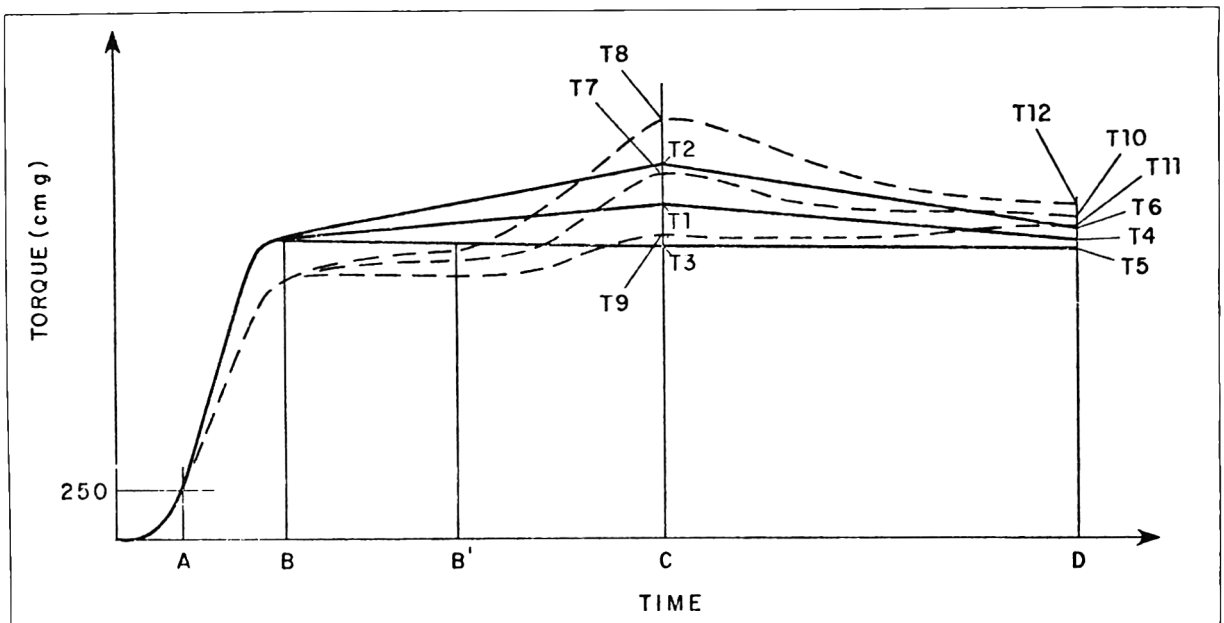
Typical torque vs time curves are shown schematically in Figure 2, in which variables y_1–y_6 are also defined. Actual midpoint time vs torque curves are shown in Figure 5.

Pasting temperatures

In the present study, the pasting temperature is considered to be the temperature at which 250 cm-g torque is reached. This torque value is equivalent to 1250 B.U. of the standard Amylograph. Inspection of Bean et al. (1974), in which curves were obtained by using the standard Amylograph under similar conditions, shows that rise in consistency is very rapid. The torque vs time curves are almost vertical. Thus the error in time or temperature, which is implied by using the 1250 B.U. torque as the observation point, is negligible. All curves in the present study were rapidly rising at this torque value, and thus the observation points are sharply defined. The observed pasting temperatures are given in Table 3.

Star point notation is a reference to the fact that there is no duplication, and thus no calculation of standard error. The numbers refer to the levels of coded variables x_1, x_2, and x_3, respectively. These variables determine the individual recipes.

There were two cases in which the pasting temperatures were not within the limits of the standard error for a particular concentration of sugar. In both cases the lower pasting temperature is associated with a higher fraction of starch in flour ingredients, and the higher temperature with a lower fraction of starch. This probably indicates a tendency toward more rapid or intensive release of the exudate with higher levels of



A	*Torque defined as the pasting limit = 250 cm-g*
AB	*Region of fast consistency development associated with the first pasting stage of starch, with rapid swelling and release of the starch exudate into the media.*
BB'	*The region of slow linear rise in consistency in flour experiments.*
BC	*The almost linear rise in consistency to a maximum in the experiment with flour components witnout gluten.*
B'C	*The rapid nonlinear rise in consistency to a maximum in flour exoeriments.*
CD	*The slow, almost linear decrease in consistency after consistency maximum.*
T1	*Mean torque at torque maximum (y_3).*
T2–T3	*The amplitude of the torque spread, the "torque envelope" at the torque maximum (y_4).*
T4	*The mean torque at the end of the experiment (y_1).*
T5–T6	*The "torque envelope" at the end of the experiment (y_2).*
T7	*Mean torque at the torque maximum in the flour experiment (y_3).*
T8–T9	*The "torque envelope" at the torque maximum in flour experiment (y_4).*
T10	*Mean torque at the end of a flour experiment (y_1).*
T11–T12	*The "torque envelope" at the end of a flour experiment (y_2).*
T8	*Maximum torque at torque maximum in flour experiment (y_6).*
T2	*Maximum torque at torque maximum in flour component experiment (y_6).*
T12	*Maximum torque at the end of the flour experiment (y_5).*
T6	*Maximum torque at the end of the flour component experiment (y_5).*

Fig. 3–Schematic presentation of torque vs time (Solid line, cooking without gluten; broken line, cooking from flour).

starch. However, additional data are necessary to confirm this trend. The pasting temperature data for flour were not significantly different from the data obtained in the model system containing flour components.

The best-fit regression line by the method of least squares was:

$$T = 53.2 + 0.89C_s \quad (1)$$

where T is the pasting temperature in °C, and C_s is the percentage of sugar in the sugar-water solution (w/w).

Eq (1) has a correlation coefficient to the observed data of r + 0.873, and the statistically unbiased estimate of the standard error is 1°C.

The pasting temperature vs percentage of sugar has been plotted in Figure 6, which also shows the best-fit regression line.

No statistically significant correlation could be found between the percentage of flour in the flour-water mixture and the pasting temperatures observed. The correlation coefficient between them was r = 0.462, with the standard error of 4.7. This indicates that in this experimental range, the pasting temperature can be viewed as a function of the concentration of sugar in the sugar-water mixture. This supports the suggestion of Bean and Yamazaki (1973) that in pasting the influence of sugar solution exceeds others. Therefore, in relation to pasting temperature, the sugar solution should be viewed as a single medium in which the starch content of flour becomes a paste.

Delay of starch gelatinization in sugar solutions

Bean and Yamazaki (1973) and Bean et al. (1974) suggested that sugar delays the gelatinization of starch in aqueous solutions, rather than inhibiting it. The results of the present work seem to support this suggestion. Berry and White (1966) analyzed the delay in gelatinization in two aspects: the physical reduction of the accessibility of water to starch, and competition of other substances with starch for the available water.

The pasting of starch occurs at a level of water activity

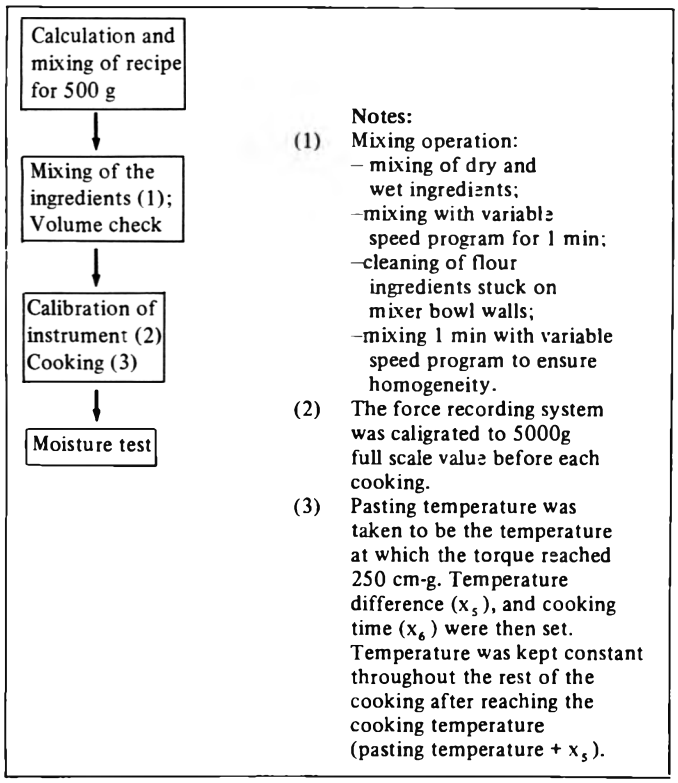


Fig. 4—Flow diagram of experimental run.

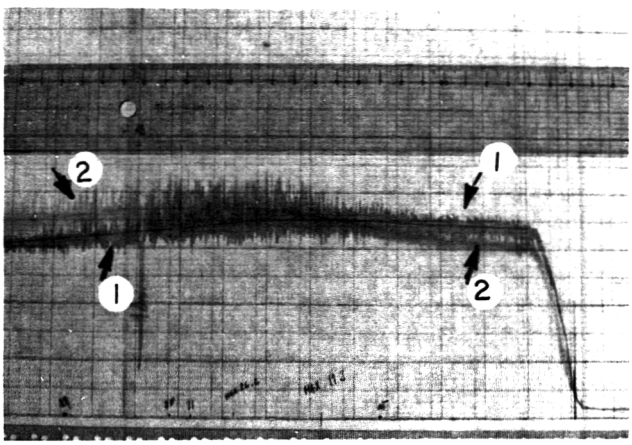


Fig. 5—Midpoint torque vs time curves: (1) Cooking without gluten; (2) Cooking with flour including gluten.

Table 3—Pasting temperatures of flour ingredients and flour in sugar solutions

Sugar (%)	Flour (%)	Pasting Temp (°)	Std error	Note
38.62	25.32	87.0	–	Star point
41.15	29.11	89.7	0.9	1 or −1, 1, 1
41.48	31.90	89.5	–	Star point
42.02	26.30	89.6	0.8	1, −1, 1
42.02	26.30	91.4	0.1	−1, −1, 1
43.28	29.12	91.0	0.6	Midpoint
44.96	26.11	92.5	–	Star point
45.12	32.56	93.3	0.5	1 or −1, 1, −1
46.00	29.56	94.0	0.4	1, −1, −1
46.00	29.56	96.6	0.8	−1, −1, −1
48.04	33.42	97.0	–	Star point
38.62	25.31	88.5	–	Flour
41.48	31.90	89.0	–	Flour
43.28	29.12	90.9	0.2	Flour midpoint
44.96	26.11	94.0	–	Flour
48.04	33.24	95.0	–	Flour

Table 4—Pasting temperatures of starch in sugar solutions and their respective water vapor pressures in pascals (X 10^3) from Eq (2)

% Sugar	0	30	35	40	45	50	Source[a]
T(°C)	–	–	84.3	88.8	93.2	97.7	(1)
P_w	–	–	5.559	6.750	7.851	9.433	(1)
T	53.8	82.0		91.5		102	(2)
P_w	1.5006	5.134		7.423		10.916	(2)
P_i	–	4.502		6.003		7.503	(2)
T	67.0			100.33		104.11	(3)
P_w	2.7347			10.137		12.085	(3)
P_i	–			10.939		13.674	(3)
T	56.0	66.0		78.0		85.0	(4)
P_w	1.6512	2.615		4.365		5.783	(4)
P_i	–	4.954		6.605		8.256	(4)

[a] (1) Present work; (2) Berry and White (1966); (3) Cakebread (1969); and (4) Bean et al. (1974)

available for hydration of starch. Increasing the sugar content decreases the activity of available water in solution, but increasing temperature can restore it to the level required for pasting.

For the sake of simplicity, the water vapor pressure could be viewed as an indication of the activity level of the water in the pasting process. Furthermore, water vapor pressure can be considered mainly as a function of the sugar concentration, since sugar is the major soluble ingredient. Eq (2), developed in this study and similar to that expressing Raoult's Law, may be used as a first approximation to determine gelatinization temperature from the water vapor pressure of the system.

$$P_i = P_0(10x_s) \qquad (2)$$

where P_i = water vapor pressure at the pasting temperature of the system; P_2 = water vapor pressure at the temperature at which starch gelatinizes in pure water; and x_s = weight fraction of sugar in the sugar-water mixture, $0.20 \leq x_s \leq 0.50$.

In Table 4, data from the present work and from three other sources have been collected. The table shows the observed pasting temperatures (t), the water vapor pressures at these temperatures (P_w), the water vapor pressures (P_i) calculated from Eq (2). The water vapor pressure data used are from Longcin (1969). These values are plotted in Figure 7. In the figure, solid lines connect the experimental data and dotted lines connect the values calculated from Eq (2). The values calculated from Eq (2) do not agree closely with the observed values. The calculated values are lower than the observed ones for Berry and White (1966) and higher than observed values for Cakebread (1969) and Bean et al. (1974). In general, the values observed in the present study approximate the calculated values of Bean et al. (1974), and the slopes are

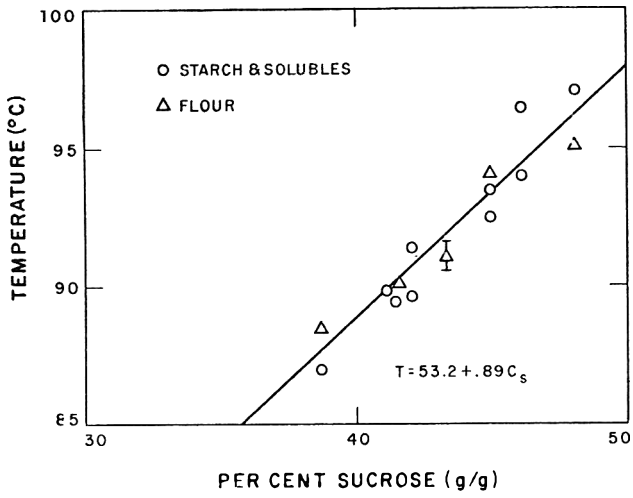


Fig. 6—Pasting temperature vs sugar concentration.

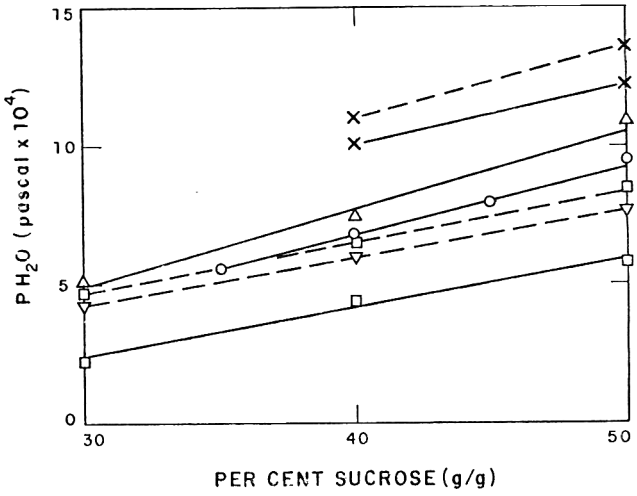


Fig. 7—PH$_2$O vs sugar concentration: o = data from present work; x = data from Cakebread; Δ = experimental data of Berry and White; ▽ = data from Eq (2); and □ = data from Bean et al. (1974).

similar despite the wide spread of values. The disagreement may partly result from the use of different methods and materials. Pasting experiments in water without sugar were not carried out in the present work.

Eq (2) does not take into account the suppression of water activity by ingredients other than sugar. But since sugar plays the predominant role in suppressing water vapor pressure in the present experiment, further attempts to refine Eq (2) were not made. It is believed that more detailed consideration is not meaningful in the present work, in view of the complexity of the recipe and incompleteness of information in the literature. More experiment is necessary before the relation between the water activity and pasting temperature can be established. The mechanism of pasting in terms of reactivity of water on a thermodynamic basis also needs further study.

Analysis of the cooking curves

The second order polynomials are given in Eq (3) to (8). The correlation coefficient between the data and the calculated values, r, and the estimate of standard error, S.E., are given with the equations. The equations for variables measured at the maximum consistency are:

$$y_3 = 1.813 + 0.154x_2 - 0.164x_3 - 0.027x_{11}^2 - 0.045x_3x_5 + 0.037x_4x_5 \quad (3)$$
$$(r = 0.890;\ S.E. = 0.113)$$

$$y_4 = 0.400 + 0.264x_2 - 0.281x_3 - 0.100x_4 - 0.39x_3^2 + 0.057x_1x_2 - 0.097x_2x_3 - 0.068x_3x_5 - 0.064x_4x_6 \quad (4)$$
$$(r = 0.888;\ S.E. = 0.227)$$

$$y_6 = 1.985 + 0.047x_1 + 0.266x_2 - 0.305x_3 - 0.073x_4 + 0.076x_3^2 - 0.079x_3x_5 \quad (5)$$
$$(r = 0.886;\ S.E. = 0.215)$$

The equations for the variables measured at the end of the cooking are:

$$y_1 = 1.551 + 0.093x_2 - 0.078x_3 - 0.062x_4 - 0.074x_5 - 0.049x_6 - 0.029x_1^2 - 0.026x_1x^3 - 0.063x_2x_5 - 0.065x_3x_6 - 0.058x_5x_6 \quad (6)$$
$$(r = 0.883;\ S.E. = 0.102)$$

$$y_2 = 0.174 + 0.185x^2 - 0.216x_3 - 0.077x_4 + 0.057x_2^2 + 0.089x_3^2 + 0.034x_6^2 - 0.100x_2x_3 - 0.047x_2x_5 - 0.097x_5x_5 \quad (7)$$
$$(r = 0.889;\ S.E. = 0.176)$$

$$y_5 = 1.648 + 0.037x_1 + 0.203x_2 - 0.186x_3 - 0.073x_4 - 0.072x_5 - 0.072x_6 + 0.032x_2^2 + 0.030x_3^2 - 0.043x_2x_5 + 0.042x_2x_6 - 0.043x_3x_5 \quad (8)$$
$$(r = 0.902;\ S.E. = 0.151)$$

The sum of squares accounted for by Eq (3) to (8) is significant in all cases. But at the same time the sum of squares for the lack of fit is significant at the 1% level. This shows that improvement over the second order polynomial should be made to represent the consistency during cooking. However, given the significance of part of the model and the acceptable correlation coefficients, the equations in their present form are useful in understanding the phenomena which occur during the cooking.

The cooking curves for several of the star point cookings, showing the effects of changes in individual variables, are shown in Figures 8–15. Inspection of these figures reveals the following facts about the effects of the individual variables.

The increase in starch content of flour solids, x_1, seems to lower consistency during cooking (Fig. 8). When the flour content in the flour-sugar mixtures, (x_2), is increased, the torque increases significantly (Fig. 9). This is due to larger amounts of flour material which swell and form networks of exudate in media of low sugar concentration. Also, since the pasting temperature of the low sugar (high flour) mixture is lower, the increase in consistency may be partially due to an effect of lower temperature.

Water, (x_3), has a strong effect on the consistency of the paste (Fig. 10 and 11). Processing at low moisture content does not provide sufficient water to hydrate the flour ingredients fully. The mixture becomes a stiff paste, and complete swelling of the starch is not achieved. However, the partially swollen granules themselves contribute significantly to the increase in consistency.

Shear or rpm, (x_4), does not seem to have a significant effect on the end-of-cooking consistency in the experiment with flour (Fig. 12). For flour ingredients, there seems to be only a small effect of shear (Fig. 13). The consistencies at high shear cooking are lower, indicating a mechanical breakdown. At higher rpm consistency develops more rapidly until maximum consistency is reached. This can be attributed to increased mechanical breakdown which releases exudate and starch. However, this effect disappears before pasting is achieved. In these experiments the effect of shear is not visibly demonstrated because the shears were high and the range of rpm was small.

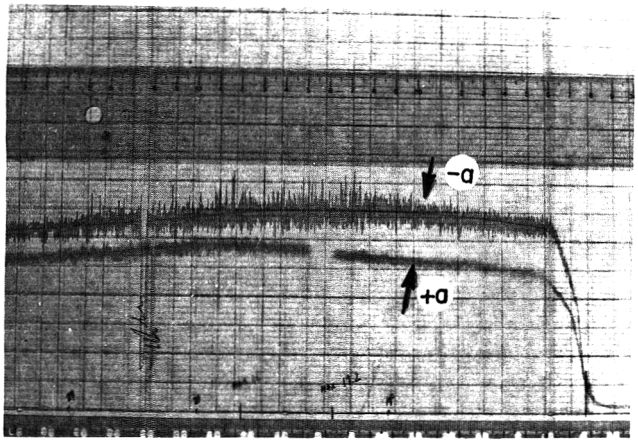


Fig. 8—Flour component experiments with x_1 (starch content of flour ingredients) on levels +a and −a.

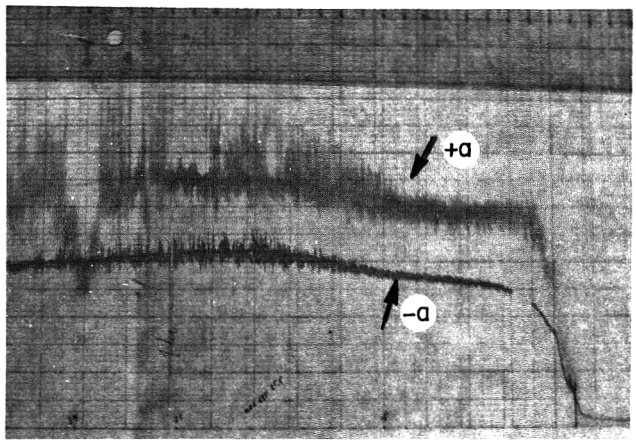


Fig. 9—Flour experiments with x_2 (flour content of flour-sugar mixture) on levels +a and −a.

The temperature difference above the pasting temperature, x_5, affects the consistency (Fig. 14 and 15). Initially, only small influences are apparent, with the torque curves running close together up to the maximum. Thereafter, the large difference in temperature has a marked viscosity reducing effect. Therefore, phenomena taking place before maximum consistency are not dependent on the temperature difference. If the slow rise of consistency to its maximum is an indication of the second stage in pasting, it seems to occur even before the boiling point of the medium is reached. This seems to contradict the conclusions of Bean et al. (1974). Thermal breakdown after the maximum is evident. This can be partially due to the thermal degradation of starch, and partially to the stronger effect of mechanical work (shear) at the higher temperature.

The effect of time on the consistency is also evident. The consistencies drop continuously when the cooking is prolonged. This indicates the mechanical and thermal breakdown of the swollen starch particles and of the exudate networks.

Inspection of the maximum consistency Eq (3, 4, 5) indicates that the flour content in the solids (x_2) and the water content in the mixture (x_3) have the greatest effect on the consistency.

Increasing the flour content greatly increases the consistency, and increasing the water content lowers it. The increase in rpm does not affect the average consistency, but it does lower the consistency spread amplitude. The starch content of flour ingredients slightly increases the maximum consistency, which seems to occur (second-order effect) within the range for the average consistency, but has no direct effect on the consistency spread amplitude. This appears to be a combined effect of temperature and the flour content of solids. The cross-product of the temperature difference and flour content of solids (x_5 and x_2), which appears in all of these equations, indicates the effect of the temperature on the flour. The decreasing flour content (x_2) accompanies the increase in the sugar concentration, which is associated with higher pasting temperature. If the higher temperature difference (x_5) is added to the higher pasting temperature, the actual cooking temperature is also higher. The combination of these interactions can be viewed as an indication of the effect of thermal breakdown of the flour components.

For the end-of-cooking values, the effects of time and of temperature differential (x_6 and x_5) start to play a more prominent role. Increases in both of these variables have a consistency lowering effect. This is also true for rpm (x_4). The effect of flour content of solids (x_2) in combination with the temperature difference (x_5) is also evident. These factors combined reflect the thermal and mechanical breakdown of the

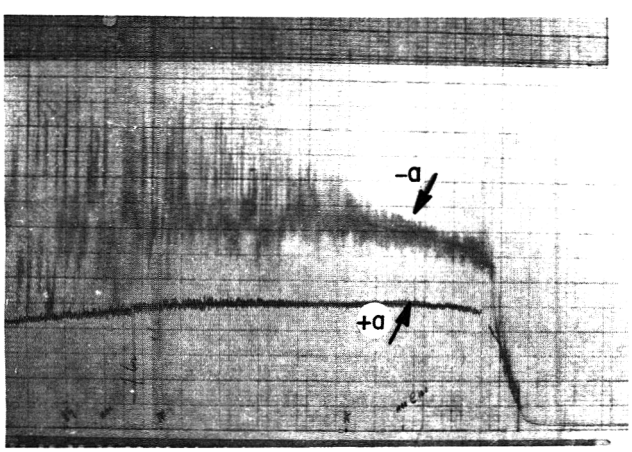


Fig. 10—Flour experiments with x_3 (water content) on levels +a and −a.

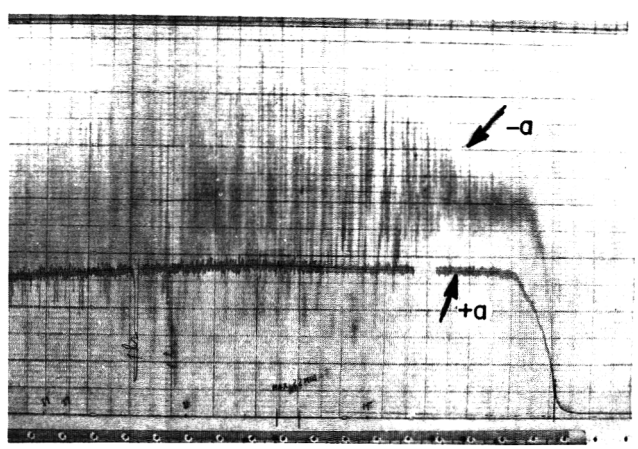


Fig. 11—Flour component experiments with x_3 (water content) on levels +a and −a.

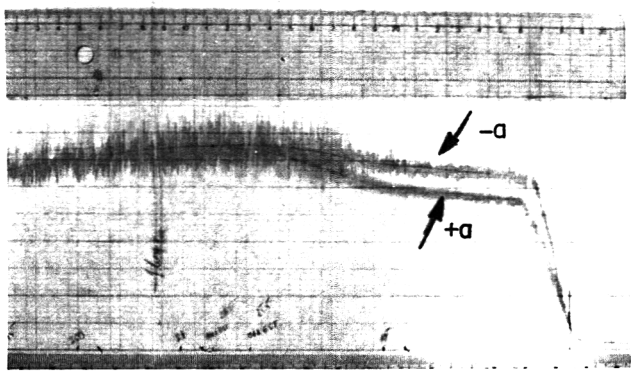


Fig. 12—Flour experiments with x_4 (rpm) on levels +a and −a.

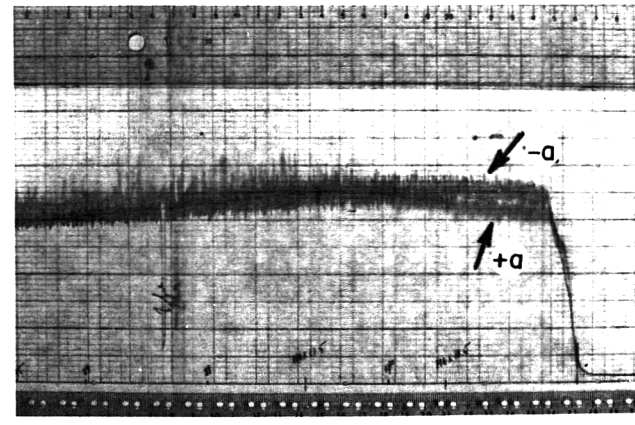


Fig. 13—Flour component experiments with x_4 (rpm) on levels +a and −a.

structures of granules and of the exudate networks during prolonged cooking.

Some general explanation on pasting can be given from Figure 3. In the case of experiments with flour ingredients without significant amounts of gluten, there is a rapid initial increase in consistency, which is usually followed by a slower, almost linear increase. Assuming a rapid initial release of exudate at the first stage, followed by a slow continued release, these changes are in good agreement with observations of Jaska (1971) concerning water and starch mobility. In experiments with flour, the gluten seems to have a shielding effect during the initial rise in consistency, but in time this effect diminishes and a more rapid rise in consistency occurs in the second stage. Thereafter, the higher consistency is observed in those model systems containing flour ingredients without gluten. This may be due to the effect of a physical barrier of gluten, which in flour is on the surfaces of the granules. However, part of the effect may be due to commercial wheat starch granules already being ruptured during the preparation of starch from flour, and thus being more susceptible to hydration. Proteins may also interact with starch during the gelatinization of the flour systems and the model systems (Hwang, 1963; Grant, 1968; Stalder, 1964). In wheat flour the gluten has not been denatured before cooking. However, the protein in wheat flour solubles has been subjected to a drying operation, and can be at least partly denatured. If so, the difference observed between systems containing wheat flour and those containing flour ingredients may be explained by observations made with milk proteins (Hwang, 1963; Grant, 1968; Stalder, 1964). Further research on this phenomenon is needed before the mechanism can be fully understood.

It is worth noting that the second small rise in viscosity takes place even in the cases with the low temperature differential treatment. This indicates continued starch release into the medium at a rate independent of the temperature differential, even if the boiling point of the medium is not reached. Thus, if it is assumed that the slow rise in viscosity is associated with the second pasting stage of starch, it would seem to take place whether or not the boiling point of the medium is reached. This contradicts the claim of Bean et al. (1974). It is apparent at least that whatever the cause of the rise in viscosity, the process continues uninterrupted once the initial pasting stage has taken place.

The effect of the prolonged cooking on the visual structure is shown in the scanning electron micrographs in Figure 16. These micrographs show a cloudlike structure of swollen starch particles imbedded in a mass of sugar-starch intermedia. When the cooking, i.e., the thermal and mechanical breakdown, is continued, the number of particles decreases and forms a more homogeneous intermedium with the structure showing some layering effect.

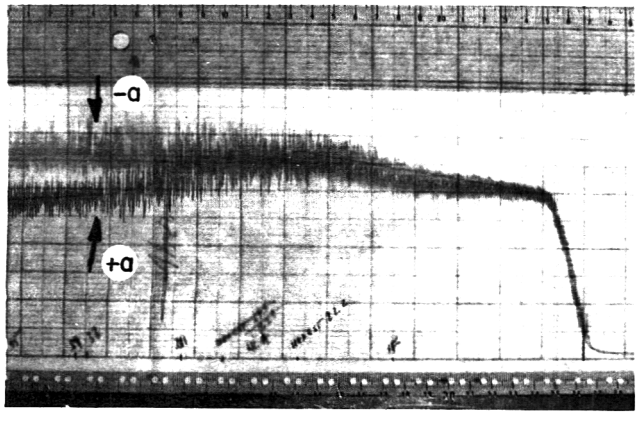


Fig. 14—Flour experiments with x_5 (temperature difference above pasting temperature) on levels +a and −a.

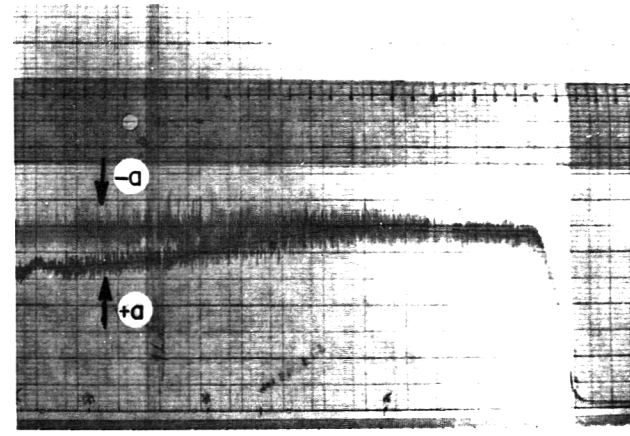


Fig. 15—Flour component experiments with x_5 (temperature difference above pasting temperature) on levels +a and −a.

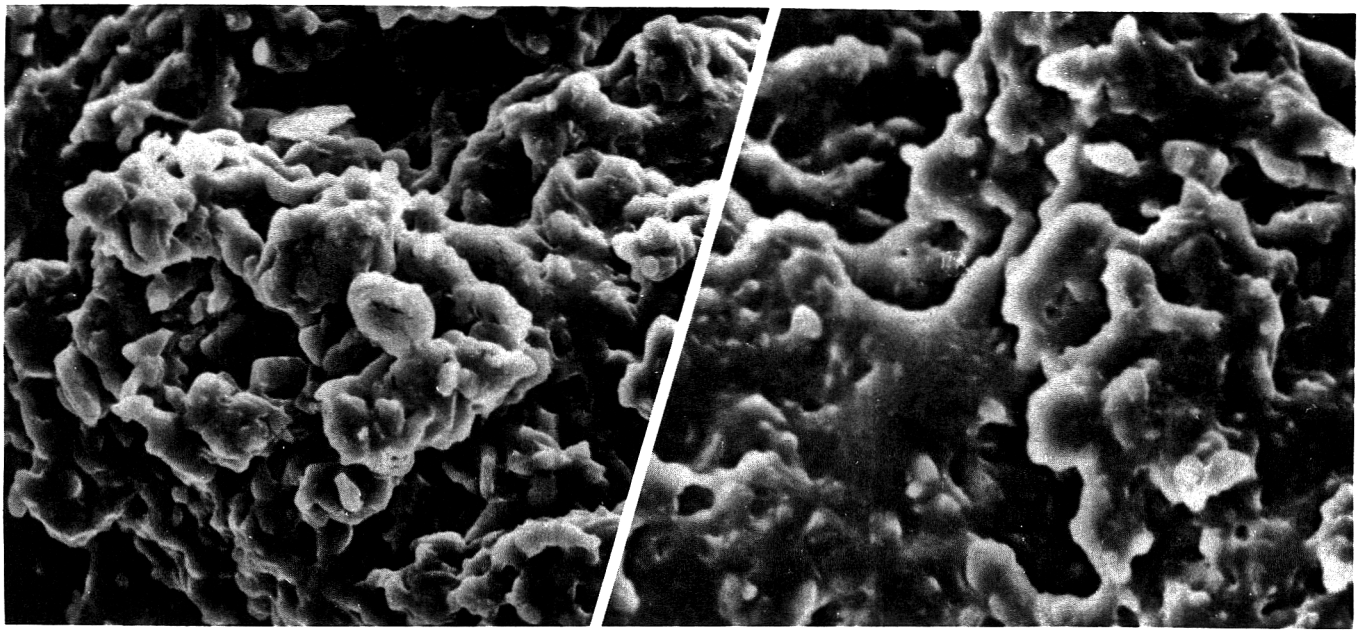


Fig. 16—Scanning electron micrograph of midpoint cooking at 31 min (left) and at 59 min (right).

Moisture loss during cooking

When cooking in an Amylograph, a certain amount of moisture is lost. This moisture loss is considerable, although it may not affect the progress of the various steps during cooking. Some addition of water during cooking might result in more accurate processing. The moisture loss for the apparatus used in these experiments (in per cent w.b./min) is given in Eq (9),

$$y_7 = 0.208 - 0.028x_3 - 0.018x_4 + 0.029x_5 + 0.031x_1x_2 - 0.026x_3x_5 - 0.012x_4x_5 \quad (9)$$
$$(r = 0.721; \text{S.E.} = 0.048)$$

The analysis of variance shows that the equation is significant on a 1% level, and that the lack of fit is not significant at the 5% level.

Eq (9) shows that a larger temperature differential (x_5) increases the moisture loss. The combined effect of starch content of flour and flour content of the flour sugar mixure (x_1 and x_2) seems to indicate that the increasing amount of starch binds more water and reduces moisture loss. Increasing water also seems to reduce moisture loss, which is probably due to the fact that the pasting temperature is lowered when flour content increases and sugar content decreases. The combined effect of water and temperature differential (x_3 and x_5) also appears to be associated with the overall temperature of the cooking. The increased shear (x_4) seems to reduce moisture loss, which can be partially explained in terms of the effects of shear (rpm) on the initial rise in viscosity. In this case, more starch is released to bind water. Reduction of moisture loss by increasing shear may also be partially due to the geometrical arrangements of the apparatus. Similar explanations can be given of the combined effects of rpm and temperature differential (x_4 and x_5). The absence of time from this equation is a direct consequence of the units used.

CONCLUSIONS

(1) The second order polynomial equations expressing the relationship between ingredient and processing parameters are developed for the flour-sugar-water model system of high solids content.

(2) A second order model did not accurately predict the means or spreads of consistency during cooking from the ingredient and processing parameters.

(3) Water content, fraction of flour in solids, and starch content in flour all affect the consistency of the flour-sugar-water paste.

(4) The pasting temperature of the mixtures of starch or flour in sugar solution is a function of the sugar concentration in the sugar-water mixture.

(5) Differences in results obtained with flour and flour ingredient systems do appear at certain parameter levels.

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PAUL R. MATHEWSON and Y. POMERANZ
U. S. Grain Marketing Research Center, USDA, ARS
1515 College Ave. Manhattan, KS 66502

HOT-PASTE VISCOSITY AND ALPHA-AMYLASE SUSCEPTIBILITY OF HARD RED WINTER WHEAT FLOUR

ABSTRACT

Alpha-amylase is recognized as a major contributor to the results of viscometric tests on wheat flour. The use of a simple, rapid colorimetric α-amylase assay to routinely predict amylograph viscosity was studied. Particle size and overall composition of malt fractions did not affect the colorimetric test; and protein content of wheat flours did not consistently affect amylograph viscosity. Equivalent levels of α-amylase from different malts had similar effects on amylograph viscosity of a single flour; however, a single malt produced various peak viscosities in different flours. The data suggests that the viscosity may be influenced by environmental conditions which alter the starch properties and thereby effect viscosity. Consequently, the colorimetric α-amylase assay did not reliably predict amylograph viscosity of flours from various locations and environments.

INTRODUCTION

THE AMYLOGRAPH has been used, along with the Falling Number test, as a means of measuring the viscosity of a buffered flour-water suspension. A decrease in viscosity to a predetermined level (i.e., 500 B.U.) is interpreted as an index of α-amylase supplementation, and also as an indication of the baking characteristics of the tested flour. Although α-amylase is a major contributor to the viscometric results of these tests, it is not the only one. Several studies have sought to identify those properties of the flour which were responsible for viscosity. Selman and Sumner (1947) proposed the use of the amylograph in controlling flour malt supplementation. They reported that milling damage to starch was a possible source of variation but pointed to α-amylase as the "prime factor" in the amylographic evaluation of the malt. Anker and Geddes (1944), using starches from different classes of wheat, stated that differences in amyloclastic susceptibility were due, at least in part, to varying degrees of mechanical damage to the starches during milling. This observation was also made by Brown and Harrell (1944) and supported a conclusion that peak viscosity could not necessarily be interpreted as a direct index of amylase activity. While these results were of interest, they did little to define the cause or extent of the variations observed.

Proceeding on the assumption that α-amylase was the "prime factor" contributing to the viscometric results from the amylograph, we developed a colorimetric technique for quantitatively measuring α-amylase in wheat flour. We hoped that accurate determination of α-amylase would allow prediction of amylographic results within an acceptable range. This technique was useful as a reproducible, accurate measure of α-amylase activity, but the variation between α-amylase and viscosity was unacceptably large. We then began a study to define the origin and extent of this variation.

MATERIALS & METHODS

Materials

Untreated straight grade flours were experimentally milled (Allis) from four cultivars of hard red winter wheat (KS 619042, CI 12995, Plainsman V and KS 644) harvested in 1973 and 1974, and from a composite grist of many hard red winter wheat varieties harvested at many locations throughout the Great Plains in 1974 and 1975 (Regional Baking Standard, RBS 74 and 75).

Thirteen wheat samples were received from the Southern Regional Performance Nurseries (SRPN). Each sample was a blend of 23 cultivars of wheat, all of which were grown at 13 locations in the Great Plains. From these 13 samples, two SRPN samples from the 1971 crop year (SRPN-1-71 and SRPN-2-71) and one from 1973 (SRPN-3-73) were used in the study of the effect of protein content on amylograph viscosity. The remaining ten SRPN samples from the 1975 crop were each from 10 locations and were used to determine whether growing conditions affected the amylograph viscosity. All samples were experimentally milled (Allis) into flour at our research center. A composite of the ten 1975 SRPN wheat flours was prepared by mixing 100g of each of the flours.

Six straight grade flours (of about 73% extraction) were milled on a Miag Multomat from hard red winter wheat cultivars harvested in 1976 from several locations in the Great Plains. These flours were treated with a barley malt to produce a 500 B.U. reading on the amylograph. The protein content of the flours ranged from 12.7 to 14.1% (14% m.b.).

The barley malt flour was a commercial product (Amylomalt) manufactured as a diastatic supplement for breadmaking. This malt was used both to treat the Multomat flours and to prepare nine air-fractionated malt samples. These nine fractions were described elsewhere (Pomeranz et al., 1976). Check samples of malted barley (G-75 and H-75) were from the Check Sample Service, American Society of Brewing Chemists, Madison, WI.

Methods

Percent moisture, protein, ash and Amylograph tests were performed according to the methods of the American Association of Cereal Chemists (AACC, 1969).

The determination of α-amylase in flour was basically the same procedure described in a previous report (Mathewson and Pomeranz, 1977) for detection of sprout damage in wheat. In the case of flour, however, the incubation time was extended to exactly 20.0 min.

Cereal malts were assayed in the manner that was described for flour except that 10 mg of material was suspended in 50 ml of extraction solution. One ml of filtrate was added to a test tube containing 8 ml of extraction solution. Incubation with Phadebas was for 5 min at 50°C.

The amount of malt necessary to achieve a 500 B.U. reading on the amylograph for the ten 1975 SRPN flours was determined using 100g samples (14% m.b.) of the composite flour. The 500 B.U. reading was achieved at level corresponding to 0.28% of 40 D.U. malt. One "D.U." is defined by the American Society of Brewing Chemists (ASBC, 1958) Methods as "the quantity of α-amylase which will dextrinize soluble starch in the presence of an excess of β-amylase at the rate of one gram per hour at 20°C." This was a total (inherent plus malt) α-amylase level of 0.220 mDU/mg. From these data, the individual flours were then supplemented with malt, depending on the initial α-amylase content of the flour, so that all flours had a total α-amylase content of 0.220 mDU/mg.

A sieve analysis was performed on six of the 1975 SRPN flours. Particle size of the flours was determined by sieving 100-g samples on an 8 inch diameter Ro-Tap Testing Sieve Shaker (U.S. Tyler Co.) for 30 min. Two Carmichael cleaners with nylon brushes were placed on each sieve to assist in separation. The samples were sieved through the following standard Tyler sieves:

Tyler sieve mesh:	115	150	170	200	250
Sieve opening (μm)	125	105	88	74	63

Average particle size was determined as described in the *Agricultural Engineers Yearbook* (Anon., 1975).

Standard calibration lines were prepared for 5 and 20 min incubation times using the barley malt check samples. Activities of these malts had been determined according to the standard method (ASBC, 1958).

The malts were used to prepare α-amylase concentrations over a desired range. For the 5 min line, six levels in the 0–90 mDU range were used. For the 20 min line, the range was 2–18 mDU in 4 mDU increments. The correlation coefficients for comparison of calculated values for α-amylase vs experimental results for the 5 min and 20 min lines were 0.995 and 0.975, respectively.

RESULTS & DISCUSSION

ONE DRAWBACK of the amylograph method is that it does not measure the α-amylase directly, but rather measures the *effects* of the enzyme. Though it is known that several factors influence the viscosity of the flour suspension, α-amylase is considered the major one. Therefore, we wanted to develop a method by which the effect of α-amylase, as measured by the amylograph, could be predicted from the α-amylase concentration in the flour. Though some differences were expected, the extent of the effects of other factors was unknown.

We first had to establish that α-amylase could be quantitatively determined in wheat flour. To determine the effects of malt composition, including α-amylase concentration, on the colorimetric assay method, nine fractions of air-fractionated barley malt representing a wide range of α-amylase activity and protein content were assayed according to the procedure for malts. The values obtained with Phadebas agreed well (r = 0.990) with the corresponding values obtained by the standard ASBC method (Table 1). RBS flour was then supplemented to 0.25% by weight with each of these nine malt flour fractions. The thoroughly mixed flours were assayed for α-amylase concentration. Agreement was satisfactory (r = 0.979) and indicated no detrimental effects due to peculiarities of any malt fraction (Table 2).

The malt level in the amylomalt supplemented flours from the Multomat mill was selected to lower the amylograph peak viscosity to 500 B.U. These malted samples and the malt were assayed by the colorimetric method to determine the α-amylase content in each. Since the percentage of malt used for each flour was known, the actual α-amylase concentration was calculated and compared to the experimental results (Table 3). The linear correlation coeffecient (r = 0.888) was statistically significant at the 1% level. This somewhat low value probably resulted from the narrow range of amylase values in this series. These data indicate that α-amylase can be quantitatively determined in wheat flour using the colorimetric technique.

As stated previously, one drawback of the amylograph is that since it measures only the effect of α-amylase, several trial and error determinations may be required to reduce the viscosity to the 500 B.U. level. However, if by use of the colorimetric method the α-amylase in an untreated flour is determined and the desired level of supplementation is known, the amount of malt required to achieve the proper α-amylase concentration could be calculated.

If this procedure is to be practical, two requirements must be met. First, the desired level of supplementation must be known prior to the test, and second, it must be shown that this level of α-amylase, when added to the flour, will give a viscosity close to the accepted 500 B.U. value.

Wheat flour is commonly supplemented with an amount equivalent to 0.25% by weight of a 40 D.U. malt. In the first series of tests, 100g (14% m.b.) of RBS-75 flour was supplemented with a 36 D.U. barley malt (G-75). A reading of 500 B.U. was obtained at a level equivalent to 0.26% of 40 D.U. malt. We then used two different malts, H-75 and Amylomalt, with activities of 32 and 51 D.U., respectively, as determined by the colorimetric method, and added an amount equivalent to 0.26% of 40 D.U. malt to the RBS-75 flour. Resultant peak viscosities were again about 500 B.U. (Table 4).

Having established that equal quantities of α-amylase from three malts (G-75, H-75 and Amylomalt) lowered the viscosity of a single flour (RBS-75) to approximately 500 B.U., we then investigated the effect of α-amylase from a single malt (G-75) on different flours. Three flours with different protein contents were selected. As shown in Table 4, an equal amount of α-amylase produced different effects in the flours. Flours CI 12995 and KS 644 had viscosities of 575 and 555, respectively. The KS 619042, however, had a viscosity of 660. We wanted to know whether this exceptionally high value was the result

Table 1—Some characteristics of barley malt and its fractions

Amylomalt[a] and fractions	Protein[b] (%)	ASBC α-amylase D.U./g	Phadebas α-amylase D.U./g
Amylomalt	10.6	54.1	51.0
0-150, T-115	14.2	67.4	65.0
0-170, T-150	13.5	69.8	66.5
0-200, T-170	12.4	73.8	70.5
0-250, T-200	11.5	63.0	59.5
Thru 250	9.1	43.6	42.5
B-1	10.5	67.4	63.5
B-2	7.6	36.2	39.0
B-3	10.8	41.7	42.0
	Average	57.4	55.5

[a] Number following Over (O) and Thru (T) refers to Tyle sieve meshes/inch; B-1, B-2, B-3 are abbreviations for the coarse, medium, and fine Bahco fractions of the 250 thrus. B-3 is the effluent fraction from the first throttle setting of 14, which yields a residue of B-2 plus B-1. B-2 is the effluent fraction from the second throttle setting of 8, which yields the residue B-1.
[b] 14% moisture basis

Table 2—α-Amylase content of RBS-75 flour supplemented with air-fractionated malts

Amylomalt[a] fraction	mDU/ml Experimental	mDU/ml Calculated
Amylomalt	1.52	1.52
0-150, T-115	1.86	1.79
0-170, T-150	2.00	1.91
0-200, T-170	1.88	1.89
0-250, T-200	1.80	1.62
thru 250	1.00	1.20
B-1	1.76	1.76
B-2	1.00	1.11
B-3	1.04	1.23
Average	1.54	1.56

[a] For designation, see Table 1.

Table 3—α-Amylase content of Multomat flours supplemented with Amylomalt

Multomat flours	mDU/ml Experimental	mDU/ml Calculated
a	1.56	1.42
b	1.28	1.29
c	1.36	1.28
d	1.68	1.49
e	1.48	1.43
f	1.52	1.49
Average	1.48	1.40

of the low protein or was a characteristic of this particular flour.

To determine the effect of protein on amylograph viscosity, we selected a series of wheat flours covering a wide range of protein values. The flours were supplemented with equal amounts of G-75 barley malt. The results (Table 5) show that the protein content did not consistently affect the viscosity ($r = -0.261$).

The two RBS flours differed widely in viscosity (Table 5). Both should represent standard flours for wheats grown in the Great Plains, but viscosity differed between 1974 and 1975. For the straight grade flours milled from single varieties for 1973 and 1974, the viscosities within each year are close. These data suggest that for a particular crop year, these wheats developed similarly, at least with respect to their amyloclastic susceptibility. This observation agrees with data of Meredith and Simmons (1975) in which they also note a seasonal effect on the viscosity of wheat flour.

A seasonal effect is an environmental effect. If environmental conditions affect a total crop in a given season, these effects may also be manifested on a regional scale.

To test this hypothesis, we obtained samples of wheats from the Southern Regional Performance Nurseries (SRPN) that were blends of the same hard red winter varieties grown in ten locations in the Great Plains in 1975. Since these samples were blends of 23 different varieties of wheat, differences among the samples due to variety would be masked. As a result, only those differences due to locational variation would show up. Ten flours from the blends and a composite flour made by mixing equal portions of the ten flours were tested by the colorimetric method, for α-amylase activity, prior to malt supplementation.

The composite flour was used as the base flour to determine the amount of malt necessary to lower the amylograph viscosity to 500 B.U. The ten flours were supplemented so that each had the same total α-amylase concentration, and they were tested in duplicate on the amylograph using 100-g samples (14% m.b.). The data show that flour viscosity varied even though equal amounts of α-amylase were present (Table 6).

The amylographic results in Table 6 were evaluated statistically using Duncan's multiple range test which establishes a set of significant differences for comparing treatment means (Steel and Torrie, 1960). The results of this analysis are shown in Table 6. It can be seen that on the basis of viscosity, four groups of related values are discernible. These groups correspond to roughly defined geographic regions. The wheats with the highest viscosity were grown in central Oklahoma and together are similar, yet distinct from the other flours. The Columbia (MO) flour stands alone while Goodwell, in the Oklahoma Panhandle forms a group with Hutchinson and Hays, Kansas. The fourth group includes Clovis, NM., Colby and Garden City, Kansas. Considering the distance between the New Mexico and Kansas locations, this grouping appears atypical. An examination of the weather data (Table 7) indicates that the two regions were different with respect to precipitation and average temperature difference. These weather data lend support to the suggestion that environmental conditions affect the viscosity of wheat flour on a regional scale. These weather data are not conclusive by themselves since no individual condition, i.e., rainfall or temperature, will correlate precisely to viscosity. The environmental effect is the result of all such factors combined. The data presented were compiled using daily reports for the 15 days prior to harvest.

These results corroborate findings by Hagberg and Olered (1975) which indicated that starch granules in rye are subject to reversible physical and chemical changes during the last stages of ripening. The data presented here might well explain the findings of Anker and Geddes (1944) and Selman and Sumner (1947) since the different environmental factors that

Table 4—Protein and amylograph data for RBS-75 and HRW flours supplemented with equal amounts of α-amylase[a] from barley malt

Flour and malt	Flour protein[b] (%)	Amylograph peak viscosity (B.U.)
RBS-75 flour	12.4	
+ G-75 malt		505
+ H-75 malt		490
+ Amylomalt		530
G-75 malt		
+ KS 619042 flour	11.2	660
+ CI 12995 flour	14.0	575
+ KS 644 flour	13.7	555

[a] Determined by the colorimetric method; equivalent to 0.26% of 40 D.U. malt.
[b] 14% moisture basis

Table 5—Protein and ash content and amylograph viscosity of wheat flours supplemented with equal amounts of α-amylase[a] from G-75 barley malt

Flour and year	Protein[b] (%)	Ash (%)	Amylograph peak viscosity (B.U.)
Composites			
SRPN-1-71	9.0	0.38	650
SRPN-2-71	17.5	0.48	600
SRPN-3-73	8.0	0.44	640
RBS-74	12.4	0.43	670
RBS-75	12.4	0.44	505
Single varieties			
CI 12995 (1973)	14.0	0.42	575
KS 644 (1973)	13.7	0.35	555
KS 619042 (1974)	11.2	0.42	660
Plainsman (1974)	16.1	0.43	650

[a] Determined by the colorimetric method; equivalent to 0.26% of 40 D.U. malt.
[b] 14% moisture basis

Table 6—Protein, mean particle diameter and amylograph data on SRPN (1975) wheat flours

Flour source	Protein[a] (%)	Mean particle diam (μm)	Amylograph peak viscosity (B.U.)[b]
Clovis, NM (dryland)	12.4	65.5	405
Clovis, NM (irrigated)	13.2	75.6	415
Colby, KS	9.3	-	430
Garden City, KS	10.0	-	440
Goodwell, OK	12.1	-	475
Hutchinson, KS	11.1	-	475
Hays, KS	11.7	65.2	500
Columbia, MO	8.9	-	545
Altus, OK	14.4	64.2	610
Lahoma, OK	12.9	66.8	635
Composite	11.8	64.4	500

[a] 14% moisture basis
[b] Values showing no significant difference at the 1% level are connected by lines.

Table 7—Environmental data for SRPN (1975) locations

Location	Date of harvest	Precipitation (inches)	Avg temp max (°F)	Avg temp min (°F)	Avg temp (°F)	Temp difference (°F)
Clovis, NM	June 24, 30	0.83	89.8	53.9	71.8	35.9
Colby, KS	July 3	1.05	85.0	58.9	71.9	26.1
Garden City, KS	June 27	2.37	86.7	59.8	73.2	26.9
Goodwell, OK	June 30	1.51	90.7	64.1	77.4	26.6
Hutchinson, KS	June 24	2.75	86.0	60.2	73.1	25.8
Hays, KS	July 4	3.19	86.8	64.4	75.6	22.4
Columbia, MO	June 23	3.06	81.7	62.6	72.2	19.1
Altus, OK	June 9	2.25	86.9	61.9	74.4	25.0
Lahoma, OK	June 18	2.03	86.1	58.5	72.3	27.6

affect starch would in turn affect the milling properties of wheats. We have previously shown (Table 5) that varietal differences in protein content were not correlated with amylograph peak viscosity. The data shown in Table 6 also indicate that no such correlation exists for samples composited across varieties and ranging in protein content from 8.9 to 14.4%.

The difference in viscosity could result from a difference in particle size as a result of different milling characteristics. The difference in particle size might well influence the rate and degree of hydration which would affect the ability of α-amylase to attack the starch. To define these possibilities more clearly, a sieve analysis was done to determine particle size of those flours which had the highest, lowest, and intermediate viscosity values. The sieve analysis did not show any significant correlation between particle size and viscosity (Table 6). This does not preclude the possibility that major differences in particle size may affect viscosity.

This points to the possibility that there are real differences in the starch itself. The question remains whether the differences in viscosity were due to the inherent qualities of the starch, or to the susceptibility of the starch to α-amylase. To distinquish between these two effects, 60-g samples (14% m.b.) of each of the ten unsupplemented flours (identified in Table 6) were run on the amylograph. We found no significant correlation between viscosity values of the native flours compared to the values obtained from the malt-supplemented flours. This indicates that the variations, likely, are due to characteristics of the starches which alter their susceptibility to attack by α-amylase.

In summary, from the data in Tables 1–3, it is apparent that α-amylase can be quite accurately determined by the colorimetric method. However, considering all the data, it seems that the variations in viscosity ostensibly resulting from regional differences in environmental condiditions make the routine application of the α-amylase assay impractical for predicting amylograph viscosity. Thus, this method would be useful only for testing flour from a single crop year and a limited local environment.

The viscosity of a flour-water suspension is the result of many factors, of which α-amylase is but one. It is important to understand what is being measured by viscometric methods since the technique is used throughout the world as a means of determining malt supplementation, sprouting and evaluation of breadmaking properties of rye flours. We have shown that the viscosity of a wheat flour cannot necessarily be interpreted as a direct index of α-amylase activity, and that the viscosity is probably affected by environmental factors in the region where the crop was grown.

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V. S. RAO, U. K. VAKIL and A. SREENIVASAN[1]
Biochemistry and Food Technology Division
Bhabha Atomic Research Centre, Bombay 400 085, India

EFFECTS OF GAMMA-IRRADIATION ON COMPOSITION OF WHEAT LIPIDS AND PUROTHIONINES

ABSTRACT

The effects of gamma-irradiation on chemical composition of wheat lipids and on the changes in chemical properties of lipoprotein complex, were studied. No appreciable changes in total lipids in wheat, irradiated up to 1 Mrad, were observed. However, a significant increase in free lipids with concomitant decrease in bound lipids was observed. Separation of nonpolar and polar lipids into microconstituents did not show significant variations. Purothionines, extracted from lipid fractions of wheat flour exhibited several protein bands on electrophoretic separation; most of them disappeared at high dose level (1 Mrad). This may result in the disruption of lipid-purothionine complex. When bread dough was allowed to rest for 2 hr, aggregation of low molecular weight protein entities took place; this could strengthen the structure of lipoprotein complex during resting and baking.

INTRODUCTION

THE FUNCTIONAL ASPECTS of individual components of wheat lipids and their effects on rheological properties, have been indicated by several fractionation and reconstitution studies (Tao and Pomeranz, 1968; Hoseney et al., 1969). Defatting of wheat flour deteriorates its bread making quality (Pomeranz, 1966) and this can be restored by addition of polar but not by nonpolar lipids (Daftary et al., 1968). The transitional complexes formed by interactions of lipids with proteins, greatly influence the rheological properties of wheat dough and the baking performance (Grosskreutz, 1961). Hoseney et al. (1970) have visualized the gas retaining complex in the dough as gliadin and glutenin units, bound to polar lipids simultaneously by hydrophilic and hydrophobic bonds, respectively. Several low molecular weight wheat proteins, designated as purothionines have been isolated from lipid fractions and characterized as 'fast-moving globulin doublet' (Fisher et al., 1968; Redman and Ewart, 1973; Nimmo et al., 1974).

The main effects of radiation on fat are the formation of peroxides (Nawar, 1972) and volatile carbonyl compounds (Merritt, 1972), which are mainly responsible for imparting rancidity and off-flavor to irradiated foods. However, lipids in cereals are degraded generally only at very high dose levels (Tipples and Norris, 1965). No significant effects of irradiation on iodine value, acidity or color intensity of wheat flour lipids have been observed (Chung et al., 1967).

The present work relates to the effect of gamma-irradiation at different doses, on lipid composition as determined by chemical analysis and thin-layer chromatography. Further, certain purothionine-lipid complexes were isolated from wheat flour and changes in their behavior during dough making as well as after a 2-hr resting period were studied. Two low molecular weight purothionines, associated with wheat polar lipids, were isolated from control and irradiated wheat and characterized.

[1] Present Address: Gautam Apartments, Pali Hills, Bombay 400 050, India

MATERIALS & METHODS

AUTHENTIC LIPID samples were purchased from Sigma Chemical Co. (St. Louis, MO). All solvents were of Analar (British Drug House, U.K.) quality and redistilled before use. Silica gel G (chromatographic grade) was obtained from E. Merck (U.S.A).

Irradiation of wheat

'Vijay' variety of wheat, procured from Niphad Agricultural Research Experimental Station, Maharashtra, was used in the present studies. Samples (500g) were packed in polyethylene bags, heat sealed and irradiated in ^{60}Co gamma cell 220 (Atomic Energy of Canada Ltd.) having a flux of 25 krad/min at 0.02–1 Mrad dose levels. Absorption of radiation was checked with ferrous and ceric sulfate dosimetry (Weiss, 1952). The overdose ratio was about 30%. Samples were stored for 2–3 wk before milling.

Lipid composition

Total lipids in wheat flour (500g) were extracted repeatedly with chloroform:methanol (C:M) mixture (2:1 V/V) and traces of carbohydrates and proteins were removed from the crude extract by Folch's (Folch et al., 1957) method. The purified extract was dried in vacuum to constant weight. The residue was dissolved in chloroform and stored under N_2 at 0°C. Iodine absorption number was measured by the titration method (AOAC, 1970). To estimate phospholipids, an aliquot of lipid extract was digested with concentrated HNO_3 and a few drops of perchloric acid until a clear digest was obtained. Phosphorus (P) liberated was measured colorimetrically (Fiske and Subba Rao, 1925), and phospholipids (P × 25) were calculated. Triglycerides were estimated by the method described by Van Handel (1961). Saturated and unsaturated fatty acids were separated from saponified (4% alcoholic KOH for 30 min) lipid samples, following lead acetate (20%) precipitation (AOAC, 1970).

Free and bound lipids

Total free lipids (TFL) in wheat flour were extracted with petroleum ether (60–80°C) for 16 hr and dried under N_2 at 0°C. The defatted residue was re-extracted with water saturated butanol (WSB) for 16 hr to obtain bound lipids.

Total free lipids were further separated into nonpolar and polar lipids by column chromatography. A glass column (2 × 25 cm), packed with activated (at 118°C for 1 hr) silicic acid under constant pressure of 30 mm Hg, was used (Daftary and Pomeranz, 1965). Nonpolar and polar lipids were eluted with chloroform and methanol, respectively. Excess of solvents were evaporated under N_2 and the residues weighed to constant weights.

Thin-layer chromatography (TLC)

Nonpolar and polar lipids were further fractionated by TLC on glass plates (20 × 20 cm), coated (10 mm thickness) with silica gel G-water (1:2 W/V) slurry, dried and activated at 130°C for 90 min (Daniels et al., 1966). An aliquot (≡ 100 γ) of lipids was applied and a petroleum ether:diethyl ether:acetic acid (85:20:1), and chloroform:methanol:water (85:20:2) mixtures were used as solvent systems for fractionation of nonpolar and polar lipids, respectively. The plates were sprayed with saturated $K_2Cr_2O_7$ in 50% H_2SO_4 and charred at 130°C for 10 min. Authenic samples of monoolein, diolein, pure coconut oil, tripalmitate, oleic acid (FFA), cholesterol, lecithin, cephalin and digalactosyl diglycerides were run simultaneously. TLC plates, developed similarly, were sprayed separately with ninhydrin (0.3% in n-butanol) or Dragendorff's reagent for the detection of cephalin and lecithin, respectively (Dittmer and Lester, 1964). The plates were photographed under ultraviolet light.

Table 1—Effect of gamma-irradiation on lipid composition of wheat[a]

Wheat lipid component	Radiation dose (Mrad) 0	0.02	0.2	1
Total lipids (g%)	1.78	1.84	1.72	1.74
Total free lipids (TFL) (g%)	1.20	1.25	1.42	1.44
Bound lipids (g%)	0.50	0.52	0.28	0.27
Non-polar lipids (% of TFL)	60.00	60.00	57.00	56.00
Polar lipids (% of TFL)	48.00	47.00	45.00	45.50
Nonpolar/polar lipids	1.25	1.19	1.26	1.22

[a] Total, free and bound lipids were extracted from wheat flour and weighed to constant weights as described in the text. Polar and nonpolar lipids were separated from TFL by silicic acid column chromatography and eluted with methanol and chloroform, respectively. Results are averages of three determinations.

Table 2—Lipid constituents in irradiated wheat[a]

Lipid constituent	Radiation dose (Mrad) 0	0.02	0.2	1
Iodine number (mg I_2 absorbed/g fat)	114	114	105	–
Saturated fatty acids (g%)	0.32	0.31	0.32	0.31
Unsaturated fatty acids (g%)	1.21	1.17	1.10	1.03
Phospholipids (% of total lipids)	12.0	11.9	12.0	11.9
Triglycerides (% of total lipids)	31	31	30	30

[a] Lipid composition was determined by methods described in the text. Results are averages of three independent estimations, carried out in triplicate.

Identification of lipoproteins

Wheat flour (100g) was extracted several times with chloroform: methanol (2:1) mixture. An aliquot from the lipid extract was spotted on a filter paper strip, dried and dipped in Amido black 10 B (1%) solution for 5 min. The presence of proteins was indicated by a dark spot. For further isolation of total purothionines, an aliquot of wheat lipids was extracted with 10 parts of WSB solution and kept at room temperature for 5 min. The white precipitates formed, were filtered, washed with WSB and designated as purothionine I. The supernatant was reprecipitated with acetone; the precipitates were washed with ethyl acetate and labelled as purothionine II (Hoseney et al., 1970). Protein content was estimated colorimetrically using bovine albumin as standard (Lowry et al., 1951). Purothionines I and II were fractionated by polyacrylamide gel electrophoresis and their protein distribution patterns compared with water- and 6% salt-soluble wheat proteins. Tris-glycine buffer (pH 8.3) containing 3M urea was used and electrophoresis carried out at 30 mamp for 1 hr at 27 ± 1°C. The gels were stained with 1.0% Amido black 10 B, and destained with 7% acetic acid (Davis, 1964).

Purothionines in dough

Wheat flour (100g) was mixed with water (62 ml) and part of the dough was allowed to rest for 2 hr at 30°C. The samples were freeze dried. Extraction of lipids, spot test, and isolation of purothionines were carried out as described above.

RESULTS

Lipid composition of irradiation wheat

Results on the contents of total, free and bound lipids, extracted with C:M mixture, p-ether and WSB, respectively, from control and irradiated (at 0.02–1.0 Mrad) wheat, are summarized in Table 1. Total lipid content in wheat was not changed due to radiation treatment. However, a significant increase (20%) in total free lipids and a decrease (46%) in bound lipids were observed in samples irradiated at 1 Mrad. Free total lipids constituted 48% polar and 60% nonpolar lipid fractions (Table 1). The distribution pattern and their rations were not affected by radiation treatment up to 1 Mrad. Similarly, results on iodine absorption number revealed no significant effect of irradiation (Table 2). Wheat samples contained about 32% saturated and the rest unsaturated fatty acids. Though the levels of saturated fatty acids were not affected, slight decrease in unsaturated fatty acids was observed at 1 Mrad (1.03%) as compared to control (1.21%).

Thin-layer chromatography

TLC patterns of nonpolar components in free lipids of wheat are shown in Figure 1. The spot at the solvent front was not identified. The other following spots were tentatively identified as triglycerides, free fatty acids, diglycerides and monoglycerides, respectively. Visual observations showed no significant difference in the distribution of nonpolar components in control or irradiated wheat up to 1 Mrad. Quantitative analysis (Table 2) showed that wheat contained about 30% triglycerides. Radiation treatment up to 1 Mrad, had no significant effect on the distribution of different nonpolar lipids.

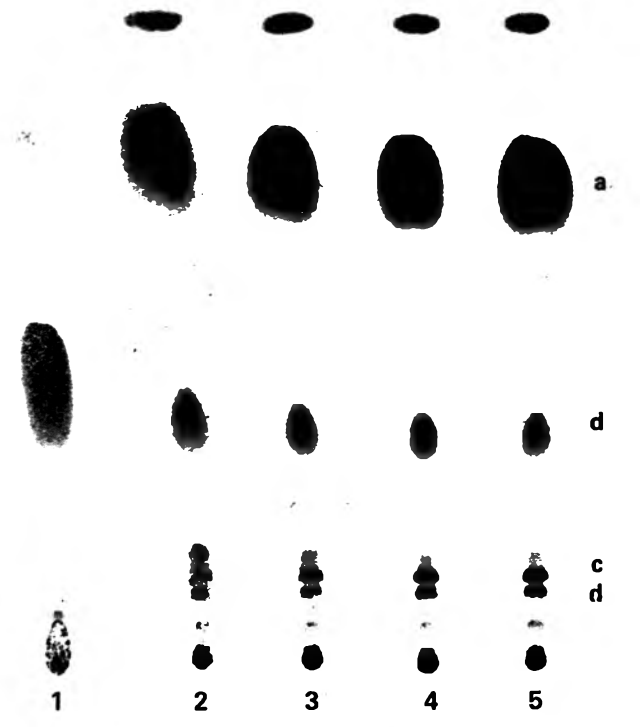

Fig. 1—TLC of nonpolar lipids. Aliquots (≡ 100 γ) of free lipids were applied on TLC plate and developed using diethyl ether:p. ether:acetic acid (20:85:1) as a solvent system. Spots were visualized and photographed under UV light. (a) triglycerides; (b) free fatty acids; (c) diglycerides; (d) monoglycerides. (1) Control; (2) 0.02 Mrad; (3) 0.2 Mrad; (4) 1.0 Mrad; and (5) mixture of authentic samples.

TLC patterns of the polar components in free lipids of wheat are shown in Figure 2. The main polar components, identified either from available authentic samples or from reported RF values, include: phospholipids such as lysolecithin, lecithin, cephalin etc. and glycolipids like mono- and

di-galactosyl diglycerides. Relatively low concentrations of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, and some unidentified compounds were also present. No significant differences in concentrations of individual polar lipids from control and irradiated samples (up to 1 Mrad) were observed. This was further confirmed by quantitative analysis of total phospholipids (Table 2) by colorimetric method.

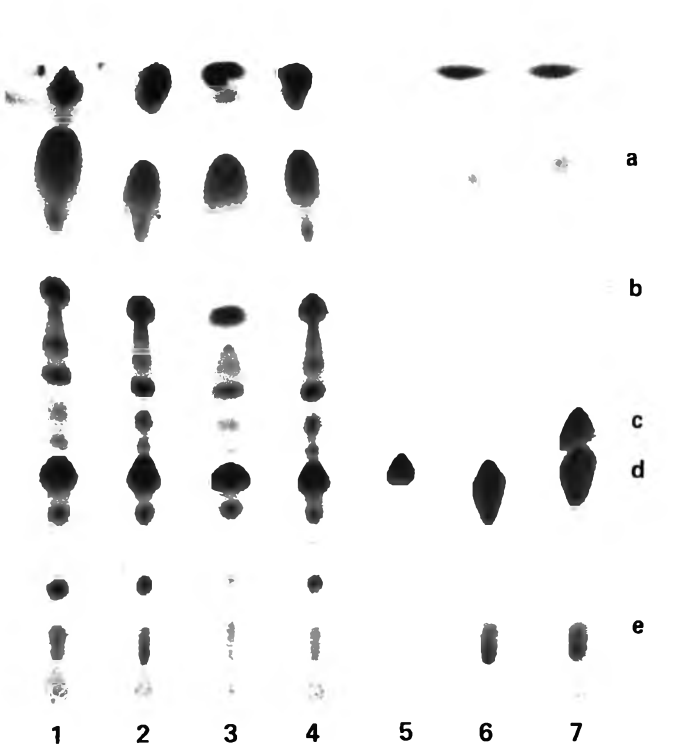

Fig. 2—TLC of polar lipids. TLC plates were developed using chloroform:methanol:water (85:20:2) as a solvent system. The spots were identified as: (a) unresolved nonpolar lipids; (b) monogalactosyl diglycerides (DGDG); (c) digalactosyl diglycerides; (d) lecithin; and (e) lysolecithin. (1) Control; (2) 0.02 Mrad; (3) 0.2 Mrad; (4) 1.0 Mrad; (5) DGDG; (6) lecithin; and (7) mixture of authentic samples.

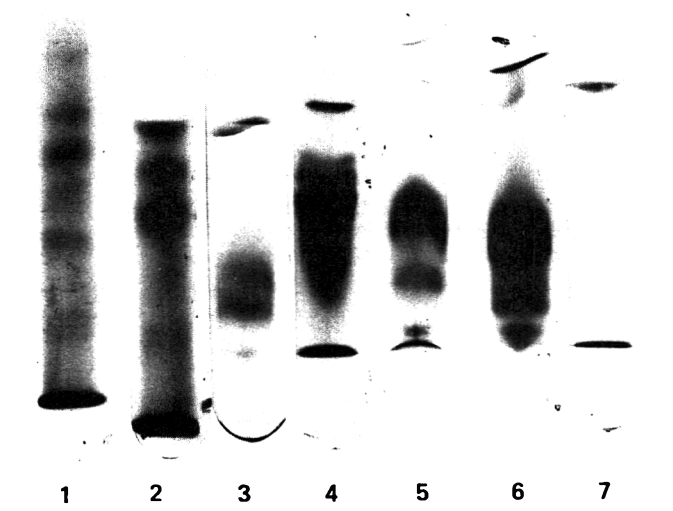

Fig. 3—Polyacrylamide gel electrophoretic pattern of purothionine I fraction, separated from total wheat lipids. Electrophoresis was carried out as described in the text. (1) Water soluble wheat proteins; (2) Salt- (6%) soluble proteins; (3) Salt- (2%) soluble proteins; Purothionine I from (4) Control; (5) 0.02 Mrad; (6) 0.2 Mrad; and (7) 1 Mrad.

Effect of gamma-irradiation on purothionine

Results on the effect of radiation on the distribution of purothionines, isolated from lipid fractions are given in Table 3. Wheat contained about 6 mg purothionine I and 3 mg purothionine II per g of total lipids. At lower doses of gamma irradiation, the distribution of purothionines in these two fractions was not affected, but at 1 Mrad, there was a decrease in purothionine I with a concomitant rise in the more soluble purothionine II fraction.

Further, the polyacrylamide gel electrophoretic pattern of purothionine I was compared with those of water-soluble and salt-soluble wheat proteins (Fig. 3). This indicated the heterogeneity of this protein and its distribution pattern was comparable to salt-soluble, rather than water-soluble wheat proteins. The main constituent corresponded in electrophoretic mobility to the fast moving globulin doublet, which was predominantly present in the salt-soluble fraction. In the control sample, two bands, corresponding to water-soluble proteins were noticed. However, they disappeared at 0.2 Mrad. At higher dose (1 Mrad), except one low molecular weight protein band, all others disappeared. A distinct band at the origin may indicate the presence of high molecular weight nonpurothionine contaminant; this was not affected by radiation treatment. Purothionine II fraction in control exhibited a single homogenous band in the very fast moving region corresponding to low molecular weight protein (Fig. 4). On the other hand, two bands appeared in samples, irradiated at 0.2 and 1.0 Mrad.

A preliminary investigation was also carried out with untreated control wheat to study the process of changes, if any, in lipid-protein association during doughing and resting. It was observed that the solubility of flour lipids in C:M mixture and hence their extractability was significantly affected in the resting dough. Even from quickly frozen and lyophilized dough, only 25% of the total lipids could be extracted. The proteins extracted from lipid fraction (with C:M (2:1) mixture) of fresh and rested (2 hr) doughs were separated by gel electrophoresis (Fig. 5). They were showing only one or two bands in the low molecular weight region, suggesting that they were no longer extractable or were bound to other proteins. However, on resting, several bands in the faster moving region, which resembled gliadin on electrophoresis, were observed along with a distinct band similar to that of glutenin.

DISCUSSION

NO GROSS CHANGES in chemical composition of wheat lipids, including distribution pattern of nonpolar and polar lipids, were observed when wheat kernels were irradiated up to 1 Mrad (Tables 1 and 2). We have earlier observed adverse effects on baking quality at 1 Mrad (Vakil et al., 1973). However, this could not be fully attributed to lipids in general and to free and bound lipids in particular, which have a decisive role in governing the bread quality (Hoseney et al., 1969). The phos-

Table 3—Distribution of purothionines in irradiated wheat[a]

Radiation dose (Mrad)	Total lipids (g/100g wheat flour)	Purothionine I (mg protein/g lipid)	Purothionine II (mg protein/g lipid)
0	1.2	6.0	3.0
0.02	1.2	6.0	3.0
0.2	1.2	5.0	3.0
1.0	1.3	4.5	4.0

[a] Proteins were isolated from total lipids, extracted with C:M mixture and dissolved in WSB. Insoluble fraction was designated as purothionine I and soluble as purothionine II. Values are averages of three experiments.

Table 4—Protein-lipid association in wheat flour dough[a]

Sample	Lipids extractable with C:M (g%)	Proteins in lipid extract (g%)
Wheat flour	1.32	1.21
Dough (0 hr)	0.35	0.31
Dough (2 hr)	0.41	0.32

[a] Flour lipids were extracted with C:M (2:1) solvent mixture and proteins were estimated. The dough was prepared by mixing flour with an appropriate amount of water and incubated at 30°C for 2 hr. Results are averages of three experiments.

pholipid contents which show high correlation with the texture of bread (Pomeranz, 1968) were also not affected by radiation treatment. However, measurement with very sensitive and refined methods like infrared spectroscopy, revealed some modifications in lipids such as the appearance of conjugated triene and an increase in oxidation products in wheat, irradiated even at 0.2 Mrad dose level (Deschreider, 1966). Tipples and Norris (1965) reported a significant decrease in linoleic and linolenic acid contents of irradiated (10 Mrad) wheat.

A considerable amount of lipids was bound to proteins during dough mixing and the amount of extractable lipids was reduced about 25% (Table 4). Chiu et al. (1968) have attributed the slight increase in free lipids during fermentation to their release by dough development. Mann and Morrison (1974) have shown that most of the nonpolar and almost all of the polar lipids were bound on dough mixing; binding was nonselective with regard to fatty acid composition.

A definite correlation has been shown between lipid binding and protein quality in bread making (Wooten, 1968). Grosskruetz (1961), on the basis of X-ray scattering and electron microscopic studies, has postulated a lipoprotein model, which occupies about 2–5% of elastic gluten structure, and shown that this complex is capable of providing plasticity, necessary for optimum baking characteristics. In view of the importance of lipoprotein complex in governing the functionality of wheat flour, information on whether or not its structure remains intact during dough mixing in irradiated wheat, is essential for interpretation of dough behavior. Purothionines present in lipid extracts (Fisher et al., 1968), were identified as low molecular weight proteins (Redman and Fisher, 1968). The changes in the distribution of purothionines I and II in lipid fraction (Table 3) may affect their association with specific lipid fraction (Daniels et al., 1966). Again, the disappearance of some purothionine bands at higher doses (Figures 3 and 4), suggests that association and dissociation of wheat proteins with lipids to form an effective lipoprotein complex during dough mixing and fermentation, are markedly affected by radiation treatment. This might have affected adversely the gas retention capacity of the dough during fermentation and baking, which results in the observed low loaf-volume of bread, prepared from wheat irradiated at high dose (1 Mrad) level.

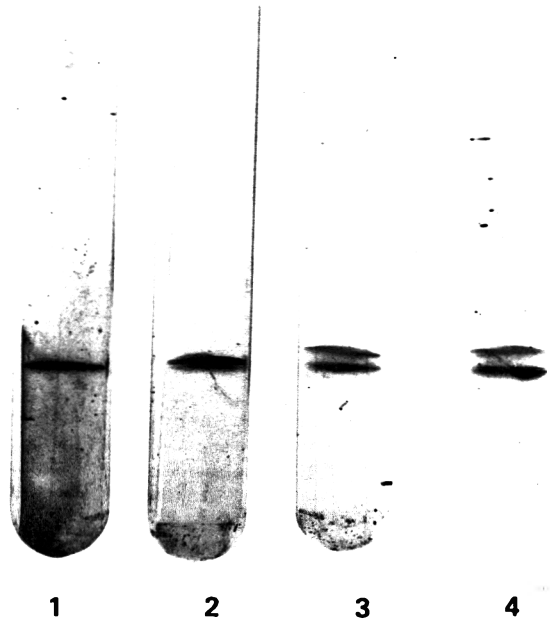

Fig. 4—Polyacrylamide gel electrophoretic pattern of purothionine II: (1) Control; (2) 0.02 Mrad; (3) 0.2 Mrad; and (4) 1 Mrad.

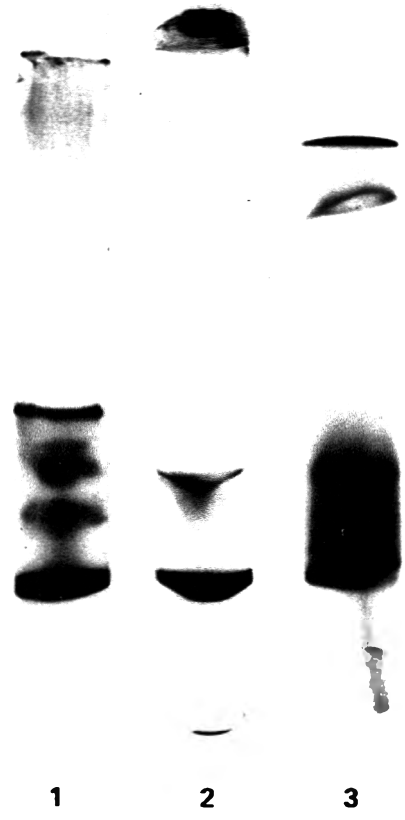

Fig. 5—Gel electrophoretic pattern of total crude purothionine fraction isolated from unirradiated wheat: (1) Wheat flour; (2) mixed dough; and (3) fermented dough (2 hr).

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V. S. RAO, U. K. VAKIL, C. BANDYOPADHYAY and A. SREENIVASAN[1]
Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Bombay-400 085 India

EFFECT OF GAMMA-IRRADIATION OF WHEAT ON VOLATILE FLAVOR COMPONENTS OF BREAD

ABSTRACT

Comparative sensory and objective evaluations of bread prepared from wheat flour, irradiated at different doses, have been carried out. The preference of bread decreases with higher radiation dose (1 Mrad) due to increase in off-flavor intensity. Total carbonyl contents are increased in irradiated products. A significant inverse correlation between consumer preference and total carbonyls as well as GLC headspace vapor analysis, is established. An attempt has been made to postulate a mechanism for the excessive formation of volatiles, imparting off-flavor in bread from irradiated wheat. It is suggested that they may arise from the volatile degradation products of amino acids and proteins or by their interaction with reducing sugars, the ultimate radiation-induced breakdown product of starch.

INTRODUCTION

THE COMPLEX chemical and enzymatic reactions occurring during fermentation and baking give rise to several volatile (Johnson et al., 1966) and nonvolatile (Pence, 1967) compounds, which react synergistically or otherwise and contribute to the flavor of the finished bread. The assessment of the flavor of food products is usually done by taste panels, but this determines only the overall perceptive response and appreciation of the consumer. The differences imparted by more than one flavor to the test product, therefore, cannot be distinguished accurately by panelists. Several volatile flavor components of wheat flour and bread such as carbonyls, organic acids, alcohols etc., have been identified using temperature programming by gas-liquid chromatography (GLC) (MacWilliams and Mackey, 1969). Attempts have been made to establish the significance of each one to the formation of the total flavor complex (Ng et al., 1960; Figureiredo, 1964).

Our earlier results (Vakil et al., 1973) on sensory evaluation of breads prepared with irradiated wheat (0.02–1.0 Mrad), revealed that at higher doses, consumer preference was low due to dark crust and peculiar irradiation off-flavor. Organoleptic properties of cakes, bread and biscuits prepared from gamma-irradiated wheat flour are also adversely affected (Milner and Yen, 1956; Webb et al., 1961; Miller et al., 1965). In the present study, qualitative characterization of volatile components by GLC and quantitative determination of total carbonyls, reducing sugars and free amino acids by chemical methods, were carried out. An attempt was made to correlate the objective instrumental measurement of aroma with the subjective, organoleptic evaluation of breads and changes therein due to irradiation.

MATERIALS & METHODS

WHEAT SAMPLES (*Vijay* Hard Winter variety) were procured from Niphad Agricultural Research Station, Maharashtra. These were divided into different lots of 1 kg, packed in polyethylene bags and irradiated at 0.02–1 Mrad dose levels in ^{60}Co-gamma cells 220 (Atomic Energy of Canda Ltd), having a flux of 25 Krad/min and overdose ratio of about 30%. Samples were stored for 3–4 wk and milled in Buhler's experimental Mill having three break systems and three reduction rolls. The solvents (Analar) were procured from B.D.H., U.K.

Baking test

Breads were prepared by straight dough lean procedure. Ingredients were: 100g wheat flour, 2.5g yeast, 2.5g sugar, 4g skimmed milk powder, 4g shortening, 1.5g salt and 10 ppm potassium bromate. Water was added as required for optimum dough consistency (500 B.U.), determined in a Brabender Farinograph. The dough was fermented for 2½ hr at 30°C with punching at 45 and 100 min. It was scaled (100g), molded and proofed for 60 min in a slightly greased pan. Baking was done at 210°C for 20–25 min (Rao et al., 1976). Loaf volume was measured by the mustard seed displacement method (AAAC, 1970).

Sensory evaluation

Sensory evaluation of breads was conducted by preferential rating techniques with trained panel members (Amerine et al., 1965). A nine-point hedonic scale was used to differentiate the samples on the basis of overall acceptability, and a five-point intensity scale was applied for scoring off-characteristics such as aroma, taste and color.

Total carbonyl content

The solvents were treated with 2,4,-dinitrophenyl hydrazine (DNPH) and redistilled before use to remove the traces of carbonyls present. Carbonyls were extracted from 5g of wheat flour or pieces of bread, with 20 ml benzene for several times and the extracts were combined (Henick et al., 1954). Aliquots were reacted with 0.05% DNPH reagent and 4.3% TCA, at 60°C for 30 min and cooled. Ethanolic KOH (4%) was added and the final volume (10 ml) was adjusted with ethanol. Absorbancy was measured after 10 min at 480 nm in a Bausch and Lomb Spectronic-20. Acetophenone was used as a standard.

Head space analysis

Direct headspace vapors were collected essentially by the method described by Bandyopadhyay et al. (1970). Wheat flour (75g) or bread crumb and crust pieces (50g) were mixed with water (150 ml) into a lump-free slurry. This was put into a conical flask, fitted with an adapter with a side tube having a stopcock and a small sealed aperture at the top. The flask was kept for 2 hr in a pre-heated oven at 60°C. Vapor samples (10 ml for wheat flour and 2 ml for bread), trapped in the headspace, were taken by inserting a hypodermic syringe (60°C) through a septum and injected in a glass column (6 ft × 1/4 in. o.d.). This was packed with 10% Carbowax–20M supported on acid-washed chromosorb W (60–80 mesh) and pre-equilibrated at 75 ± 5°C. A gas chromatograph, equipped with a flame ionization detector (Model BARC) and fabricated in this Research Centre, was used. The carrier gas was nitrogen with a flow rate of 15 ml/min. Chromatograms were obtained on a recorder with a sensitivity of A × 10^{-10} and a chart speed of 0.5 in./min. A few internal standards such as formaldehyde, acetaldehyde, valeraldehyde, butyraldehyde and ethanol were run simultaneously. Retention times in minutes were recorded and the peaks were tentatively identified from GLC profiles of reference compounds. Gas chromatograph peak area was determined by multiplying 1/2 peak height by peak width.

Reducing sugars

Total reducing sugars were determined in aqueous extracts of wheat flour using maltose as a standard (AOAC, 1970). Freshly prepared alkaline $K_3Fe(CN)_6$ (0.1N) solution was added to an aliquot (5 ml) and immersed in a boiling water bath for 20 min. After cooling, acetic acid-salt mixture (20 ml) and 1 ml of 1% starch-KI solution (as an internal indicator) were added. The mixture was titrated with 0.1N $Na_2S_2O_3$, up to complete disappearance of blue color.

To measure diastatic activity, wheat flour (10g) was suspended in 0.1M acetate buffer (48 ml), pH 4.8 and incubated at 30°C for 1 hr

[1] Present address: Gautam Apartments, Pali Hill, Bombay-400 050 India

Table 1—Effect of irradiation on baking properties of wheat[a]

Radiation dose Mrad	Specific loaf vol cc/g	Crust color % Transmittance	General appearance
0	3.0	98	Satisfactory
0.02	3.5	95	Satisfactory
0.20	3.8	90	Satisfactory
1.00	2.7	76	Not satisfactory

[a] Specific loaf volume was measured by mustard seed displacement method. Bread crust was extracted with 70% ethanol and color intensity measured at 550 nm in a Bausch and Lomb Spectronic-20. General appearance of bread was assessed by subjective evaluation by panel members. Results are averages of three experiments.

with frequent shaking. Reaction was stopped with 10% TCA and reducing sugars were determined in the supernatant.

Total free amino acids

Wheat flour (5g) was extracted thrice with 70% ethanol. Extracts were pooled and passed through Dowex-50 H^+ column (0.9 x 10 cm), eluted with 2N NH_4OH and lyophilized. The residue was taken up in 0.2M sodium citrate buffer, pH 2.2. An aliquot was used for quantitative separation of amino acids on a Beckman Unichrome Automatic Amino Acid Analyzer (Gerritsen et al., 1965).

RESULTS

Sensory evaluation

Results on baking properties (Table 1) show that breads made from wheat irradiated at higher dose levels, had dark crust color and the general appearance was also not satisfactory. Average preference scores for breads are given in Table 2. The panel evaluation revealed that aroma and taste of breads became less desirable with increasing irradiation dose. Off-flavor intensity was very high (above moderate) with breads prepared from wheat irradiated at 1 Mrad.

Carbonyl compounds

Results on the effect of irradiation on total carbonyl levels in wheat flour and breads are summarized in Table 3. In wheat flour, these were not appreciably affected up to 200 Krad; however, it increased significantly (33%) at 1 Mrad. Relatively low levels of carbonyl compounds in control bread (132 ppm) compared to that in wheat flour (240 ppm) may be attributed to initial volatilization or oxidation during fermentation and baking. However, an increase by 22–97% was noticed in breads prepared from wheat irradiated at 0.02–1.0 Mrad dose levels, compared to control.

Head space analysis

Though sub-ambient programmed temperature operation (from −180°C to 200°C) of the gas chromatographic column, greatly enhances the ability to separate a mixture of flavor components (Merritt et al., 1966), this cannot assure complete separation. Since this was a comparative study, only a Carbowax 20 column operated at 75–80°C was used to separate volatile compounds.

Data presented in Table 4 revealed that total peak area, occupied on GLC headspace profile increased gradually with irradiation dose. At 1 Mrad, this was about 38% more compared to control samples. Acetaldehyde, isobutyraldehyde, butyraldehyde and valeraldehyde, were the main volatile components tentatively identified in wheat flour. Compounds with similar retention time were identified, in all the samples, with the main difference in quantity, this being more in irradiated samples.

Results on headspace area distribution on GLC profiles of flavor components, separated from breads, are presented in Table 5. Total peak area was decreased slightly in irradiated

Table 2—Sensory evaluation of off-characteristics of irradiated wheat breads[a]

		Avg points scored on intensity scale		
		Irradiated (Mrad)		
Off-characteristic	Control	0.02	0.2	1.0
Discoloration	1.2	1.6	2.6	4.9
Off-odor	1.0	1.3	2.0	3.7
Irradiation flavor	1.0	1.3	2.0	4.9
Burnt flavor	1.0	1.6	2.0	4.0
Texture (undesirable)	1.0	1.2	2.0	3.5
Taste (objectionable)	1.0	1.2	2.0	3.5
Overall acceptability (Avg ± SE on hedonic scale)	7.3 ±0.4	7.5 ±0.4	6.2 ±0.3	4.6 ±0.4

[a] Off-characteristics in bread were evaluated using an intensity scale of 5 points, and a 9-point hedonic scale was used for overall acceptability. Results are averages of points scored by three independent panels, each of 12 members. Intensity scale: 1 = none; 2 = slight; 3 = moderate; 4 = above moderate; 5 = strong.

Table 3—Effect of irradiation on total carbonyls in wheat flour and bread[a]

Radiation dose (Mrad)	Total carbonyl content (ppm)	
	Wheat flour	Bread
0.0	240 ± 2.23	132 ± 4.36
0.02	240 ± 2.23	162 ± 5.69
0.2	260 ± 2.50	194 ± 6.59
1.0	320 ± 8.00	260 ± 5.70

[a] Acetophenone was used as a standard. Results are averages of six determinations ± SE.

Table 4—GLC headspace profile of wheat flour[a]

		Headspace area (cm^2)		
		Irradiated at (Mrad)		
GLC peaks	Control	0.02	0.2	1.0
Acetaldehyde	0.20 ± 0.01	0.42 ± 0.02	0.50 ± 0.05	0.63 ± 0.07
Isobutyraldehyde	0.25 ± 0.03	0.37 ± 0.03	0.41 ± 0.03	0.47 ± 0.02
Butyraldehyde	2.78 ± 0.24	3.56 ± 0.28	3.86 ± 0.35	4.21 ± 0.36
Valeraldehyde	0.28 ± 0.50	0.28 ± 0.12	0.32 ± 0.08	0.37 ± 0.09
Total peak area	3.52 ± 0.78	4.63 ± 0.45	5.19 ± 0.51	5.68 ± 0.54

[a] Results are averages of four experiments ± SE.

Table 5—Effect of irradiation on the composition of volatile flavor components of bread[a]

		Headspace area (cm^2) From wheat irradiated (Mrad) at		
GLC peaks	Control	0.02	0.2	1.0
Formaldehyde	0.20 ± 0.01	0.22 ± 0.0	0.23 ± 0.02	0.24 ± 0.06
Acetaldehyde	0.20 ± 0.04	0.26 ± 0.06	0.29 ± 0.05	0.32 ± 0.09
Ethanol + methanol	24.0 ± 7.90	23.10 ± 1.92	20.90 ± 6.40	20.5 ± 6.60
Hexanone	0.48 ± 0.03	0.63 ± 0.02	0.63 ± 0.17	0.69 ± 0.10
Butanol	0.22 ± 0.03	0.30 ± 0.10	0.35 ± 0.05	0.36 ± 0.05
Total peak area	25.11 ± 8.01	24.51 ± 2.08	22.4 ± 6.69	2.11 ± 6.90

[a] GLC profiles of flavor volatiles separated from bread (50g) were obtained as described in text. Results are averages of four experiments.

samples compared to control. Alcohols were not detected in wheat flour but constituted the major components of bread flavor volatiles. Bread prepared from wheat irradiated at 1 Mrad, contained a lower percentage of both ethanol and methanol, but relatively higher contents of formaldehyde and acetaldehyde than the control. About 45% increase at 1 Mrad in hexanone, the main ketone identified in bread, was also noticed. However, furfural, reported as one of the constituents of bread volatiles (Rothe and Thomas, 1959) was not separated by this technique.

Reducing sugars

Results on the effect of irradiation on reducing sugars and diastatic activity of wheat flour are given in Table 6. Levels of initial water-soluble reducing sugars in wheat irradiated at 0.02 to 1 Mrad, was increased by 5–92% compared to control. Diastatic activity, expressed as 'maltose value,' was significantly more in irradiated samples after 1 hr incubation.

Free amino acids

Data on free amino acids of control and irradiated (0.02–1 Mrad) wheat samples (Table 7) revealed no appreciable effect of radiation treatment at low doses. However, at 1 Mrad, there was an overall increase of about 9% in total free amino acids, mostly accountable to appreciable release (23–35% higher values, as compared to control) of isoleucine, tyrosine, valine and alanine.

Table 6—Reducing sugars and diastatic activities in irradiated wheat[a]

	Initial reducing sugars		Maltose value	
Dose level (Mrad)	mg maltose/ 10g wheat flour	% inc over control	mg maltose liberated/10g wheat flour at 30°C for 1 hr	% inc over control
0	90	–	150	–
0.02	95	5.5	172	40.6
0.2	125	38.2	211	78.0
1.0	172	91.1	259	78.0

[a] Aqueous extracts of wheat flour were analyzed for total reducing sugars. 'Maltose value' was measured as described in text. Results are averages of three independent experiments.

Table 7—Effect of irradiation on free amino acid content of wheat[a]

	Wheat irradiated (Mrad) at						
	0	0.02		0.2		1.0	
Amino acid	(mg/g N)	(mg/g N)	% inc over control	(mg/g N)	% inc over control	(mg/g N)	% inc over control
Aspartic acid	4.03	3.97	–	3.97	–	4.01	NS
Threonine	0.37	0.35	–	0.39	5.4	0.39	NS
Serine	3.47	3.51	1.1	3.35	–	3.28	–
Glutamic acid	3.04	2.88	–	2.77	–	2.90	–
Proline	0.44	0.45	2.2	0.47	6.8	0.46	4.5
Glycine	0.84	0.83	–	0.86	2.3	0 87	3.5
Alanine	1.92	2.11	9.8	2.36	22.9	2 6	35.4
Valine	0.76	0.78	2.6	0.82	7.7	0.84	15.2
Methionine	0.33	0.34	3.0	0.34	3.0	0.37	12.1
Isoleucine	0.52	0.56	7.6	0.53	1.9	0.04	23.0
Leucine	0.62	0.64	3.2	0.66	6.4	0.65	4.8
Tyrosine	0.48	0.49	2.0	0.54	12.5	0.60	25.0
Phenylalanine	0.47	0.49	4.2	0.50	6.3	0.51	9.0
Lysine	0.46	0.45	–	0.45	–	0.47	2.1
Histidine	0.22	0.21	–	0.23	4.5	0.21	–
Arginine	1.73	1.79	3.4	1.77	2.3	1.80	4.0

[a] Results are averages of three determinations.

DISCUSSION

RADIATION TREATMENT effectively disinfests wheat (Nicholas et al., 1958) but it also brings about certain changes in the flour. The loaf volume was increased up to 0.2 Mrad dose levels when bread was prepared with lean straight dough formula (Table 1). Similar observations have been reported by Lai et al. (1959) and Nicholas et al. (1958). However, at doses above 500 Krad, irrespective of the baking formula, loaf-volume and other bread qualities were deteriorated (Miller et al., 1965). This was attributed to damaged gluten by Rao et al. (1975), from their studies on fractionation and reconstitution of different wheat components. Though no off-characteristics are exhibited at disinfestation dose level (20 Krad), unpleasant irradiated flavor and dark crust were detected at higher doses (Tables 1 and 2).

A direct relationship between the panel evaluation data and the content of carbonyl compounds, the principal flavor components of bread (Thomas and Ronneback, 1960) has been observed (Table 3). As the carbonyls increased in irradiated samples, the bread aroma and taste became less desirable and acceptability of the product decreased. Higher total GLC headspace area in irradiated wheat flour also paralleled the observed off-flavor. Increase in total volatile aldehydes (Tables 4 and 5), having a direct bearing on bread flavor and taste (Wiseblatt and Kohn, 1960) would be the main factors responsible for off-flavor. Hexanone, formed only during baking, is another important flavor component of bread. Increase in hexanone at higher doses could also contribute to off-flavor (Lorenz and Maga, 1972). Decrease in alcohols in bread at higher dose (1 Mrad) could cause lesser gas production and retention, as reflected in lower loaf volume (Table 1).

We also estimated inherently related compounds like reducing sugars and certain amino acids in wheat, from which these excessively volatile irradiation off-flavor compounds (like aldehydes) are expected to form. It has been shown that the initial interaction between reducing sugars and free amino acids during baking, through successive condensation, rearrangement and polymerization reactions produce nonenzymatic Maillard browning (Johnson and Miller, 1961) and malanoidins (Mills et al., 1969). These volatile and nonvolatile compounds are mainly responsible for typical bread aroma (Johnson and El-Dash, 1967). Nonreducing sugars, however, do not produce any browning or bread aroma (Pomeranz et al., 1962). Overall increase in initial total reducing sugars (Table 6) in irradiated wheat flour would arise from step-wise and random degradation of starch (Ananthaswamy et al., 1970). The increase in 'Maltose value' during fermentation (Table 6) indicates the increased availability of these sugars to amylase action. Similarly, an overall increase (8.5%) in free amino acids in irradiated wheat at the higher dose could be attributed mainly to depolymerization of proteins (Srinivas et al., 1972). This increase is mainly due to a rise in levels of amino acids like glycine, alanine, valine methionine, lysine, isoleucine, leucine, tyrosine and phenylalanine (Table 7). Deschreider (1966) has observed a similar increase in some of the free amino acids, this being directly proportional to the doses applied. He attributed the variation in solubility of various wheat proteins to their structural modifications. Some of the off-flavor imparting compounds like benzene, phenols and sulfur compounds detected in irradiated foods (at high doses) are known to be formed from radiosensitive phenylalanine, tyrosine and methionine, respectively, by direct bound cleavage of amino acid moieties (Merritt et al., 1966).

Aldehydes, found in bread headspace profile are the by-

products of Maillard reaction. Results on model systems with amino acids and sugars, indicate their association with each other; sugar determines the browning and amino acid, the aroma (Collyer, 1964). It has been shown that during fermentation acetaldehyde, formaldehyde, valeraldehyde and phenylacetaldehyde are formed mainly from alanine, glycine, leucine and phenylalanine, respectively, by Strecker degradation (Golovnya et al., 1974); volatile components formed by reaction between sugars and valine have been identified as compounds of pyrazine, pyridine and piperazine series. Golovnya et al. (1969) have shown that increased content of pyridine in the crust adversely affects the organoleptic characteristics of bread. Thus, the strong disagreeable odor in bread may partially be attributed to considerably high free valine content in wheat at 1 Mrad.

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A. H. ROUSE and P. G. CRANDALL
University of Florida, Institute of Food & Agricultural Sciences
Agricultural Research & Education Center
P.O. Box 1088, Lake Alfred, FL 33850

PECTIN CONTENT OF LIME AND LEMON PEEL AS EXTRACTED BY NITRIC ACID

ABSTRACT

Research is described for the extraction of pectin from lime and lemon peel with nitric acid. Temperature and time were varied while extraction acidity remained constant at pH 1.6. For those extraction conditions that resulted in maximum jelly units, yield of pectin as alcohol precipitate, jelly grade and yield as 150 grade pectin were tabulated. Yields of pectin from lemon peel decreased (6.49% in July to 2.55% in November) with increasing fruit maturity. Comparative data were also obtained for pectins extracted from commercially leached peel and the corresponding rotary dried peel. Loss due to drying peel was from 7.7–10.6% in yield of 150 grade pectin.

INTRODUCTION

A NUMBER of methods have been reported on the hydrolysis of protopectin in citrus peel to water-soluble pectin and many of these have been reviewed by Kertesz (1951). Later gamma radiation (Kassem, 1969) and a combination of ion-exchange resin-hydrochloric acid (Huang, 1973) were reported to solubilize pectin in citrus peel. Commercial extraction of pectin is usually accomplished with mineral acids such as sulfurous (Wilson, 1925; Joseph and Havighorst, 1952), sulfuric (Snyder, 1970), hydrochloric (Myers and Baker, 1929) or nitric (Braddock et al., 1976; Rouse and Crandall, 1976). Nitric acid is used in Europe and to a lesser extent in this country. Approximately 4,000 tons of dry lime and lemon pomace are produced in Florida, most of which is shipped to Europe for pectin extraction. However, conditions for obtaining maximum jelly units from fresh and dried lime and lemon peel extracted with nitric acid have not been published. Generally, extraction temperatures previously reported range from 80–100°C and time of extraction from 20–60 min. The pH varied between 1.4 and 2.6.

There were three objectives in this study: (1) to present extraction conditions (temperature and time) that resulted in maximum jelly units when pectins were extracted using nitric acid from laboratory leached lime and lemon peel; (2) to show the effect of fruit maturity on pectin extracted from lemon peel; (3) to compare data for pectins extracted with nitric acid that resulted in maximum jelly units obtainable from commercially leached lime and lemon peel and from their corresponding rotary dried peel.

MATERIALS & METHODS

DURING THE 1976 SEASON samples of ground lime peel (predominantly Persian, *Citrus aurantifolia* Swing.) and lemon peel (predominantly Avon, *Citrus limon* L Brum.f.) were obtained from commercial plants. The legally mature fruit (Soule et al., 1976) was extracted on a FMC In-Line juice extractor and the peel ground through a chopper with a 3/4-inch screen. Samples of peel that were laboratory leached and extracted were obtained on the following dates: lime, Aug. 19 and 25; lemon, July 20, Sept. 22, Oct. 20 and Nov. 9. Samples of commercially leached peel and their corresponding dried peel were obtained and extracted: lime, Aug. 25; lemon, Oct. 20 and Nov. 9.

Commercial leaching procedure for peel

One part of chopped peel was mixed with three parts of tap water at 27°C in a countercurrent system having about 20 min holding time. The mix was passed through a continuous press to remove excess water. The leaching process removed soluble solids (mostly sugars) prior to the extraction of pectin.

Laboratory leaching procedure for peel and the extraction procedures that were used on each prepared wet or dried peel were as previously described (Rouse and Crandall, 1976).

Definition of terms

Yield of pectin is the amount of dry alcohol precipitate extracted from the peel.

Jelly grade denotes the quality of pectin and is estimated by measuring jelly sag of a standard 65% soluble solids jelly on a Ridgelimeter (IFT Committee on Pectin Standardization, 1959).

Yield of 150 grade pectin is a calculated value that indicates to the processor the amount of this grade pectin produced from a given peel. Commercial pectin sold to the jelly and jam trade is based upon a 150 grade product.

Jelly units are calculated by multiplying percent yield of pectin by jelly grade and indicates the peel's economic potential.

RESULTS & DISCUSSION

TO DEMONSTRATE the effect of temperature upon the extraction of pectin from peel, researchers (Myers and Baker, 1931; Gaddum, 1934) used increments of 10° and 20°C. Maximum jelly units are usually obtained at extraction temperatures between 85° and 95°C with extraction times from 30–60 min. We used temperature increments of 5°C and time increments of 15 min. Within these restricted conditions, differences in jelly units and yields of 150 grade pectin seemed small compared to previous work.

Data presented in Table 1 are results of a typical series of six extractions for leached lime peel prepared Aug. 19. In this particular series, jelly units ranged from a low of 13.4 when extracted at 85°C for 60 min to a high of 14.1 when extracted at 95°C for 30 min. Corresponding yields of 150 grade pectin were 8.92 and 9.39% on wet peel basis. At the lower 8.92% yield value, a commercial plant processing 18.1 metric tons (20 tons) of peel daily would lose approximately $102,000 over a 6-month period at the current price for 150 grade pectin.

Since an extraction temperature of 95°C for 30 min at pH 1.5 resulted in maximum jelly units, this finding then appears

Table 1—Data for a typical pectin extraction series of lime peel, samples Aug. 19, 1976, using nitric acid at pH 1.6 (Calculated to fresh-peel basis)

Extraction					
Temp °C	Time min	Yield pectin-%	Jelly grade	Jelly units	Yield calc to 150 grade pectin-%
95	45	5.16	265	13.7	9.12
95	30	5.16	273	14.1	9.39
90	60	5.12	268	13.7	9.15
90	45	4.95	274	13.6	9.05
90	30	4.86	278	13.5	9.01
85	60	4.94	271	13.4	8.92

Table 2—Extraction conditions that resulted in maximum jelly units when pectin was extracted from laboratory leached lime and lemon peel using nitric acid (pH 1.6) during the 1976 season. (Calculated to fresh-peel basis)

Sampling date	Extraction Temp °C	Extraction Time min	Yield pectin-%	Jelly grade	Jelly units	Yield calc to 150 grade pectin-%
			Lime			
8-19	95	30	5.16	273	14.1	9.39
8-25	90	60	5.29	286	15.1	10.09
			Lemon			
7-20	90	60	6.49	254	16.5	10.99
9-22	90	45	3.71	269	10.0	6.65
10-20	95	30	3.14	260	8.2	5.44
11- 9	90	30	2.55	264	6.7	4.49

Table 3—Extraction conditions that resulted in maximum jelly units when pectin was extracted from commercially leached citrus peel and the corresponding rotary dried peel using nitric acid (pH 1.6) during the 1976 season (Calculated to water-free basis)

Fruit sample	Sampling date	Extraction Temp °C	Extraction Time min	Yield pectin-%	Jelly grade	Jelly units	Yield calc to 150 grade pectin-%
				Leached peel			
Lime	8-25	90	30	33.05	273	90.2	60.15
Lemon	10-20	90	30	27.71	263	72.9	48.58
Lemon	11- 9	90	30	28.62	268	76.7	51.13
				Rotary dried peel			
Lime	8-25	95	30	32.25	250	80.6	53.75
Lemon	10-20	90	30	27.28	241	65.7	43.83
Lemon	11- 9	95	30	30.11	235	70.8	47.17

as the first entry in Table 2. Yields, as alcohol precipitates and jelly grades are also presented (Table 1) to visualize the effect of other temperatures and times of extraction upon pectin. Difference between minimum and maximum jelly units in any of the series has never exceeded 10%.

Values presented in Table 2 are also calculated on fresh peel basis for pectin extracted from the six series of laboratory leached lime and lemon peel that resulted in maximum jelly units. The data indicated that over a period of time (July through Nov.), extraction conditions varied slightly for obtaining maximum jelly units. Close proximity of jelly units (14.1 and 15.1) and yields of 150 grade pectin (9.39 and 10.09%) resulted in the two samples of lime peel extracted for pectin in August. The four lemon samples did decrease in jelly units (16.5 to 6.7) and yields as 150 grade pectin (10.99 to 4.49%) with advancing maturity. Lowest yields of 150 grade pectin reported by Rouse and Crandall (1976) were 5.98% for orange peel and 4.33% for grapefruit peel. It is apparent (Table 2) that extracted pectins from lime peel have higher jelly grades than those from lemon peel and that increasing fruit maturity did not appreciably affect the grade of extracted pectins from lemon peel.

Comparative values for pectins extracted with nitric acid that resulted in maximum jelly units and yields of 150 grade pectin from commercially leached lime and lemon peel and their corresponding rotary dried peel are presented in Table 3. Results are given on water-free basis for comparison purposes. Obviously there was a decrease in jelly grade of pectins that reflected the loss in jelly units and yields of 150 grade pectin when the peel was dried. Loss of 150 grade pectin due to drying the peel amounted to 10.6, 9.8 and 7.7%, respectively, for the lime and the two lemon peels. Commercially this would be considered a normal loss.

Maximum jelly units were obtained for the commercially leached peel (Table 3) at a temperature of 90°C and extraction of 30 minutes. Only the dried peel sample of Oct. 20 required similar extraction conditions, while the other two dried peel samples required 95°C and 30 min for maximum jelly units.

CONCLUSIONS

DATA INDICATED that any single given extraction condition (temperature and time) when pH is constant will not always result in maximum jelly units.

Jelly grades of pectins extracted from lime peel were always higher than those from lemon peel.

Jelly grades of pectins from leached fresh peels were higher than those from corresponding rotary dried peel.

Yields of 150 grade pectin from lemon peel (Table 2) decreased with increasing fruit maturity while jelly grade showed no appreciable change.

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W. J. KIM,[1] C. J. B. SMIT and V. N. M. RAO
Dept. of Food Science, University of Georgia, Athens, GA 30602

DEMETHYLATION OF PECTIN USING ACID AND AMMONIA

ABSTRACT

The kinetics of deesterification of high-ester citrus pectin, using acid, ammonia, or a treatment with acid followed by ammonia in isopropanol was studied with respect to the changes in methyl ester groups, molecular weight, and the formation of acid amide groups. It was found that: (1) Higher concentrations of acid or ammonia at low temperature resulted in less depolymerization for a certain decrease in ester groups; (2) The conversion of methoxyl groups to acid amide groups approached unity as temperature was lowered and ammonia concentration increased. A linear relationship between the increase in amide content and the decrease in esterification was found; (3) In treatments using hydrochloric acid followed by ammonia, a long acid treatment and a short ammonia treatment resulted in a maximum retention of apparent molecular weight. However, this treatment gave a smaller number of acid amide groups in the final product. Depending on the selection of ammonia concentration and temperature, a wide range of acid amide levels was possible. It was also possible to predict the conditions needed for deesterification to give certain apparent molecular weights and ratios of amide levels to ester levels.

INTRODUCTION

PECTINS have been divided into two major groups in the marketplace: those containing more than 50% esterification (high-ester pectin) and those containing less than 50% esterification (low-ester pectin). Low-ester pectins are differentiated from high-ester pectins by their ability to form gels with or without sugar in the presence of divalent cations such as calcium.

Although some low-ester pectins occur in plants, they are usually manufactured from high-ester pectins. Joseph et al. (1949) classified demethylation methods into four groups according to the agents used: (1) acids, (2) alkalies, (3) enzymes, and (4) ammonia in alcohol systems or concentrated aqueous ammonia systems. Acid, alkali or enzyme demethylation produce one general type of low-ester pectin which contains carboxyl and methoxyl groups on the no. 6 carbon of the galacturonic acid units while the fourth method, using an ammonia system, gives a different type of low-ester pectin which contains acid amide groups in addition to carboxyl and methoxyl groups on the no. 6 carbon (Joseph et al., 1949).

Among these four methods, acid or NH_4OH, or a combination of acid and NH_4OH treatments (Wiles and Smit, 1971) are commonly used to manufacture low-ester pectins. The main disadvantage of the acid treatment which hydrolyzes glycosidic bonds as well as methyl ester linkages is the slowness of the reaction (Kertesz, 1951). Speiser et al. (1945) reported that an increase in temperature increased the rate of demethylation in an almost linear relationship when the logarithm of the rate constant was plotted against the reciprocal of absolute temperature. However, increase in temperature and concentration of acid will cause severe depolymerization of pectin chains with an adverse effect on gel strength (Doesburg, 1965). Many workers showed that the rate of acid demethylation proceeds at less than 0.01 of the rate of alkaline demethylation at about the same temperature and concentration of reagents (Baker, 1948; Speiser et al., 1945; McCready et al., 1944; Black and Smit, 1972). At lower temperatures, acids tend to remove methyl ester groups with little depolymerization of the pectin. Smit and Bryant (1968) stated that this property makes it possible to prepare low-ester pectins with little chain cleavage. Nonuronide material (ballast) was decreased at about the same rate as during normal acid demethylation (Speiser et al., 1945).

Demethylation with ammonia in alcoholic systems or in concentrated ammonia-aqueous systems have a rate of reaction about half of the rate of alkaline demethylation (McCready et al., 1944; Black and Smit, 1972). During alkaline demethylation or deesterification, some of the free carboxyl groups formed are converted to acid amide groups, ammonium salts and a small amount of metal salts at carbon no. 6 (Joseph et al., 1949). The viscosity and calcium sensitivity of ammonia saponified pectin is intermediate between those of the enzyme and the acid types (Joseph et al., 1949).

In this study attempts were made to determine the best procedures to prepare low-ester pectins using acid in alcohol, ammonia in alcohol or a combination procedure using acid in alcohol followed by ammonia in alcohol. The kinetics of deesterification, depolymerization, and formation of amide groups was studied and related to manufacture of low-ester pectins.

MATERIALS & METHODS

Pectin samples

All of the high-ester pectins used for kinetic studies were supplied by Sunkist Growers Inc. (Ontario, CA). The pectins were extracted from citrus peel with sodium bisulfite solution and precipitated with aluminum hydroxide before pressing, drying and storing at 4°C. The first pectin, used for acid demethylation, had 73.3% esterification and an apparent molecular weight of 101.74×10^3. The second high-ester pectin had an ester content of 63.3% and an apparent molecular weight of 146.49×10^3. This pectin was used for NH_4OH demethylation.

For demethylation of high-ester pectin, a procedure was followed similar to that described by Smit and Bryant (1968) and Black and Smit (1972).

Hydrochloric acid demethylation

Two hundred fifty grams of ground pectin of the first high-ester sample were acid washed to remove aluminum by suspending the pectin in a mixture of 2200 ml of 65% isopropanol and 200 ml of concentrated HCl. After 30 min of mild stirring, the pectin was filtered and washed again with acid-alcohol mixture, and then finally rinsed several times with 60% isopropanol (throughout this manuscript % alcohol is on a volume basis) until free of chloride. The acid-washed and rinsed filter cake was divided into two portions. Each portion was transferred to a 4L Erlenmeyer flask and kept at 3°C overnight. Three thousand milliliters of 60% isopropanol containing 3.5N HCl was also cooled to 3°C and then added to one flask while the same volume of cooled 60% isopropanol containing 4.5N HCl was added to the other flask. The pectin solutions were stirred continuously at 3°C during demethylation. Four to five samples were taken over an 8-day period from each flask and washed with 60% isopropanol until free of chlorides. After a final rinse and filtration, the samples were washed with 95% isopropanol and finally with acetone. The acetone rinsed samples were dried overnight at 40°C before sealing in closed containers and storing at 3°C.

Sulfuric acid demethylation

Two hundred thirty grams of high-ester pectin were acid washed, rinsed and divided into two equal portions for H_2SO_4 demethylation. After adjustment of the temperature to 3°C, 3000 ml portions of 2N H_2SO_4 and 4N H_2SO_4 in 60% isopropanol at 3°C were used for the two respective flasks. Four or five samples were taken periodically from each flask over an 8-day period. These were treated in the same manner

[1] Present address: Dept. of Crop Science, University of Saskatchewan, Saskatchewan, Canada

as the samples prepared with HCl except that effectiveness of washing was judged by the absence of $SO_4^=$ ions in the filtrate.

Ammonia demethylation

For NH_4OH demethylation and amidation, the second high-ester pectin having an ester content of 63.3% and an apparent molecular weight of 146.49×10^3 was used. A 350-g portion of this pectin was acid washed and rinsed to remove aluminum. The filter cake was divided into six portions and transferred to six 1L flasks. Two flasks each were kept at 3°C. 15°C, and 23°C to adjust the temperature of the sample. At the same time, 800 ml of NH_4OH and isopropanol mixtures were prepared and stored in the following way: (a) Two solutions of 60% isopropanol containing 2.0N NH_4OH and 4.0N NH_4OH respectively were prepared and stored at 3°C. (b) Two solutions of 60% isopropanol containing 1.0N NH_4OH and 2.0N NH_4OH respectively were kept at 15°C. (c) Two solutions of 60% isopropanol containing 0.5N NH_4OH and 1.5N NH_4OH were stored at 23°C. After adjustment of samples and solutions to the desired temperature, NH_4OH demethylations were started by mixing the samples and solutions in closed Erlenmeyer flasks and keeping the reaction mixtures continuously stirred at either 3°C, 15°C, or 23°C. Small portions of samples were taken at different time intervals up to 160 min. These samples were filtered immediately and rapidly washed with 60% isopropanol, followed by acid-alcohol (1N HCl in 60% isopropanol). To minimize the time of filtration, 600 ml, medium sintered glass funnels and vacuum was used. This took less than 2 min. The filter cake was then stirred with about 300 ml acid-alcohol solution for 20 min to remove ammonia present in the salt form at carbon no. 6 of the pectin. Samples were then washed and rinsed until free of chlorides. After a final rinse with isopropanol and acetone, the samples were dried overnight at 40°C and then stored at 3°C.

Chemical analysis

Moisture, percent esterification, percent galacturonic acid, percent free carboxyl groups and apparent molecular weight by viscosity were determined as described in the National Formulary (1965) and by Smit and Bryant (1967). The measurement of amide content was carried out by suspending 0.35g of sample in about 50 ml H_2O and then transferring into a micro-Kjeldahl distillation apparatus. Fifteen milliliters of 50% NaOH were added and about 150 ml were distilled into a flask containing 20 ml 0.1N HCl and a few drops of methyl red solution. The distillate was titrated with 0.1N NaOH. The percent NH_2 was calculated as follows:

$$\% NH_2 = \frac{\text{mg } NH_2 \text{ in sample}}{\text{mg sample (moisture and ash free)}} \times 100$$

Assuming 10 galacturonide units in pectin, the percent NH_2 would be 9.4143 if all the carboxyl groups in the chain were amidated. Using this factor % NH_2 was converted to % acid amide groups as follows: % acid amide groups = (% NH_2/9.4143) X 100.

RESULTS & DISCUSSION

Kinetics of deesterification

It has been suggested that the distribution of methoxyl groups and the solubility of the demethylated pectin are not only dependent on the temperature and concentrations used during demethylation, but also on the particle size and degree of hydration of the pectin (Black, 1970). The author stated that larger particle sizes would result in slower overall reaction rates and more demethylation of the outer layers. In this experiment pectins having mesh size of 60 or less were first hydrated in 60% isopropanol overnight before demethylation.

Acid demethylation. Low temperature treatments were chosen to minimize molecular weight losses during acid demethylation. The changes of the ester content of the first high-ester pectin during acid deesterification at 3°C is shown in Figure 1. Demethylation with high concentrations of acid showed a tendency to slow down as the reaction progressed. This could be due to the availability of methoxyl groups in the pectin. With more demethylation, methyl ester groups are less available for reaction and this will eventually slow down the reaction rate. Table 1 shows reaction rate constants calculated for 200 hr of acid demethylation, using a first order reaction.

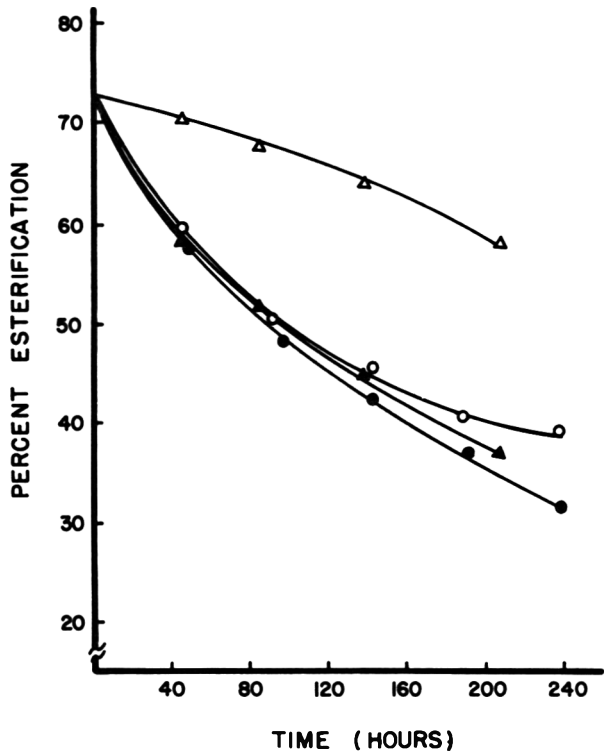

Fig. 1—Changes in percent esterification during acid demethylation of pectin at 3°C: ○ 3.5N HCl; ● 4.5N HCl; △ 2.0N H_2SO_4; and ▲ 4.0N H_2SO_4.

Table 1—Reaction rate constants of deesterification

Demethylating agents	Temp (°C)	Time	K(10^{-3} min^{-1})
2.0N H_2SO_4	3	200 hr	0.018
4.0N H_2SO_4	3	200 hr	0.055
3.5N HCl	3	200 hr	0.048
4.5N HCl	3	200 hr	0.061
2.0N NH_4OH	3	100 min	3.660
4.0N NH_4OH	3	100 min	7.704
1.0N NH_4OH	15	100 min	1.589
2.0N NH_4OH	15	100 min	3.798
0.5N NH_4OH	23	100 min	1.967
1.5N NH_4OH	23	100 min	7.303

$$K = \frac{1}{t} \ln \frac{C_o}{C}$$

Where C_o is the initial degree of esterification and C is the degree of esterification at time t in minutes. The K values in these experiments are much lower than values reported by other workers, e.g., 0.7×10^{-3} min^{-1} at pH 3 and 50°C (McCready et al., 1944), 0.190×10^{-3} min^{-1} at 30°C with 0.87N HCl (Speiser et al., 1945) and 0.1×10^{-3} min^{-1} at 25°C with 1.4N HCl (Black and Smit, 1972). The lower K values obtained in these experiments were probably due to the low temperature used for demethylation.

Figure 2 shows the decrease in apparent molecular weight during acid demethylation. It was found that treatment with higher concentrations of acid caused more depolymerization at a given time. There were no marked differences in the effects of the HCl and H_2SO_4 treatments on the rates of depolymeri-

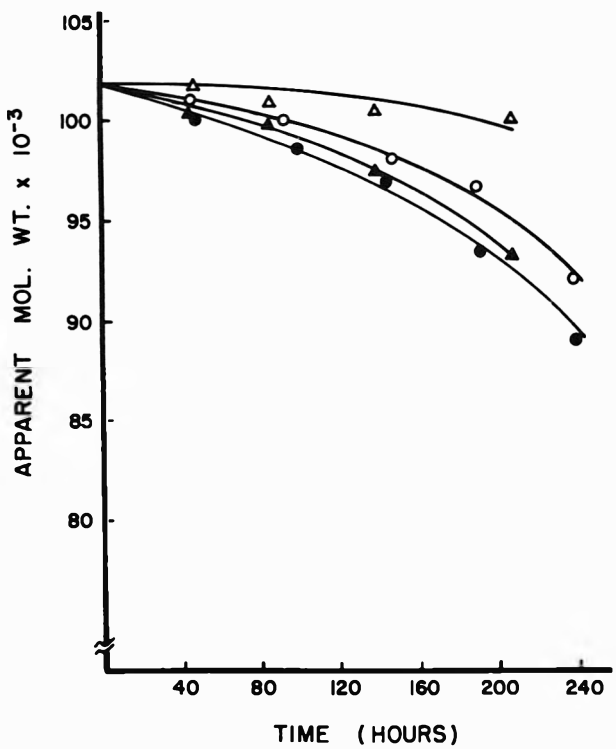

Fig. 2—Changes in apparent molecular weight during acid demethylation of pectin at 3°C: ○ 3.5N HCl; ● 4.5N HCl; △ 2.0N H_2SO_4; and ▲ 4.0N H_2SO_4.

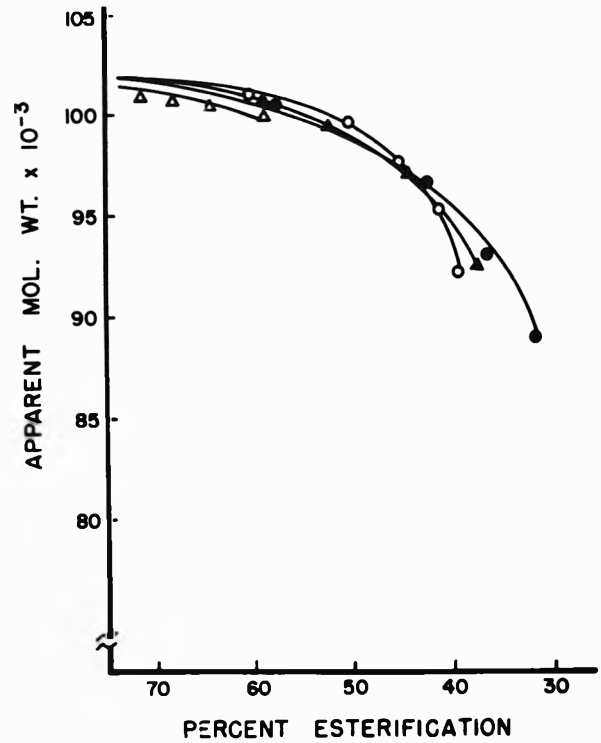

Fig. 3—The relationship between the changes in apparent molecular weight and percent esterification during acid demethylation of pectin at 3°C: ○ 3.5N HCl; ● 4.5N HCl; △ 2.0N H_2SO_4; and ▲ 4.0N H_2SO_4.

zation and deesterification. Prepared samples having ester levels below 40% were more difficult to dissolve in 1% sodium hexametaphosphate solution which indicates increasing insolubility as demethylation progressed. This result supports Black's (1970) suggestion that the surface layers of pectin particles may become highly demethylated during prolonged acid treatment and that this contributes to poor solubility.

Apparent molecular weights were plotted against the percent esterification (Fig. 3). At 3°C and low ester levels, higher concentrations of acid appeared to result in lower apparent molecular weight losses. It is also shown in this figure that apparent molecular weight decreases as deesterification progresses. Since 4.5N HCl at 3°C gave smaller decreases in apparent molecular weight at about 40% esterification than the other treatments, it was used to study the kinetics of treatments involving both acid and ammonia.

NH_4OH demethylation. The high-methoxyl pectin having 63.25% esterification and a 146.7 x 10^3 apparent molecular weight was used for demethylation with ammonium hydroxide in an isopropanol system. Figure 4 shows the changes in percent esterification of samples during NH_4OH treatment. The treatment with 4.0N NH_4OH at 3°C showed the fastest reaction rate, and this reduced the esterification from 63.3% to 24.3% in 160 min. The reaction-rate constants were calculated on the basis of 100 minutes demethylation using the formula for a first order reaction (Table 1).

These results show very similar reaction rates for treatments with 4.0N NH_4OH at 3°C and 1.5N NH_4OH at 23°C with K values of 7.704 x 10^{-3} and 7.303 x 10^{-3} min^{-1}, respectively. A higher reaction rate was observed with 2.0N NH_4OH at 15°C than with 2.0N NH_4OH at 3°C. McCready et al. (1944) reported K values of 5 x 10^{-3} min^{-1} for NH_4OH demethylation at pH 11 and 15°C, which is within the range of K values obtained in this experiment. A much higher value for K (25.1 x 10^{-3} min^{-1}) was given by Black and Smit (1972) for the treatment of pectin in solution at pH 11 and 5–7°C. The major reason for this is probably the fact that demethylation was carried out in solution and that methoxyl groups were more accessible to the ammonia.

The apparent molecular weight during NH_4OH treatment was plotted against time (Fig. 5). After 100 min, the treatment with 4.0N NH_4OH at 3°C reduced the apparent molecular weight by only 26.8 x 10^3 from an original 146.7 x 10^3 while the loss with 1.5N NH_4OH at 23°C was about 63.0 x 10^3. Comparing the apparent molecular weight loss between treatments having the same concentration of NH_4OH and very similar K values, 2.0N NH_4OH at 15°C caused a 33.7 x 10^3 reduction in apparent molecular weight while 2.0N NH_4OH at 3°C reduced it by only 20.7 x 10^3 for the same reduction in percent esterification. This agrees with Doesburg's statement (1965) that "the alkaline breakdown of pectinic acids increases more rapidly with increasing temperature than does the concurrent saponification of methyl ester groups." In contrast to acid demethylated samples, NH_4OH demethylated samples showed no solubility problems.

Figure 6 shows the increase in acid amide content during NH_4OH demethylation and amidation. Formation of amide groups on carbon no. 6 was most rapid on treatment with 4.0N NH_4OH at 3°C. In this treatment, more than 35% of the total groups were converted to amide groups in 160 min. This treatment was followed by 1.5N NH_4OH at 23°C, 2N NH_4OH at 3°C, 2N NH_4OH at 15°C, 0.5N NH_4OH at 23°C and 1.0N NH_4OH at 15°C. From the data it was not clear, though, how temperature and ammonia concentration separately affect the rate of amide formation. As the temperature decreased and NH_4OH concentration increased, the apparent molecular weight losses decreased. The most rapid depolymerization was observed on treatment with 1.5N NH_4OH at 23°C which was the lower concentration of NH_4OH and the highest temperature used. From a practical point of view, higher concentrations and lower temperatures during NH_4OH demethylation

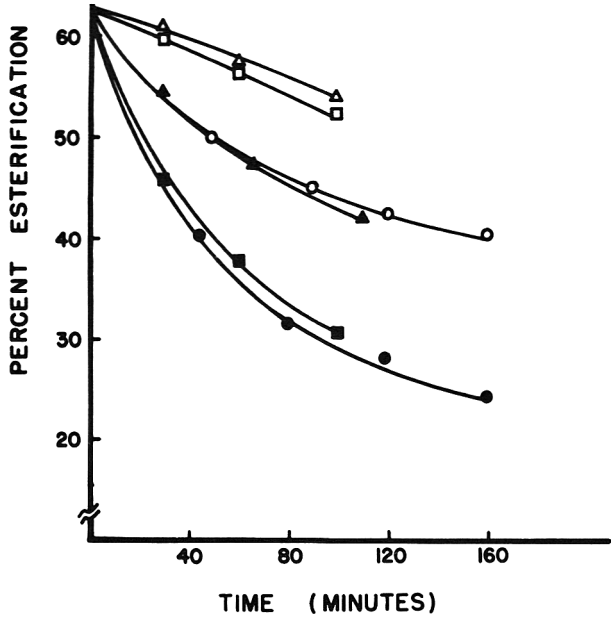

Fig. 4—Changes in percent esterification during ammonia demethylation and amidation of pectin: ○ 2.0N NH_4OH at 3°C; ● 4.0N NH_4OH at 3°C; △ 1.0N NH_4OH at 15°C; ▲ 2.0N NH_4OH at 15°C; □ 0.5N NH_4OH at 23°C; and ■ 1.5N NH_4OH at 23°C.

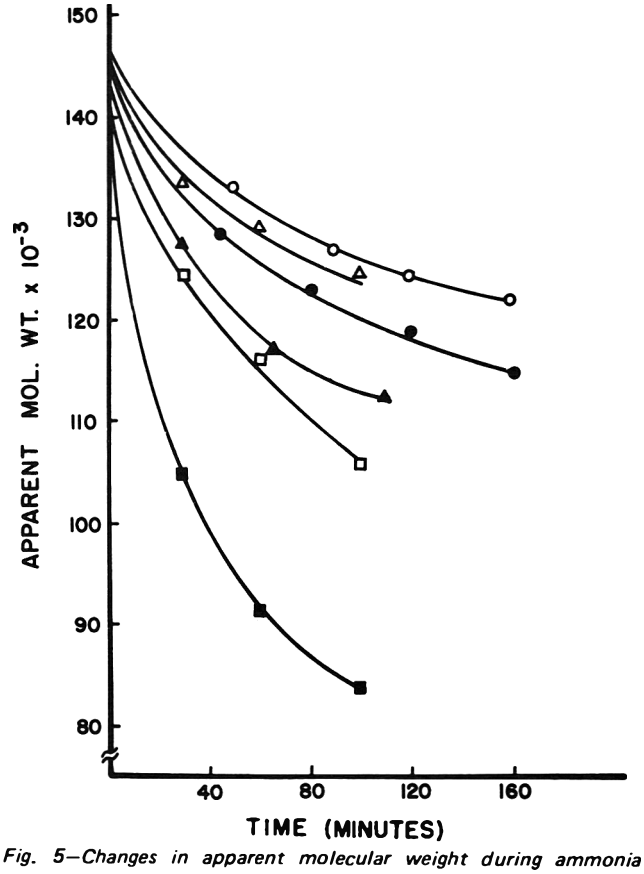

Fig. 5—Changes in apparent molecular weight during ammonia demethylation and amidation of pectin: ○ 2.0N NH_4OH at 3°C; ● 4.0N NH_4OH at 3°C; △ 1.0N NH_4OH at 15°C; ▲ 2.0N NH_4OH at 15°C; □ 0.5N NH_4OH at 23°C; and ■ 1.5N NH_4OH at 23°C.

would be recommended to minimize apparent molecular weight losses.

A similar effect of temperature and concentration of NH_4OH was observed when increase in acid amide and decrease in apparent molecular weight was compared. Deesterification with 4.0N NH_4OH at 3°C gives a maximum formation of amide groups with a minimum loss in apparent molecular weight. Further, with an increase in temperature and a decrease in NH_4OH concentration, the increase in amide levels was small in relation to the accompanying decrease in apparent molecular weight.

There was a linear relationship between the increase in amide content and the decrease in esterification. A steeper slope was also generally found at lower temperatures and higher NH_4OH concentrations except for the treatments with 1.5N NH_4OH at 23°C and $2N_4$ OH at 15°C. The slope was calculated by using the formula:

$$R = \frac{A}{C_o - C}$$

where A is percent of acid amide, C_o is initial percent of esterification and C is percent of esterification which corresponds with A. The data obtained are given in Table 2. From this it appears that the slope gets closer to unity as temperature is decreased and ammonia concentration is increased. This means that the methoxyl groups at the no. 6 carbon are more readily replaced with amide groups under those conditions. The R value of 0.895 would indicate that 89.5% of the methoxyl groups removed by NH_4OH demethylation was converted to acid amide.

Combination of acid and NH_4OH demethylation

The primary purpose of the kinetic studies was to predict the chemical composition of low-methoxyl pectin prepared by using simple or combined methods of acid and NH_4OH demethylation. The combined treatment with acid and alkali, a practice which is used commercially for the preparation of

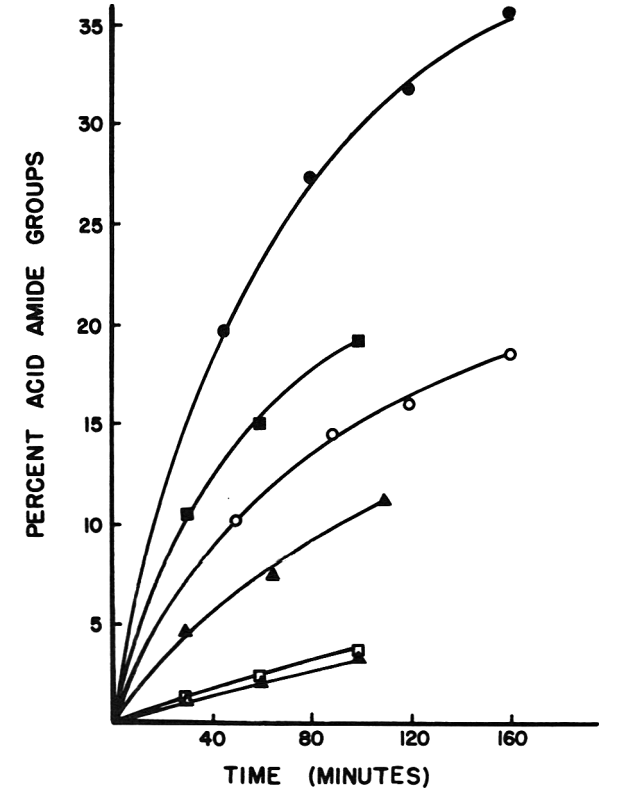

Fig. 6—Changes in percent acid amide groups during ammonia demethylation and amidation of pectin: ○ 2.0N NH_4OH at 3°C; ● 4.0N NH_4OH at 3°C; △ 1.0N NH_4OH at 15°C; ▲ 2.0N NH_4OH at 15°C; □ 0.5N NH_4OH at 23°C; and ■ 1.5N NH_4OH at 23°C.

Table 2—Ratio (R) between increase of percent acid amide groups and decrease of percent ester groups during demethylation of pectin

Conc of NH_4OH	Temp (°C)	R
2.0N	3	0.783
4.0N	3	0.895
1.0N	15	0.360
2.0N	15	0.516
0.5N	23	0.315
1.5N	23	0.595

functional low-ester pectins, is for several reasons of considerable interest. The earlier data were therefore used to obtain more information on the kinetics of such two-stage treatments. During a first-stage treatment, using a high concentration of acid at a low temperature, the ester content was reduced to different levels with a minimum effect on polymer size. These samples were then subjected to further demethylation and amidation with different concentrations of NH_4OH in isopropanol at different temperatures. The final products were expected to have a wide range of acid amide levels within a relatively limited range of ester content and molecular weight.

Figure 7 shows the theoretical relationship between the losses in apparent molecular weight and the decrease in esterification when an acid treatment with 4.5N HCl at 3°C was followed by NH_4OH deesterification and amidation with 2.0N NH_4OH at 15°C, 1.5N NH_4OH at 23°C, 4.0N NH_4OH at 3°C and 2.0N NH_4OH at 3°C. From this figure it is clear that the overall losses in apparent molecular weight during deesterification to around 40–30% may be minimized with longer acid and shorter NH_4OH treatments. However, if the acid-treated sample has a low-ester content in the neighborhood of 45–40%, the NH_4OH treatment will be short, resulting in a small amount of acid amide in the final product.

An increase in acid amide level with an accompanying decrease in molecular weight was found during NH_4OH demethylation and amidation. Since acid demethylation caused little depolymerization, the starting apparent molecular weight ranged between about 147×10^3 and 143×10^3. This indicates that a wide range of amide levels is possible depending on the selection of the NH_4OH concentration and the temperature. To obtain high amide levels in the final product, high concentrations of NH_4OH at low temperatures are required while low concentrations at higher temperatures result in low amide levels.

As discussed previously, there was a linear relationship between the increase in percent acid amide and the decrease in percent esterification. Therefore, the amide level in the final product after the second stage demethylation can be estimated. Depending on the starting acid demethylated sample, percent acid amide in the final product having an esterification of about 35% can range from zero to about 25%.

CONCLUSION

ACID DEMETHYLATION using HCl or H_2SO_4 showed that higher concentrations of acid at low temperatures gave less depolymerization during deesterification than when lower concentrations and higher temperatures were used. In a series of NH_4OH deesterifications and amidations using different ammonia concentrations at different temperatures, apparent molecular weight was retained better for a certain decrease of ester groups, and the conversion of methoxyl groups to acid amide groups approached unity, as temperature was lowered and ammonia concentration increased. A linear relationship

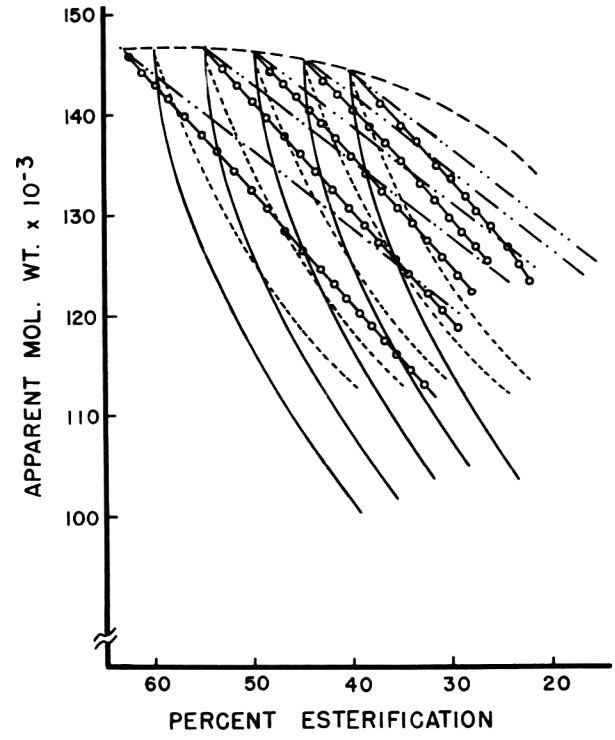

Fig. 7—Expected changes in apparent molecular weight during ammonia demethylation and amidation of acid treated pectin: ———4.5N HCl at 3°C; —o—o—o— 2.0N NH_4OH at 3°C; —••—••— 4.0N NH_4OH at 3°C; ------- 2.0N NH_4OH at 15°C; and ———1.5N NH_4OH at 23°C.

was found between the increase in amide content and the decrease in esterification.

A long acid treatment followed by a short NH_4OH demethylation would result in maximum retention of apparent molecular weight and low acid amide levels in the final product. To obtain a high amide level and also a relatively high molecular weight, higher concentrations of ammonia at low temperatures are required.

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CHERL HO LEE and CHOKYUN RHA
Dept. of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139

MICROSTRUCTURE OF SOYBEAN PROTEIN AGGREGATES AND ITS RELATION TO THE PHYSICAL AND TEXTURAL PROPERTIES OF THE CURD

ABSTRACT

The microstructure of soybean protein aggregates was examined by the optical microscope and the scanning electron microscope. The effects of heat and coagulating agents on the microstructure of the aggregates and on the physical and textural properties of the protein curd were investigated. Isoelectric point precipitation and calcium coagulation did not change the globular structure of the native soybean protein. However, heating induced the destruction of the native protein body. Heat denaturation of the protein was necessary in forming the network structure of the aggregates. When the protein aggregates were frozen, their structure became better defined and enlarged. The three dimensional network structure of the aggregate derived from heated soybean protein showed a low sedimentation rate, high curd yield, high water-holding capacity, low value of hardness and high springiness compared to the unheated precipitates of globular structure.

INTRODUCTION

THE TEXTURAL PROPERTY of soybean protein curd varies widely with the coagulative conditions, for example, temperature, pH, ionic strength, coagulation agent, protein denaturation (Aoki, 1965a, b; Aoki and Sakurai, 1968, 1969; Hashizume et al., 1975, Circle et al., 1964; Catsimpoolas and Meyer, 1970). It has been known that the curd strength is related to the interactions between protein molecules such as the hydrogen bonding, ionic bonding, disulfide bonding and hydrophobic association. The interactions between or within protein molecules would decide the microstructure of the protein network, and the microstructure integrates into the structure network which is interpreted as the mechanical properties during the processing and the textural properties upon consumption. The microstructure of soybean protein curd has been reported (Saio and Watanabe, 1968; Matsumoto, 1975); however, further study relating to the microstructure and the protein interactions to the textural properties is required for the texture fabrication process.

In the present study, two methods of coagulation for soybean protein were studied, namely, the calcium coagulation and the isoelectric point precipitation. The effects of heat and coagulants on the microstructure of soybean protein aggregates were studied by using an optical microscope and a scanning electron microscope. The sedimentation properties and water holding capacities of the aggregates and the curds were determined. The textural profile of the curd was determined by the compression test.

The objective of this study was to relate the protein interaction and the microstructure of soybean protein aggregates to the textural properties of the curd.

MATERIALS & METHODS

Sample preparation

One part defatted soybean meal (Soyafluff 200W, Central Soya) was suspended in 10 parts water. The water extract of the protein was isolated with the isoelectric point precipitation at pH 4.5. The isolate was redissolved in water at pH 7.0 and the protein level of the solution was adjusted to 5%. The solution was subjected to four different treatments to obtain aggregates.

SPI-25: The pH of the unheated protein solution was adjusted to 4.5 with dilute HCl solution.

SPCa-25: $CaCl_2$ was added to the unheated protein solution to make a final concentration of $CaCl_2$ to 20 mM.

SPI-80: The protein solution was heated at 80°C for 30 min, cooled to 25°C and the pH was adjusted to 4.5 by adding dilute HCl solution.

SPCa-80: The protein solution was heated at 80°C for 30 min, cooled to 25°C and $CaCl_2$ solution was added to make the final concentration of 20 mM Ca.

The solution was stirred with a magnetic stirrer during the addition of coagulants, continuing for 30 sec after the coagulants were added.

Optical microscopic observation

The Olympus Biological microscope, Model EM, was used. The protein precipitate was mixed with a drop of immersion oil on a slide and covered with a cover glass. Light was illuminated from the bottom and the picture was taken on Polaroid film (type 107).

Scanning electron microscopic observation

The protein precipitates were separated from the solution by centrifugation at 280G for 5 min. The specimens for the microscope were prepared by two different methods.

(1) Unfixed specimen: A small piece of protein curd was frozen in liquid nitrogen (−196°C) and lyophilized.

(2) Fixed specimen: a thin slice of protein curd was immersed in a 1.5% glutaraldehyde solution for 1 hr and then washed with water (Kalab and Harwalkar, 1973). It was frozen in liquid nitrogen and lyophilized.

The dried protein curd was cut in thin slices, mounted on an aluminum stub and coated with gold. The specimens were observed with a JSM-U3 Scanning Electron Microscope at an accelerating voltage of 15 KV. The picture was taken on Polaroid film (type 52).

Sedimentation test

The protein precipitate in solution was poured into a graduated cylinder (16 mm diam, 129 mm ht) and allowed to settle down gravitationally. The changes in the height of the interface were measured for the first 30 min and the final sediment volume was determined after 15 hr of settlement.

Water-holding capacity

The precipitate was centrifuged at 280G for 10 min to obtain the curd. The amounts of curd and the supernatant were measured and the protein concentration of the supernatant was determined by the Lowry method (Lowry et al., 1951).

Compression test

The protein precipitates were separated from solution with centrifugal forces ranging from 70−1740 × G. The protein curds obtained were cut in an identical semi-spherical size (bottom diam 25 mm, ht 10 mm). The compression force versus distance curve was obtained with the Instron Universal Testing Machine, Model 1122. The load cell scale was 0−20 kg, cross-head speed 20 mm/min, and the chart speed 200/min. The compression ratio was 0.8 (80% deformation). The compression test was repeated twice and the time lag between the first and second compression was 1 min. Each experiment was performed at least twice and the data obtained were averaged.

The three basic parameters for the GF texture profile analysis, namely, hardness, cohesiveness and springiness, of the curd were determined. The textural parameters were evaluated according to Bourne's definition (1968): Hardness being the force required for a 70% deformation, cohesiveness being 100 times the ratio of the area under the force-distance curve of the first and second bite (80% deformation) and springiness being the recovered height after the first bite (80% deformation). The initial and recovered heights of the sample were measured directly by the distance reading on the instrument.

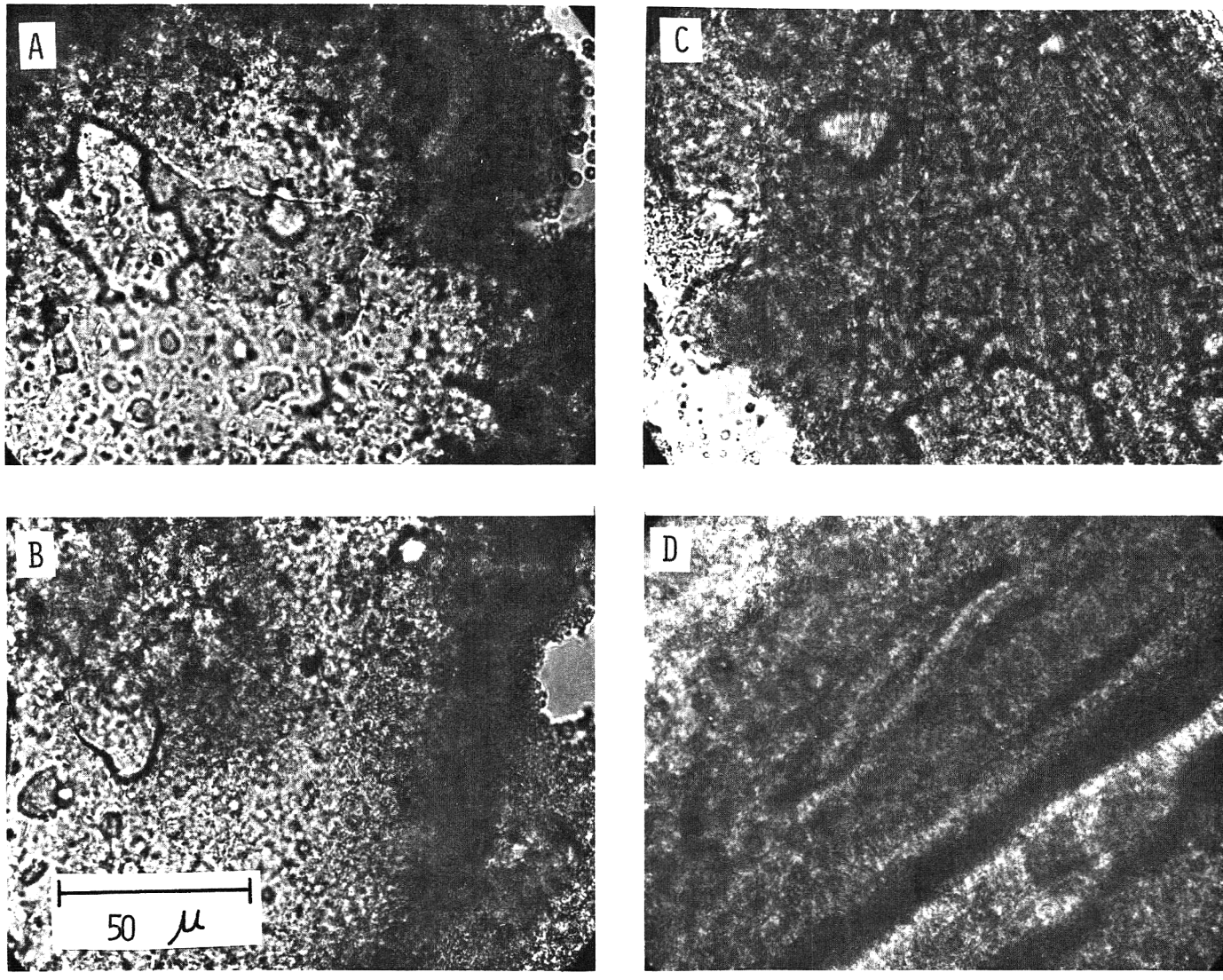

Fig. 1—Soybean protein aggregates as observed by optical microscope 540X. (A) Unheated soybean protein, isoelectric ppt curd; (B) Unheated soybean protein, calcium ppt curd; (C) Heated soybean protein, isoelectric ppt curd; and (D) Heated soybean protein, calcium ppt curd.

RESULTS

Optical microscopic structure

The optical micrograph of SPI-25 showed that the aggregates consisted of distinct small particles. These small particles were visible on the edges of the aggregate in Fig. 1-A. A similar picture was shown with SPCa-25 (Fig. 1-B). On the other hand, SPI-80 and SPCa-80 showed a fiber-like microstructure webbed evenly throughout the aggregate, as shown in Fig. 1-C and 1-D.

Scanning electron microscopic structure

Figure 2 shows the SEM structure of the four samples fixed with glutaraldehyde. The unheated protein samples, both SPI-25 (Fig. 2-A) and SPCa-25 (Fig. 2-B) had a globular microstructure which was integrated into clumps. On the other hand, the structure of heated proteins, SPI-80 and SPCa-80 were not globular. SPI-80 had a rough sponge-like surface with holes of different sizes (Fig. 2-C) and SPCa-80 showed a pumice stone structure (Fig. 2-D).

Figure 3 shows the SEM structure of the freeze-dried samples. SPI-25 still had a globular but slightly enlarged structure (Fig. 3-A). SPCa-25 showed a sheet-like flakey structure and most of the globular structure has disappeared (Fig. 3-B).

The heated protein samples showed a typical three-dimensional network structure, as shown in Fig. 3-C and 3-D. The structure was much larger than those of fixed samples. SPCa-80 showed an enlarged and well defined three-dimensional honeycomb-like network with a certain regularity.

Sedimentation property

Figure 4 shows the sedimentation curve versus the time of settling of the protein precipitates. There was a large difference in sedimentation velocity between unheated and heated proteins but no significant difference existed between acid and calcium precipitation.

The sediment volume measured after 15 hours' settling was 28% of the initial volume for SPI-25, 26% for SPCa-25, 66% for SPI-80 and 68% for SPCa-80 (Table 1).

Water-holding capacity

Water-holding capacity was much higher for the heated protein than the unheated one. The water-holding capacity of the acid precipitated curd was slightly higher than that of the calcium coagulates.

Hardness

The hardness of the curds from unheated soybean protein was slightly higher than that of the heated protein when the curd was produced by means of centrifugal force of 280 X G for 10 min. It increased proportionally with the increasing

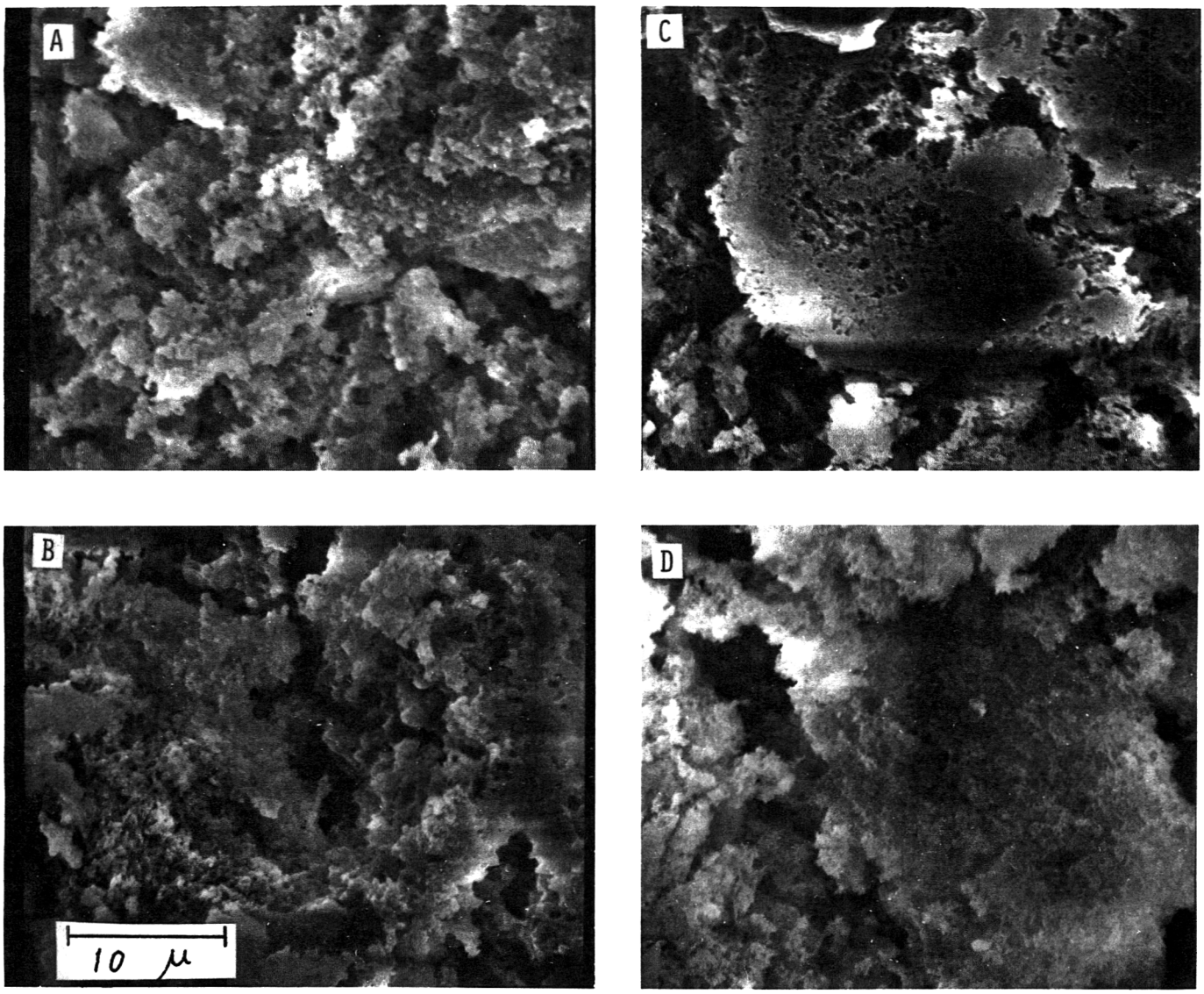

Fig. 2—Soybean protein aggregate structure as observed by scanning electron microscope fixed with glutaraldehyde solution (2300X); Sample designations same as in Fig. 1).

centrifugal force up to 1,120 X G for all samples studied (Fig. 5), but the rate of increase varied with the treatments. The hardness of the curds produced by isoelectric precipitation increased rapidly, whereas those by calcium precipitation responded slowly to the increasing centrifugal force. The heated protein curds did not increase the hardness at the centrifugal force between 1,120 and 1,740 X G while the hardness of the unheated protein curds continued to increase.

Under the experimental conditions, the curd samples did not show a breaking point nor an adhesiveness curve on the instrument.

Springiness

The springiness of the curds was not influenced by the centrifugal force up to 1,740 X G (Fig. 6). Curds from heated protein showed much higher springiness than those from unheated protein and no differences due to the coagulants employed. On the other hand, the springiness of the curds from unheated protein was influenced significantly by the coagulants; calcium coagulation resulted in a much higher springiness of the curd compared to the isoelectric precipitation.

Cohesiveness

The cohesiveness of the curds was not influenced by the centrifugal forces up to 1,740 X G, as shown in Figure 7. The heated protein curds had a cohesiveness around 55% and there was no difference between acid and calcium precipitates. In the case of unheated protein, a large difference in the cohesiveness of the curd due to the coagulants was observed; the calcium precipitates had a cohesiveness modulus of 70%, while the isoelectric precipitate showed a value of 45%.

DISCUSSION

IN THIS STUDY, two mechanisms of protein aggregation namely, isoelectric precipitation and calcium precipitation leading to coagulation were investigated. At the isoelectric point where the net charge of the protein is zero, the electrostatic repulsion between the protein colloids is minimum, then the van der Waals attractive forces become dominant. If the isoelectric point precipitation of protein is a flocculation of the protein colloids due to the van der Waals interaction, then the protein colloid appears to follow the Derjaguin, Landau, Verwey and Overbeek theory for the flocculation of the

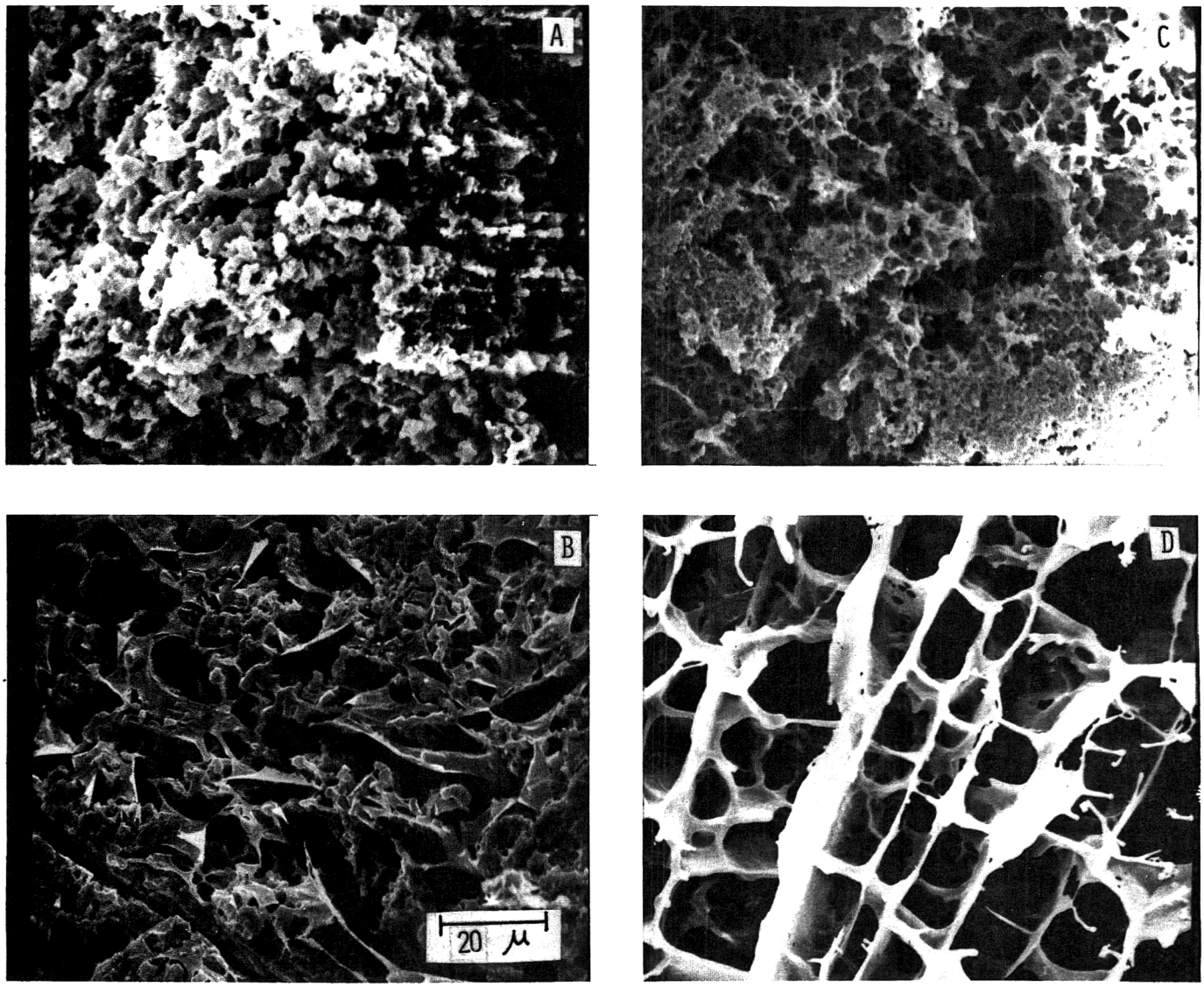

Fig. 3—Scanning electron microscopic structure of soybean protein aggregates freeze dried without fixation (800X); (Sample designations same as in Fig. 1).

lyophobic colloid system (Verwey and Overbeek, 1948; Kitchener, 1968).

On the other hand, the calcium coagulation of the protein appear mainly to be due to the cross-linking between protein molecules by calcium ions. Saio et al. (1967) postulated that the free carboxyl group of soybean protein is the major calcium binding site and phytic acid acts like a binding bridge in the formation of soybean protein calcium aggregates. Appurao and Narasinga Rao (1975) suggested that the imidazole group of histidine is the major calcium binding site of the protein. Although the site of cross-linking in the protein molecule is still under debate, the cross-link involves ionic bonding between calcium ions and the anionic groups of protein molecules.

The results of this study showed that there was little difference in the microstructure of the unheated soybean protein coagulated by isoelectric precipitation and by calcium coagulation and that a structural network was absent. Soybean protein coagulated by the above two methods still exhibited a globular structure referred to as protein bodies in the native state (Saio and Watanabe, 1966; Tombs, 1967; Wolf 1970).

The polypeptide chains of the protein in the protein body are unfolded when heated in a water dispersion (Wolf, 1972; Huang and Rha, 1974). When the soybean protein was heated or the globular structure was modified prior to aggregation, a fibrous three-dimensional network was formed. This heat treatment of the globular proteins appeared to be a prerequisite and initiating step for the three-dimensional network of the protein curd.

In order to prepare the specimen for the scanning electron microscope, the soybean protein curds were freeze dried. In order to minimize the structural changes inherent in the freeze-drying process, one set of samples was fixed with glutaraldehyde while the other remained unfixed. In both sets of samples, globular unheated protein aggregates showed little structural difference. However, heated protein aggregates which were not fixed with glutaraldehyde showed a larger and better defined network structure. Thus, the heat denaturation of the protein enhanced the structural susceptability of the curd to freeze drying. It therefore appears that the curds which are made from heated protein exhibit their structural potential upon further processing.

Although the contribution of calcium alone to the network structure formation of the unheated globular protein was not

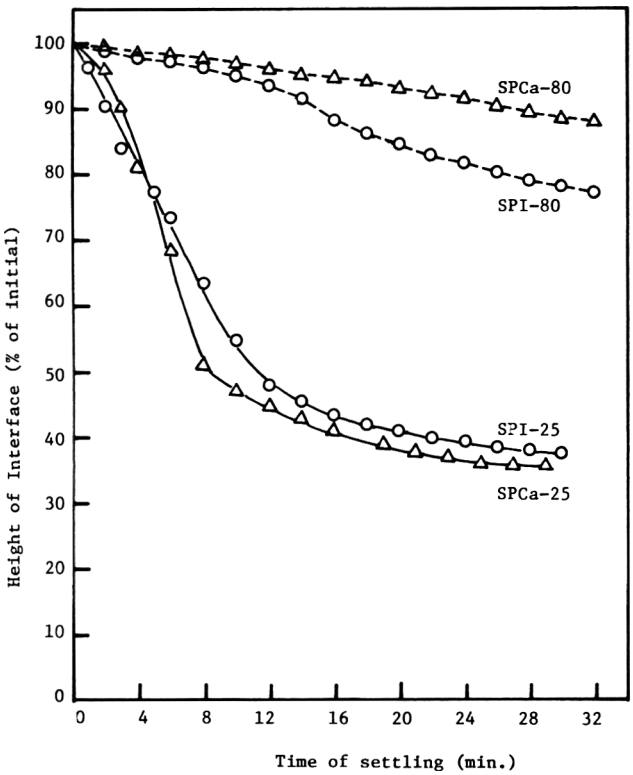

Fig. 4—Rate of sedimentation of soybean protein aggregates; (Sample designation, SPI-25, SPCa-25, SPI-80 and SPCa-80, same as in Table 1).

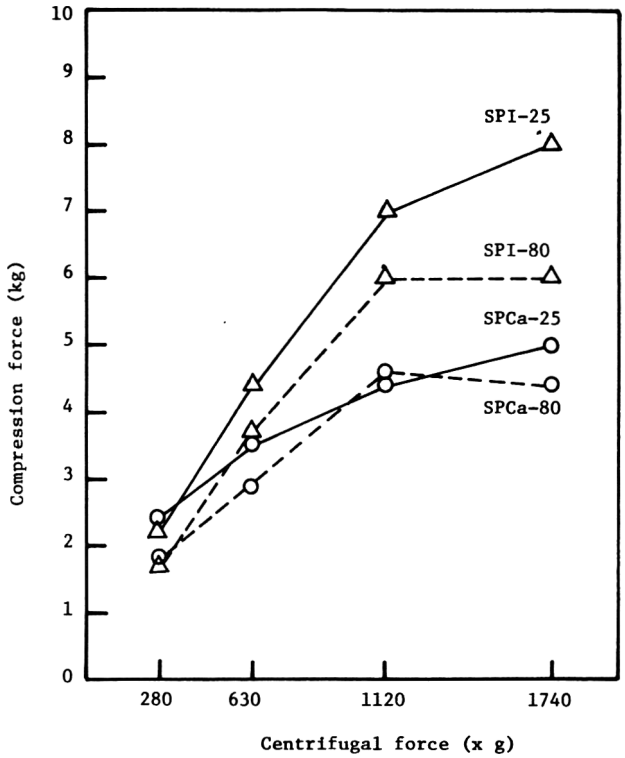

Fig. 5—Relationship of compression forces to centrifugal forces applied in curd separation; (Sample designation same as in Table 1).

evident, upon freeze drying, the globular structure was expanded two dimensionally, resulting in a sheet-like structure. Upon freeze drying, the calcium coagulum of heated protein had a larger network structure than the isoelectric precipitated curd. The difference in the microstructure between calcium coagulate and isoelectric precipitated curds appears to be due to the difference in the type of the major interaction. The interaction energy of ionic bonding is in the range of 10–20 Kcal per mole while that of van der Waals forces is 1–3 Kcal per mole (Jones, 1964). The magnitude of the structural potential at a given external stress is thus related to the interaction energy between protein molecules.

Although microscopic examination did not reveal a clear difference between the structure of the calcium coagulum and isoelectric precipitate of the unheated soybean protein, the difference was evidenced by a mechanical test. The calcium coagulated curd had a significantly higher springiness and cohesiveness. This difference in springiness and cohesiveness can be attributed to the different mode of the protein-protein interaction mechanism, the ionic bond attributing to a higher springiness and cohesiveness than the van der Walles forces (Goodman, 1963; Huang and Rha, 1974).

In both calcium coagulated and isoelectric precipitated soybean protein, when the protein was heat treated, the springiness of the curds increased significantly, indicating an increase in the flexibility and the degree of unfolding of the protein. This heating effect was dominant, and the difference in springiness due to the coagulants observed in the unheated protein was no longer significant.

On the other hand, the effect of heat denaturation on the cohesiveness of the curds prepared with different coagulants was not consistent. This indicates that the factors which control the springiness are not identical to those responsible for cohesiveness. It is likely that while the springiness depends more on a stereo structure and intermolecular reaction, the cohesiveness is related to intramolecular as well as intermolecular reaction.

Table 1—Sediment volume and water-holding property of the soybean protein aggregates

Samples	Sediment vol after 15 hr settling (% of initial)	Water holding property[a] (g/g protein)
SPI-25[b]	28	3.3
SPCa-25[c]	26	2.4
SPI-80[d]	66	5.8
SPCa-80[e]	68	5.3

[a] Obtained by centrifugation at 280 X G.
[b] Unheated soybean protein. Isoelectric point ppt.
[c] Unheated soybean protein. Calcium ppt.
[d] Heated soybean protein (80°C, 30 min). Isoelectric point ppt.
[e] Heated soybean protein (80°C, 30 min). Calcium ppt.

The hardness of the aggregates displaying a globular structure responded proportionally to the increasing centrifugal force, whereas that of the more structured curd levelled off at a centrifugal force above 1000G. This limit is an indication of the resistance of the network structure against applied force, and is therefore a measure of the structural strength of the system.

This study suggests that the textural property of protein curd can be manipulated by the selection of aggregation parameters, such as coagulating agent, temperature, and centrifugation, and thus controls the nature of the microstructure. Understanding the effect of molecular interactions on the microstructure as well as the textural properties of the system would provide the parameters useful for the texture fabrication of foods.

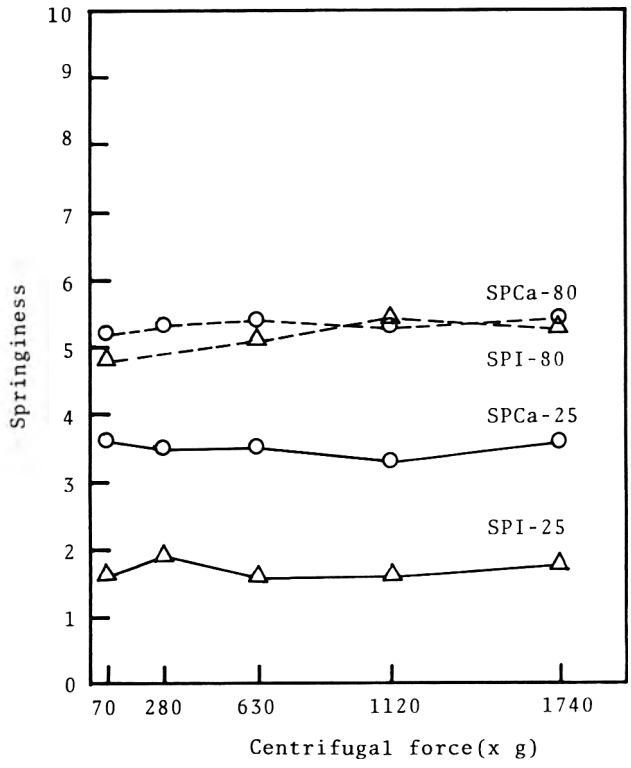

Fig. 6—Relationship of springiness to centrifugal forces applied to curd separation; (Sample designation same as in Table 1).

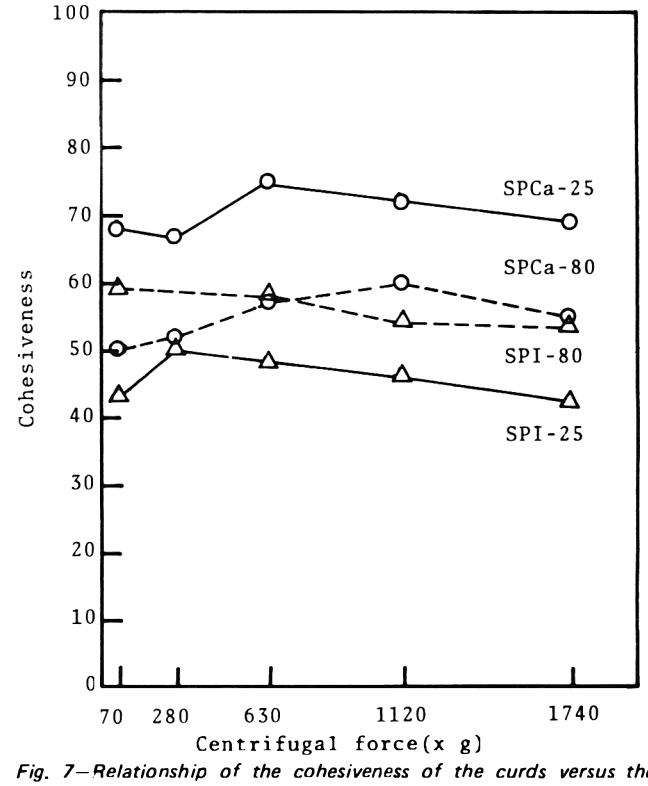

Fig. 7—Relationship of the cohesiveness of the curds versus the centrifugal forces applied for curd separation; (Sample designation same as in Table 1).

CONCLUSIONS

THE FOLLOWING CONCLUSIONS have been made from the results of this study with soybean protein.

1. Isoelectric precipitation or calcium coagulation did not alter the native globular protein body and neither of these processes gave structural integrity to the aggregates.
2. Heat denaturation or unfolding of the native protein body was necessary to form a three-dimensional network structure of the proteins.
3. The soybean protein aggregates possessed a structural potential which manifested itself upon freeze drying. The magnitude of the structural potential appeared to be governed by the degree of protein denaturation and the magnitude of the interaction energies between proteins.
4. There was a greater increase in cohesiveness and springiness in calcium coagulation than in isoelectric precipitates of unheated protein aggregates. Heat treatment of the protein increased the springiness of the curds. However, cohesiveness decreased in calcium precipitates and increased in isoelectric precipitates when protein was preheated.
5. The microstructure of protein curds and their physical and textural properties were related. The aggregates displaying a three-dimensional network structure showed a lower sedimentation velocity, higher water-holding capacity, lower value of hardness and higher springiness than unheated globular protein aggregates.

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K. GANESH KUMAR, L. V. VENKATARAMAN, T. V. JAYA and K. S. KRISHNAMURTHY
Central Food Technological Research Institute
Mysore, 570013 India

COOKING CHARACTERISTICS OF SOME GERMINATED LEGUMES: CHANGES IN PHYTINS, Ca^{++}, Mg^{++} AND PECTINS

ABSTRACT

Germinated greengram, cowpea and chickpea were studied for chemical characteristics after cooking, by estimating the changes in phytins, Ca^{++}, Mg^{++} and pectin contents. Cooking time was drastically reduced on germination in chickpea, while an inverse effect was observed in greengram and cowpea. The phytin content decreased on germination. No appreciable change in phytin P/Total P value was seen on cooking. Ca^{++} content was reduced on germination and cooking in the three legumes, while Mg^{++} content was relatively unaltered. A combined interaction of these chemical parameters, expressed as "PCMP number" was found to correlate with the cooking behavior of the legumes studied.

INTRODUCTION

IN INDIA, legumes are the primary source of dietary proteins (Patwardhan, 1961). The total production of legumes is about 99.50 million tons, cultivated in 22.02 million hectares (FAO Production Year Book 1972). Among the legumes, chickpea itself accounts for about 41% of the total area under legume cultivation. Greengram, cowpea and chickpea are commonly consumed in India. Conventionally they are also used in germinated form since the nutritional value is reported to be better (Chen et al., 1975; Ganesh Kumar and Venkataraman, 1976; Jaya et al., 1975).

One of the limitations in the use of legumes is their undesirable cooking characteristics. In USA, where peas (*Pisum sativum*) and beans (*Phaseolus vulgaris*) are used, the per capita consumption is reported to be on the decline (Rockland and Metzler, 1967). This has been attributed to the prolonged cooking period. Chemical characteristics of peas as affected by cooking have been reported to involve phytates, divalent cations, pectates and lignins (Rosenbaum and Baker, 1969; Crean and Haisman, 1963, 1964; Muller, 1967). Detailed information on the chemical characteristics affecting the cooking quality are needed to develop legumes, with desirable attributes, either by genetic manipulation or by post-harvest processing.

Information regarding the cooking quality of greengram, cowpea and chickpea are limited. While the effect of varietal difference (Muller, 1967), maturity, (Burr et al., 1968) and processing (Rockland and Metzler, 1967; Desikachar and Subramanyan, 1965) on the cooking quality have been reported, extensive literature search has not revealed any information on the effect of germination on the cookability of legumes. The present study details some of the chemical changes that affect the cooking quality in germinated legumes.

MATERIALS & METHODS

Legumes and germination

Greengram (*Phaseolus aureus*), cowpea (*Vigna sinensis*) and chickpea (*Cicer arietinum*) were obtained locally. The seeds were soaked in water for 4 hr, followed by germination on moist cloth. Germinated seedlings were removed after 24 and 72 hr and used fresh for cooking. The ungerminated legumes were cooked without prior soaking. Freeze-dried materials, powdered to 80 mesh, were used for chemical analysis.

Estimation of cooking time. Ungerminated and germinated legumes were cooked with six times their weight of distilled water. While being cooked, at definite intervals samples were drawn and evaluated by a panel for judging the uniform cooking and softness. Ten independent replicates were made to obtain the mean SCT of legumes.

Percent dispersibility. The well stirred slurry of the cooked legumes was passed through a standard 1 mm sieve. The residue was washed with hot water and dried to constant weight at 105°C. The percent ratio of the weight of fraction of the cooked sample passed through the sieve to the total weight of the sample passed was determined. The minimum time required to obtain the maximum dispersibility (98 ± 2%) was taken as the optimal cooking time.

Analytical methods.

The elemental phosphorus (P) was determined by AOAC (1965) method. Phosphates were extracted with distilled water and 0.5N hydrochloric acid. An aliquot of the extract was digested with perchloric acid, sulphuric acid and hydrogen peroxide. The total P in the digest was estimated by Allens method (1940). The procedure of Crean and Haisman (1963) was used for the estimation of phytin P.

Ca^{++} and Mg^{++} contents were estimated in the perchloric acid digest by complexometric titration with EDTA (Vogel, 1961). The interfering ions were removed previous to titration by precipitating them with zirconium oxychloride (Derderian, 1961). The procedure of Dietz and Rouse (1953) was followed for quantitating the pectins. Anhydrouronic acid content was determined by the carbazole method (McComb and McCready, 1952).

RESULTS & DISCUSSION

Cookability of legumes

The times involved for cooking ungerminated and germinated legumes are shown in Table 1. As seen in the table, chickpea was most difficult to cook, while greengram required only a short cooking time. However, the cookability pattern of germinated legumes were markedly different. Germinated chickpea was more readily cooked compared to the ungerminated ones. In this legume, the time required for cooking progressively decreased with germination time. Germinated greengram was more difficult to cook and the percent increase in cooking time was about 336.0 (Table 1). Cowpea pattern was similar to that of greengram. The time needed for cooking the various germinated legumes was different.

Table 1—Effect of germination on cooking time of legumes[a]

Legume	Period of germination (hr)	Cooking time (min) Subjective cooking time Mean ± SD	Percent[b] dispersibility Mean ± SD	Percent[c] increase/decrease in cooking time
Greengram	0	13.6 ± 1.3	14.1 ± 0.4	—
	24	22.8 ± 1.2	23.2 ± 0.2	+ 67.7
	72	59.4 ± 1.5	58.8 ± 0.6	+336.8
Cowpea	0	30.6 ± 1.4	31.2 ± 0.3	—
	24	46.4 ± 1.2	44.0 ± 0.7	+ 51.6
	72	60.4 ± 1.6	61.1 ± 0.5	+ 97.4
Chickpea	0	79.0 ± 3.9	78.6 ± 0.5	—
	24	41.6 ± 1.0	40.9 ± 0.4	− 47.4
	72	20.0 ± 0.9	20.6 ± 0.7	− 74.7

[a] Values represent mean of ten independent observations.
[b] Minimum time to get 98% dispersibility.
[c] Percent increase/decrease with reference to ungerminated seeds + increase; − decrease.

Changes in the chemical parameters affecting cookability of legumes

Phytin content. The total P content was about 0.19–0.33% of the total legumes on dry weight basis (Table 2). The inositol hexaphosphate content, determined as phytin P, varied between 37–69% of the total P, of the legumes studied. Greengram showed the highest phytin P content (∿185 mg/100g). This corresponds to 70% of the total P. The lowest amount of phytin P was found in ungerminated chickpea. The phytin P content decreased with progressive stages of germination and this trend was seen in all the three legumes.

Table 2–Phosphorus compounds of ungerminated legumes (dry wt basis)

Legume	Total P %	Phytic acid %	Phytic acid P %	Phytic acid P / Total P
Greengram	0.270	0.65	0.185	0.685
Cowpea	0.325	0.43	0.123	0.378
Chickpea	0.193	0.28	0.078	0.399

Table 3–Comparison of water and acid extractable phytins in uncooked and cooked legumes (dry wt basis)

Legume[a]	Aqueous extraction		Hydrochloric acid extraction	
	Total P (%)	Phytin P (%)	Total P (%)	Phytin P (%)
Greengram				
Uncooked	0.222	0.142	0.225	0.185
Cooked	0.175	0.080	0.190	0.150
Cowpea				
Uncooked	0.243	0.090	0.223	0.123
Cooked	0.188	0.032	0.200	0.090
Chickpea				
Uncooked	0.114	0.056	0.126	0.078
Cooked	0.103	0.036	0.121	0.075

[a] The values presented are for ungerminated material

Table 3 shows the extraction pattern of phytin P in aqueous and 0.5N hydrochloric acid media. Hydrochloric acid (0.5N) extracted the maximum amount of phytin P both in uncooked and cooked legumes compared to aqueous extraction (Fig. 1–3). The extractability of phytins in these legumes, in water, was lower compared to other types of legumes reported in literature. Lolas and Markakis (1975) have found the phytin P to be wholly water soluble in *Phaseolus vulgaris* L. In peas, the phytin P could be equally extracted both in aqueous and HCl media (Crean and Haisman, 1963). The poor water extractability of phytin P in this study may be due to the nature of phytins which may be in the form of either Na, K or Ca, Mg salts.

Cooking resulted in the decrease of both water and acid extractable phytin P, though the extent of loss of acid extractables was much less compared to water extractable ones (Table 4, Fig. 1–3). There was little change in the ratio of phytin P/total P in the uncooked and cooked legumes (HCl extract, Table 4). In the germinated cooked samples, a reduction in the phytin P/total P ratio, both in water and acid extractables was observed.

Mattson (1946) had attributed poor cookability of peas to reduction in phytin P content. The phytin P content of ungerminated legumes showed a comparable pattern, chickpea requiring a long cooking time, had the lowest amount of phytin P (∿80 mg/100g) while greengram with lowest cooking time showed the highest amount of phytin P (∿185 mg/100g). However, the extent of phytin P may not be the sole contributory factor affecting the cooking time as reported by Mattson (1946), since the pattern of germinated legumes were totally

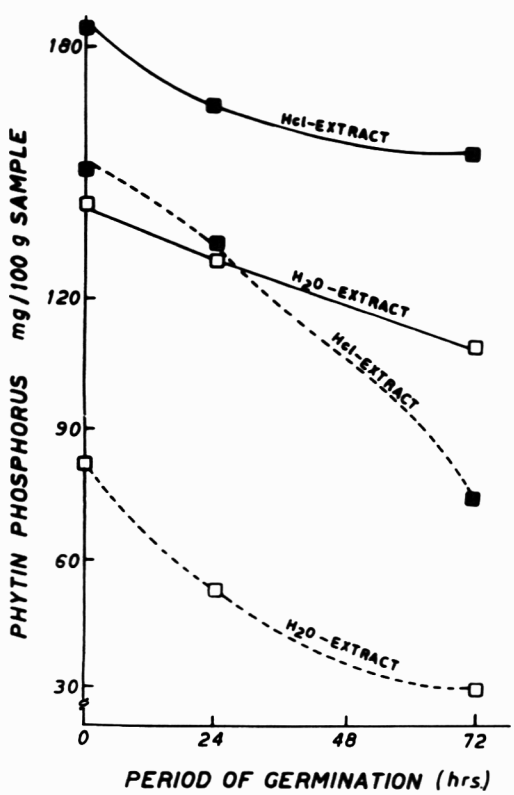

Fig. 1–Changes in phytin P content as a function of period of germination in greengram: ——Uncooked, - - - - Cooked.

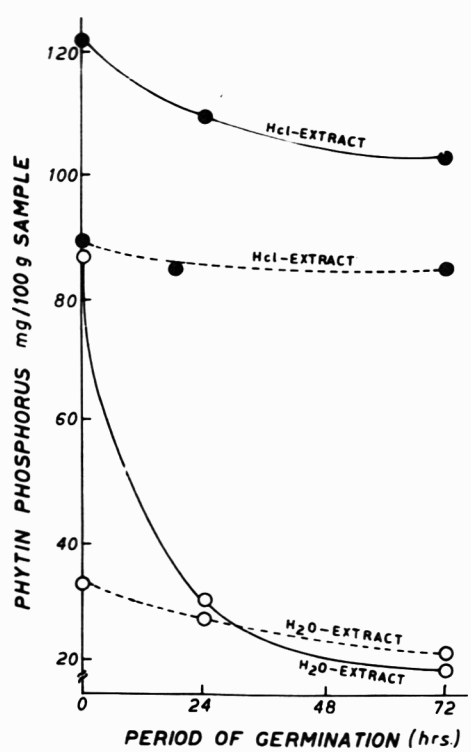

Fig. 2–Changes in phytin P content as a function of period of germination in cowpea: ——Uncooked, - - - - Cooked.

different (Fig. 1–3). Greengram which had a dramatic increase in cooking time (Table 1) did not show a corresponding change in the phytin P. This trend was comparable in cowpea and chickpea also.

Crean and Haisman (1963) had attributed the decrease in the water extractables, to the complexing of inositol hexaphosphate and calcium and magnesium to form insoluble phytates which could be extracted only with dilute acids. This may possibly explain the decrease in water extractables observed in this study. The progressive decrease in the phytin P with germination may also be due to increase in phytase activity during germination (Belavady and Banerjee, 1953; Fordham et al., 1975; Walker, 1974).

Changes in the divalent cations on cooking and germination

The Ca^{++} content of the three legumes differs (Table 5). Chickpea showed the highest value (5.54 meq/100g), followed by cowpea and greengram. A reduction in Ca^{++} content was observed in all the three legumes, as a result of cooking. The loss of Ca^{++} was low in greengram (11.6%) while in chickpea it was considerably higher (42.2%). A similar trend of mineral loss during cooking was reported in different legumes by Meiners et al. (1976). In the later stages of germination (72 hr) significant loss in Ca^{++} was found in all the legumes. On cooking the germinated legumes, the percent loss of Ca^{++} was more pronounced in chickpea compared to cowpea or greengram.

In the ungerminated legumes the Mg^{++} content was comparable (Table 5) and the loss of Mg^{++} on cooking was more noticeable in greengram. During germination the reduction in Mg^{++} content was more evident in greengram compared to chickpea or cowpea. The loss of Ca^{++} and Mg^{++} may be due to leaching during the bulk germination of seeds. Leaching of inorganic and organic compounds have been reported for legumes during germination in literature (Koller, 1972; Linhart and Pickett, 1973).

It is possible that during cooking which involves a complex reaction system, where the Ca^{++} which is concentrated in the parenchyma cells (Crean and Haisman, 1964), migrates into the cell content complexing with phytate ions. Further the permeability of cell wall is altered by the presence of Ca^{++} and Mg^{++} ions. More soluble Ca^{++} salts might be formed during these changes which get leached out.

Changes in pectins during cooking and germination

No noticeable change in free pectin (FP) content was observed in ungerminated chickpea on cooking while cowpea and greengram showed considerable reduction (Table 6). The FP content in germinated legumes was strikingly different. There was a marked increase in the FP of greengram and cowpea at 72 hr of germination, while in chickpea the FP content was reduced.

Decrease in FP may be due to the synthesis of newer polysaccharides which are incorporated in the acidic pectic substances resulting in a more branched structure. A decrease in demethylating activity could be envisaged at this stage resulting in the consequent reduction in FP (Barnes and Patchett, 1976; Matheson and Saini, 1977). A reverse trend is possible, as in greengram with depletion of polysaccharides from the acidic pectic substances, (Matheson and Saini, 1977) and increase in demethylating activity, particularly when the seeds are metabolically active due to enhanced germination.

PCMP number as an index of cooking pattern

Since any of the parameters, viz., phytates, Ca^{++}, Mg^{++} and FP content cannot individually account for the cooking pattern in legumes, Muller (1967) has suggested the cumulative effect of these as PCMP number in the following mathematical formula:

$$\text{PCMP number} = \text{Free pectin} + (Ca^{++} + \tfrac{1}{2} Mg^{++}) - \text{Phytin}$$

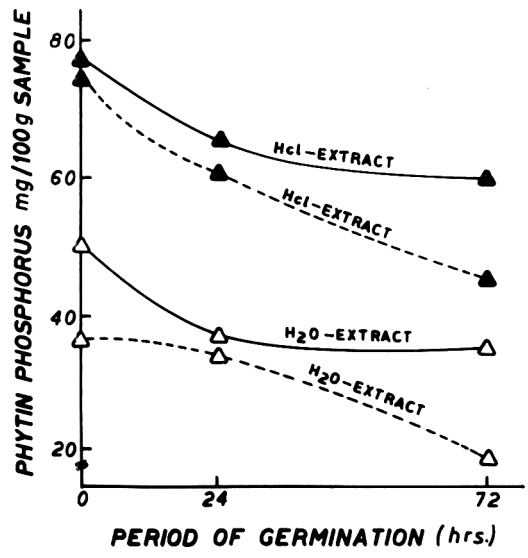

Fig. 3—Changes in phytin P content as a function of period of germination in chickpea: ——Uncooked, - - - - Cooked.

Table 4—Changes in phytin P/total P ratio during cooking and germination

Legume	Period of germination (hr)	Phytin P/total P (extracted)			
		H_2O extract		HCl extract	
		Uncooked	Cooked	Uncooked	Cooked
Greengram	0	0.64	0.46	0.82	0.79
	24	0.60	0.30	0.73	0.68
	72	0.53	0.16	0.69	0.42
Cowpea	0	0.37	0.17	0.55	0.45
	24	0.16	0.12	0.48	0.45
	72	0.08	0.06	0.45	0.42
Chickpea	0	0.44	0.35	0.62	0.62
	24	0.42	0.34	0.56	0.50
	72	0.34	0.16	0.52	0.36

Table 5—Changes in Ca^{++} and Mg^{++} content on cooking and germination of some legumes[a] (dry wt basis)

Legume	Period of germination (hr)	Ca^{+} content (meq/100 g)			Mg^{++} content (meq/100 g)		
		Uncooked	Cooked	% loss on cooking	Uncooked	Cooked	% loss on cooking
Greengram	0	2.24	1.98	11.6	8.07	6.35	21.4
	24	1.60	1.44	10.0	8.00	5.28	34.0
	72	1.37	1.29	6.0	4.52	4.48	0.92
Cowpea	0	2.40	1.76	26.7	7.06	6.41	9.3
	24	2.08	1.60	23.0	6.26	6.00	4.1
	72	1.27	1.06	16.5	5.91	5.80	1.9
Chickpea	0	5.54	3.20	42.2	7.38	6.41	13.2
	24	5.16	2.27	47.3	7.27	6.08	16.3
	72	4.32	2.40	44.5	6.45	5.58	13.6

[a] Mean values based on four independent observations

Table 6—Changes in pectin content on cooking and germination in some legumes[a] (dry wt basis)

Legume	Period of germination (hr)	Anhydro galacto uronic acid content (meq/100g)	
		Uncooked	Cooked
Greengram	0	6.19	2.58
	72	11.86	6.96
Cowpea	0	6.70	2.58
	72	8.76	6.44
Chickpea	0	3.35	3.40
	72	1.85	1.55

[a] Mean values based on four independent observations

Table 7—"PCMP number" as an index of cooking quality of the legumes[a]

Legumes	Period of germination (hr)	Pectins (meq/100 g)	Ca^{++} + ½ Mg^{++} (meq/100 g)	Phytins (meq/100 g)	PCMP no.[b]
Greengram	0	6.19	6.28	5.87	6.60
	72	11.86	3.63	5.16	10.33
Cowpea	0	6.70	5.93	3.87	8.76
	72	8.76	4.23	3.37	9.62
Chickpea	0	3.35	9.23	2.52	11.06
	72	1.80	7.55	2.10	7.25

[a] The values presented are for the uncooked material
[b] PCMP number = Free pectin + (Ca^{++} + ½ Mg^{++}) — phytins

The concept of PCMP number as applied to both ungerminated and germinated legumes used in this study were shown in Table 7. Muller (1967) has shown in peas that the hardness of the seed can directly be correlated to higher PCMP number. A similar trend has been found in this study also. Greengram which was easily cookable had the lowest PCMP number while chickpea requiring prolonged cooking, had higher PCMP value. The shortening of cooking time in germinated chickpea and the lengthening of cooking period in greengram and cowpea could be directly correlated with corresponding changes in PCMP number (Table 7).

Work on the chemical characteristics affecting cooking had been primarily on peas and beans (Rosenbaum and Baker, 1969; Crean and Haisman, 1964). While it is relatively easier to explain the chemical characteristics contributing to hardness or softness of dormant or stored seeds (Burr et al., 1968) germinated ones present a more complex system with the interplay of enzymes particularly phytase and pectinase (Belavady and Banerjee, 1953) which affect the solubility of pectins and phytins with a corresponding change in divalent cations like Mg^{++} and Ca^{++}. Though correlation could be derived by the application of PCMP number to explain the sum total of major chemical characteristics, the validity of this concept would need more critical study.

The relative affinity of divalent Ca^{++} and Mg^{++} to phytic and pectic acids perhaps play a primary role in affecting the cookability of legumes. The change in pH, degree of hydration, stability of the complex salts and the cell wall uronic acids may also play a role in the cooking process. Since greengram and chickpea in germinated and ungerminated forms present a totally contrasting cookability pattern, they may be considered as a model system for more detailed study on the interaction of various chemical principles involved in the cooking process.

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M. H. LOVE[1] and L. R. DUGAN JR.
Food Science & Human Nutrition Department
Michigan State University East Lansing, MI 48824

PHYSICOCHEMICAL ASSESSMENT OF SHORT TERM CHANGES IN THE QUALITY CHARACTERISTICS OF STORED INSTANT NAVY BEAN POWDER

ABSTRACT

Lipid oxidation, changes in soluble protein, reducing sugar content, extent of nonenzymatic browning, and product color were monitored for instant navy bean (*Phaseolus vulgaris*) powder during 1 month of accelerated storage at 37°C. Below the monolayer, (a_w 0.11), greater lipid oxidation occurred in neutral lipids and phospholipids of air-packed samples than in nitrogen samples. Indices revealed that monolayer samples browned more and lost more soluble protein than samples stored below the monolayer. Nitrogen-packed samples lost more reducing sugars and had darker colors than air-stored samples at each a_w.

INTRODUCTION

CONSUMER INTEREST in convenience foods and an erosion of the dry bean market spurred researchers to provide an acceptable convenient form of dry beans (Rockland, 1966; Hoff and Nelson, 1966; Bakker-Arkema et al., 1966). Among the products developed was an instant dry navy bean powder which was processed by retort cooking followed by spray drying (Bakker-Arkema et al., 1967). This product was similar to that developed by Morris (1961).

The instant bean powder produced by the drum dehydration process had good reconstitutability, flavor acceptance, a light tan or brown color (rated highly acceptable), and negligible free starch content (Bakker-Arkema et al., 1966). Since the development of instant legume powders, numerous potential markets have opened for their use (White and Kon, 1972) and commercial scale production of instant legume powders have been studied (Kon et al., 1974).

Storage stability studies have had two major foci. One has centered on organoleptic assessment of storage changes (Boggs et al., 1964; Burr et al., 1969; Guadagni et al., 1975). Other studies have focused upon the effects of processing and storage on the rates of physicochemically measured quality deterioration. The latter studies have been limited in scope. Counter (1969) measured changes in four parameters: lipid content, color, protein solubility and total reducing sugar content for retort and atmosphere cooked beans, while Miller et al. (1973) monitored the effects of processing and storage on retention of thiamin, niacin, pyridoxine and folacin. Kon et al. (1971) reported on the effects of processing on in vitro and in vivo digestibility and protein efficiency ratio of various legume products. Kon et al. (1974) reported upon the effects of processing on the quality and vitamin content of legume powders (pinto and California small white beans).

Numerous quality factors are susceptible to change on storage including solubility, color, texture and flavor. Inhibition of these changes is important in the development of an optimum storage environment, temperature, atmosphere and equilibrium moisture content (a_w), for the freshly processed product. This investigation was initiated to measure the effect of storage atmosphere and a_w on related quality parameters. Under accelerated storage conditions (37°C), physicochemical measures were made of changes in the color, protein solubility, reducing sugar content, and lipid content in instant navy bean powder stored in air or nitrogen environment at two a_w's.

EXPERIMENTAL

Beans and bean powder

Michigan Navy Beans, Michelite (*Phaseolus Vulgaris*) were supplied for this study by the Michigan Dry Bean Shippers Association. They were processed in the pilot plant in the Food Science Building into instant bean powder by the retort cooking and drum dehydration process of Counter (1969).

Moisture analysis

Following the AOAC, 13.003 (1960) procedure, a method was established for measuring the moisture content of the instant bean powder. This method (2g sample, 5 hr, 100°C) was used routinely for measuring the moisture content of the instant bean powder.

Protein analysis

Soluble protein was measured by dispersing 5.0g amounts of powder into 10.0 ml of 8M Urea: 1N NaOH (1:1, v/v) which was contained in a 50 ml polypropylene centrifuge tube. After 30 min, the dispersions were centrifuged (10,000 × G for 15 min) and 0.5 ml aliquots of the supernatant were analyzed for protein content by the Biuret procedure (Leggett-Bailey, 1967; Gornall et al., 1949). The percent soluble protein was calculated from this value.

The micro-Kjeldahl method, AOAC, 42.014 (1970) was also routinely used to measure the nitrogen content of whole beans and fresh bean powder. Protein content was calculated by using the factor 6.25.

Lipid analyses

Lipid extractions were made routinely using the procedure of Takayama et al. (1965). The extraction utilized a chloroform: methanol solvent system (2:1, v/v). This procedure produced the total lipid extract which was further analyzed after concentration on a rotary evaporator under reduced pressure. Gravimetric estimates were performed using the 18 hr continuous extraction procedure of Takayama et al. (1965), which utilized benzene:ethanol (68:32, v/v). Values from these analyses provided a check on the total lipid procedure for the completeness of extraction with chloroform and methanol.

Total lipid extracts were separated into major classes by preparative scale thin-layer chromatography on silica gel G (solvent system $CHCl_3$:MeOH: H_2O:NaOH: 65:35:4:0.5; Del Rosario, 1970). The location of the neutral lipid band was facilitated by the use of ultraviolet light and the phospholipid bands were visualized by using Dragendorff's Reagent (Spray #97, Stahl, 1969) for phosphatidylcholine (PC) and ninhydrin (Spray #178, Stahl, 1969) to locate the phosphatidyl ethanolamine (PE). These bands were scraped from the plates, eluted from the solid support with $CHCl_3$:MeOH (4:1, v/v) and the eluates were made to 25 ml in a volumetric flask. Appropriate aliquots of each class were methylated according to the procedure of Morrison and Smith (1964). The fatty acid methyl ester patterns were analyzed for each class on a Varian Aerograph Model 200 gas chromatograph. The analyses were run isothermally over a 0.32 cm × 210 cm stainless steel column containing 15% DEGS on Chromosorb W as a solid support. The pattern distributions were claculated using the height × width at 1/2 height method of area measurement, and the ratio of unsaturated to saturated fatty acid content was calculated for each class. Aliquots of the total lipid extract were analyzed for

[1] Present address: Food & Nutrition Dept., Iowa State University Ames, IA 50011

Table 1—Physicochemical characteristics of freshly prepared instant navy bean powder

Characteristic	Value
Total lipid (%)	2.99[a,b]/2.89[a,c]
Relative fluorescence of total lipid extract	26
Total reducing sugars (%)	0.20[a]
Protein Micro-Kjeldahl (%)	23.2[a]
Browning index	11.1
Color[d]	45.3
Moisture content (%)	6.83

[a] Dry weight basis
[b] $CHCl_3$; MeOH (2:1, v/v)
[c] Continuous extraction—Benzene: Ethanol (68:32, v/v)
[d] Agtron 500M Reading—Relative reflectance measured in blue mode.

Table 2—Gas chromatographic analysis of the fatty acid methyl-esters in three lipid classes of freshly processed instant bean powder

Fatty acid	Percentage Composition by Class[a,b]		
	Neutral lipid (NL)	Phosphatidyl choline (PC)	Phosphatidyl ethanolamine (PE)
16:0	9.81	25:70	29.84
18:0	1.83	2.70	2.43
18:1	11.71	15.70	12.76
18:2	26.15	30.04	33.54
18:3	50.92	25.34	21.43
>18:3	0.55	0.86	1.23

[a] Condit ons: Varian Aerograph 200; 10% DEGS, 0.32 cm X 210 cm stainless steel column; Isothermal analyses; (Temperatures) Injector—220°C; Column—180°C, and Detector—195°C; Gas flow: Air—250 ml/min; hydrogen—30 ml/min; nitrogen—35 ml/min; Flame setting 1, Chart Speed 0.5 cm/min.
[b] Distrib ution by class 2/3 NL, 1/3 PC + PE with PC 1.5 X greater than PE.

phosphorus according to the procedure of Rouser et al. (1966). (The greatest care was exercised to completely evaporate all solvent from the aliquots due to the *EXPLOSIVE* nature of perchloric acid used in the digestion for phosphorus analysis!)

Fluorescence and infrared analysis

Total lipid extracts were routinely monitored for relative fluorescence on an Aminco Bowman Spectrophotofluorometer. The excitation wavelength was set at 350 nm. The emission spectra were scanned from 390 nm to 550 nm. Bands of fluorescing material were separate from the total lipid extract using the thin-layer chromatographic system of Braddock (1970). These bands were analyzed on the spectrophotofluorometer for excitation and emission wavelengths, and their relative emission fluorescence was measured after standardization of the instrument with quinine sulfate. This value was taken as the relative fluorescence intensity (RFI).

Reducing sugars

Sugar extracts from instant bean powder were performed according to AOAC, 2.041 (1960). A 5.0-g sample was used in these analyses, and the quantities of reagent were reduced by one-tenth. Aliquots from the extract were analyzed for reducing sugar content according to the colorimetric procedure of Furuholman et al. (1964).

Browning index

The formation of water soluble brown products was measured according to the method of Choi et al. (1949) as modified by Fishwick and Zmarlicki (1970). The trypsin (1–100, hog pancreas, 4 × pancreatin, Nutritional Biochemical Corp., Cleveland, OH) released the maximum levels of colored products at a concentration of 100 mg trypsin/ml of water. The Browning Index was derived by multiplying the A_{390nm} of the clarified filtrate by 100. Increases in this index implied the formation of water soluble nonenzymatic browning products.

Color analyses

Product color changes were monitored on an Agtron Reflectance Spectrophotometer M-500-A following the procedure of Johnson (1966). A 5.0-g sample of powder was analyzed, and the 44 and 56 disks were used to zero and standardize the instrument. Deterioration in the color of the product was measured as decreases in the blue mode relectance.

Storage conditions

Two relative humidities were selected for the storage study. These were 11% and 23%. At equilibrium, these produced a_w's which were respectively below and at the monolayer moisture value for instant navy bean powder (Love, unpublished). These relative humidities were maintained by use of saturated salt solutions (Rockland, 1960) which had been equilibrated at the storage temperature of 37°C before the study. Under these conditions the target moisture value of 4.0% was reached in the 11% samples by day 4 of the study. The target moisture value of 5.5% was reached in the 23% samples on day 12 of the study.

The product was processed and dried to a final moisture content of 7%. Two lots of fresh powder were randomly selected and placed on large glass dishes into the storage desiccators (vacuum, pyrex, 250 ml) at the appropriate relative humidites. Air and nitrogen atmospheres were compared at each a_w. The desiccators containing an air atmosphere were placed directly into storage. The desiccators containing a nitrogen atmosphere were evacuated with a vacuum pump and returned to atmospheric pressure using compressed prepurified nitrogen. This process was repeated once. The desiccators were then placed immediatedly into storage at 37°C. A control sample was prepared from the same freshly processed powder and held at -20°C under nitrogen during the study.

Design of the study

Initial quality parameters were measured on the freshly prepared product. During storage, the moisture content, soluble protein, browning index, relative reflectance (color), and fluorescence were measured at 4-day intervals. Representative samples were taken from an individual desiccator and the desiccator immediately returned to storage. The nitrogen atmosphere was replenished in the two desiccators before they were returned to storage. At the conclusion of the study, phosphorus, unsaturated to saturated fatty acid (U/S) ratios for all three lipid classes, and reducing sugar analyses were performed on all treatments and compared to the control and initial values.

RESULTS & DISCUSSION

Composition of fresh instant bean powder

Before initiating the storage study, base line values were measured using the methods to be employed during the storage period. These values are presented in Table 1. The protein values and other composition data agree with that of Counter (1969). The lipid values by either extraction technique are slightly lower than those reported by Takayama et al. (1965); however, these values were measured on whole beans which had been processed into powder while the literature values were reported on extracts of only the cotyledonous portions of the beans.

Lipid analyses

The fatty acid patterns for the various classes of lipids are presented in Table 2. The unsaturated fatty acid content was very high in linolenic acid (18:3) which is typical of bean lipids. The neutral lipid fraction contained greater than fifty percent linolenic acid. This fact agrees with a report by Buttery (1975). Takayama et al. (1965) did not find $C_{18:3}$ fatty acid in the phospholipid fraction from the Michelite beans he extracted. They proposed that the phospholipases in these legumes destroyed the linolenic acid content during a soaking process which had been employed to remove the seedcoats. There was no evidence that linolenic acid had been

Table 3—Characteristics of instant navy bean powder lipids after storage at 37°C

Sample	Days in storage	U/S ratios NL[a]	PC[b]	PE[c]	μg P/g powder in (TL) total lipid extract DWB	Relative Fluorescence intensity Highest (Day)	Final
Fresh	0	8.55	2.11	2.52	298	26(0)	–
0.11, Air[d]	28	7.49	2.03	2.25	274	31(4)	23.4
0.11, N_2	28	8.09	2.09	2.37	284	30(4)	24
0.23, Air	29	7.26	1.91	2.46	266	50(23)	23
0.23, N_2	29	7.50	2.07	2.48	287	42.5(23)	27
Control	28	8.57	2.11	2.50	288	–	26

[a] Neutral lipids
[b] Phosphatidyl choline
[c] Phosphatidyl ethanolamine
[d] a_w, Atmosphere

Table 4—Changes in physicochemical parameters of instant navy bean powder during storage at 37°C

Sample	Days in storage	Relative Reflectance[a,d]	Soluble protein[b,d]	Reducing sugar content[b]	Browning index[d]
Fresh	0	45.3	23.9	0.202	11.1
0.11, Air[c]	28	33.8	21.8	0.153	13.0
0.11, N_2	28	29.8	21.4	0.141	12.3
0.23, Air	29	35.0	19.9	0.136	12.6
0.23, N_2	29	32.5	22.1	0.069	15.2
Control	28	45.1	24.0	0.203	11.0

[a] Agtron 500M values measured in blue mode
[b] g/100g powder, DWB
[c] a_w, Atmosphere
[d] Average of five samples

destroyed in the processing of the bean powders used in this study.

The ratio of unsaturated to saturated fatty acids (U/S) was used as an index of oxidative deterioration of the lipids in stored bean samples (Table 3). This is a modification of the polyunsaturated to saturated ration (P/S) developed by Buttery et al. (1961). These data indicate that lipid oxidation occured in the neutral lipid of all stored product. The samples stored in a nitrogen atmosphere were slightly protected at the lower a_w. There was more extensive oxidation of the neutral lipids at the higher a_w. At both a_w's the PC in air was oxidized to the greatest extent. The P data follow the same trend as the U/S ratio. Buchanan (1969) showed a similar loss of extractable phospholipid during storage studies on dry leaf protein concentrate. Insufficienct numbers of replicates were analyzed (only 2/treatment) to comment on the statistical significance; nevertheless, this trend is indicated by the data.

Spectral data

Relative fluorescence intensity (RFI) analysis of oxidation products revealed that lipid soluble browning products formed in all samples as shown by increases in the RFI for each treatment. Chromatographic separation of the extract revealed two families of fluorescing compounds. One band with R_f of 0.58 had an emission of 450 nm, while the other band with a R_f of 0.91 had an emission of 445 nm.

Infrared analyses of these products showed only minor peaks. These bands corresponded to 1748 cm^{-1}, 1710 cm^{-1}, 1565 cm^{-1}, 1465 cm^{-1}, and 1385 cm^{-1} which are part of the IR spectral characteristics of fluorescing Schiff base compounds. The 1710 cm^{-1} and the 1565 cm^{-1} bands were cited as part of the condensed Schiff base system. The 1748 cm^{-1} band is for carbonyl stretching. These data are consistent with structural features reported by Malshet and Tappel (1973), and the reports of Braddock (1970) and Dugan and Rao (1972) indicating the development of fluorescing condensed Schiff base compounds extracted from autoxidizing dry model systems of lipid and protein.

The rates for the development of maximum RFI imply that autoxidation was more rapid at the lower a_w which implies a possible protection of the lipid by the water phase of the powder. At both a_w's the extent of RFI development was slightly greater in the air-stored samples.

Quality parameter changes

A composite of changes in the indices for the 30 days of the study are presented in Table 4. Deterioration of the quality of the product was observed as measured by all indices. The products browned as shown by decreases in relative relectance data and increases in the browning index for all treatments. In agreement with the reports of model system studies by Labuza et al. (1970) and the studies of Lea and Hannan (1949) and Lea and White (1948), samples stored at the higher a_w browned to a greater extent than those at the lower a_w. The greater amounts of water at the higher a_w likely served as a solvent to promote the reactions producing ultimately, brown pigments. The samples at higher a_w had a higher browning index for samples stored under nitrogen. This extent of browning under a nitrogen atmosphere is not surprising considering the browning values reported by Fishwick and Zmarlicki (1970) for freeze-dried turkey at 5.0% moisture.

The data on reducing sugars indicate that the greatest losses occured in treatment 0.23, N_2. Both samples at this a_w had greater losses of reducing sugar than the samples stored at 0.11 a_w. These trends agree with those reports of Labuza et al. (1970); Lea and Hannan, (1949); and Lea and White, (1948) and support the concept of water promoting the reaction between carbonyl compounds and free amino groups (e.g. ϵ-NH_2 of lysine).

Measurable losses of soluble protein were recorded during the study. The treatment at 0.23 a_w in air showed the greatest losses of soluble protein. The combined effects of greater lipid oxidation and sugar amine browning likely were the factors contributing to these losses. The greater losses of reducing sugar in the 0.23, N_2 treatment are likely the source of the higher Browning Index value for this treatment; however, these processes were not as detrimental to protein solubility as were the carbonyls from lipid oxidation. Clark and Tannenbaum (1970, 1974) have shown that numerous reducing sugars are associated with the amine groups of browned protein (ratio NH_2 : sugar, 1:4). These products would likely be more soluble in the aqueous media than condensation products formed from nonpolar, lipid derived carbonyls.

The ultimate concern of this study was the consequences of these measured changes on the quality of stored instant bean powder. These data substantiate the organoleptic analyses of Guadagni et al. (1975); Boggs et al. (1964) and Burr et al. (1969). In those studies, the air-packed samples were also less stable than nitrogen-packed samples. Off-flavors were reported in samples stored for comparable storage periods at 37°C. Buttery et al. (1961) reported similar changes in U/S ratios for products stored under comparable conditions.

Water activity, as shown here, was important in affecting the stability of the powder. At 5.23% moisture and an a_w of 0.23 there was extensive protein insolubilization, lipid oxidation, and browning during storage in air at 37°C for a month. Storage below the monolayer at 4.0% moisture (a_w0.11) resulted in lipid oxidation but less sugar amine browning.

The use of an inert atmosphere (i.e. nitrogen) and a storage temperature of 22°C was shown by Burr et al. (1969) to provide stability for at leat 1 yr, when an antioxidant (butylated hydroxytoluene) was also used. Nitrogen atmospheres were not capable of totally protecting the samples in this study. Guadagni et al. (1975) theorized that flavored legume based soups with Q_{10} values of 1.4 for nitrogen-packed samples would be stable for 1 yr at 37°C. Based on data in this study, the flavorings must be a significant factor in preventing the perception of off-flavors.

Data in this short-term study reveal that changes in product quality of instant navy bean powder begin at an early stage in storage and that lipid oxidation and browning reactions are dominant in the chemical changes which prevail. It appears that these products should be maintained at low moisture levels and the use of suitable antioxidants should be considered in order to minimize quality changes.

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M. L. SWARTZ,[1] R.A. BERNHARD and T.A. NICKERSON
Dept. of Food Science & Technology, University of California, Davis, CA 95616

INTERACTIONS OF METAL IONS WITH LACTOSE

ABSTRACT

Binding between lactose and metal salts was investigated. When lactose combines with a salt in aqueous solution, a complex is formed with a specific rotation different from that of pure lactose. Lactose combined with the metal salts in a ratio of one to one for all the cations studied (Ca^{++}, Ba^{++}, Sr^{++}, Fe^{++}, Mg^{++}, Mn^{++}, Zn^{++}, Na^{+} and Li^{+}) except for K^{+} and NH_4^{+} where there is no apparent binding. The specific rotation of complexes was determined by saturating a lactose solution with salts until 100% of complex was formed, then the amount of complex in various mixtures was calculated from the observed rotation. Equilibrium constants were found to be 0.205 for lactose with Ca^{++}, 0.379 for lactose with Li^{+}, and 0.527 for lactose with Na^{+}. Variation in pH from 2.0–6.5 had no effect on the amount of complex formed. Complex formation decreased with increased ionic strength.

INTRODUCTION

THE PRESENCE OF SALTS alters certain properties of sugar solutions. One such property is the increased solubility of lactose when salts are present. Herrington (1934a) hypothesized that the increased solubility of lactose when $CaCl_2$ is added is due to the formation of a lactose-$CaCl_2$ complex which he was later able to isolate in crystalline form. This complex is more soluble than lactose alone (Jensen et al., 1940). Formation of soluble calcium-lactose complexes also has been suggested as a hypothesis to explain the enhanced intestinal absorption of calcium that is observed in the presence of lactose (Bergeim, 1926; Ali and Evans, 1973).

Several crystalline lactose-salt complexes have been isolated and described: lactose-$CaCl_2 \cdot 7H_2O$ (Cook and Bugg, 1973; Herrington, 1934b). lactose-$CaBr_2 \cdot 7H_2O$ (Bugg. 1973: Bugg and Cook, 1972), and lactose-$CaI_2 \cdot 3H_2O$ (Jensen et al., 1940). Though the stoichiometric ratios of the complex can be determined for the crystalline forms, the same ratios are not necessarily present in solution. Complexing would be expected to alter the properties of both the lactose and the salts in solution.

Determination of the stoichiometric ratio of the sugar-salt complex

Job (1928) devised a method to determine the ratio in which individual components combine to form a complex in solution. The principle as described by Rendleman (1966) is as follows: "If equimolar solutions of two complexing solutes are mixed in different proportions, the concentration of the complex is generally a function of the proportion in which the solutions have been mixed. The concentration of the complex is maximal when the solutions are mixed in the proportion as that in which the simple components are present in the complex. The position of the maximum is a function of the molar ratio of salt to complexing agent and is independent of both the combined concentration and the concentration of the original solutions (prior to mixing)."

In order for this method to be useful, the complex must have a measurable quality which is different from the components alone. Although there are many qualities that could be used, Job used the change in the refractive index as a result of mixing the components. Wilkund (1955) used the change in the rotation of plane polarized light to find the most favored combinations of sucrose with a salt.

Using the polarimeter to study complex formation

Heinrich Trey (1897) was probably the first to study extensively the effects of salt on the rotation of light by lactose. All the salts he studied influenced the rotation of lactose; some causing an increase in rotation ($BaCl_2$, $Ca(NO_3)_2 \cdot 4H_2O$, KI, $NaNO_3$, $MgCl_2 \cdot 6H_2O$, $BaCl_2 \cdot 2H_2O$, $HgCl_2$, NaCl and NaBr) and others a decrease (KCl, $K_2SO_3 \cdot 2H_2O$ and NH_4Cl).

Herrington (1934a) found that increasing either the lactose or the salt concentration subsequently gave greater and greater deviations from the expected optical rotation. He considered that this supported the theory that lactose forms compounds with salts in solution. Later, Vavrinecz (1962) demonstrated that there is a regular pattern in the change in rotation when salts are present which agrees with Herrington's theory on complex formation.

Effect of cations and anions on rotation

Both the cation and anion of a salt affect the formation of a carbohydrate-salt complex. With sucrose it appears that the stoichiometric ratio of the complex is determined by the cation. Of the salts tested with sucrose by Job's method, salts with similar cations peaked at the same mole fraction of sucrose indicating similar combining ratios. The anions had varied effects on the rotation of the solutions, therefore they probably play an important role in the amount of complex formed (Wilkund, 1955). Jensen et al., (1940) showed that some anions were more effective than others in increasing the solubility of lactose. Therefore, if increased solubility is due to compound formation (as Jensen et al. postulated), then it can be deduced that the anion of the salt influences the amount of lactose complex formed.

Ramaiah and Vishnu (1959) determined approximate specific rotation values for sucrose- and fructose-salt complexes. They mixed the sugar with increasingly higher concentrations of salt. Eventually a point was reached where the addition of more salt to the sugar solution caused no further change in rotation. This suggested that all the sugar had been complexed. From these data, Ramaiah and Vishnu determined the specific rotations of the sugar-salt complexes.

In milk and milk products and in food systems in general, lactose is present along with calcium and other soluble salts. Complexing of the lactose and the metal ions would affect the properties of the lactose and the activities of the ions, which could in turn affect other food constituents, e.g., proteins. The purpose of this study was to determine whether lactose-salt complexes could be demonstrated and quantitatively estimated in aqueous solutions.

EXPERIMENTAL

Preparation of lactose solutions

The required amount of U.S.P. grade lactose was weighed out and then transferred to a volumetric flask. The flask was partially filled with hot distilled water (70–90°C) in order to dissolve the lactose rapidly and to assure that mutarotation equilibrium would be established quickly. The following day, 18 hr minimum, the flask was made up to the mark with distilled water at 25°C. The solution was thoroughly mixed before using.

[1] Present address: Dynapol, Palo Alto, CA 94304

Preparation of salt solutions

Twelve different salts were used in this study: $CaCl_2$, $Ca(NO_3)_2 \cdot 6H_2O$, $BaCl_2 \cdot 2H_2O$, $SrCl_2 \cdot 6H_2O$, $MgCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $ZnCl_2$, $FeCl_2 \cdot 4H_2O$, NaCl, LiCl, KCl and NH_4Cl. These salts were commercial reagent grade chemicals. Salt solutions were prepared using distilled water. All the salts formed a clear solution within a few minutes except for $ZnCl_2$ which forms a hydroxide which precipitates at approximately pH 6. To dissolve this precipitate, sufficient HCl was added to adjust the pH to 3. After the clear salt solution was prepared, it was made up to volume with distilled water.

Optical rotation measurements

The optical rotations of all solutions were measured using a Perkin-Elmer Model 141 Auto-Polarimeter with an accuracy of ±0.002°. The temperature was maintained at 25°C. Duplicate measurements of each solution were made. If these values were within 0.004° of each other, they were averaged. If the two values did not agree within these limits, the measurement was repeated until the agreed limits of precision were achieved.

pH Measurements

The pH of all solutions was measured using a Beckman Model 4500 digital pH meter immediately following the polarimetric determinations.

Equilibration of the complexing reaction

Equal parts of 0.6M solutions of lactose and salt were mixed. This solution was immediately put into a polarimeter tube and readings were taken every 5 min for an hour. Hourly readings were then made until it appeared that the solutions had equilibrated.

Stoichiometric ratio

Solutions of 0.6M lactose and 0.6M salt were mixed in suitable ratios to give eleven solutions from zero to 1.0 mole fraction in 0.1 mole fraction intervals. Six replicates were prepared for each solution.

Specific rotation of the lactose-salt complex

Increasing quantities of each salt were added to small amounts of lactose to achieve 100% complex formation (Table 3). Water was added so that the flask was partially filled. The flasks were swirled until the salt dissolved, then additional water was added to bring the solution to the mark. The solutions were thoroughly mixed and allowed to remain overnight at 25°C. The optical rotation was measured the following day.

Equilibrium constant

Mixtures were prepared from 0.6M lactose and 0.6M salt solutions. Three replicates were made for each of the determinations. The optical rotation measurements were made the following day.

Effect of pH and ionic strength on complex formation

Specific solutions were prepared to determine whether differences in pH or ionic strength of the solutions affected complex formation as measured by optical rotation. To one series of flasks containing 50 ml of 0.6M lactose and 3.33g $CaCl_2$ varying amounts of 0.1M HCl or 0.1M KOH were added to adjust the pH between 2.0 and 6.5. In another series various amounts of KCl were added. The solutions were mixed thoroughly and polarimeter readings made the following day.

Quantitative information

When lactose and a salt are mixed, it may be postulated that they combine to form a complex. This reversible reaction can be stated as

$$mL + nS \rightleftharpoons p(L_mS_n) \quad (1)$$

Where L stands for free lactose, S for free salt, LS for the complex, L_t for the total lactose, and S_t for the total salt. The combining ratios are expressed by m,n and p. When this reaction is at equilibrium,

$$\frac{[L_mS_n]^p}{[L]^m[S]^n} = K \quad (2)$$

is true where the concentrations of the components are in moles per liter and K is the equilibrium constant. Eq (2) can be restated as

$$\frac{[L_mS_n]^p}{([L_t] - [L_mS_n])^m([S_t] - [L_mS_n])^n} = K \quad (3)$$

In Eq (3), there are three unknowns: the equilibrium constant, the stoichiometric ratio and the concentration of the complex at a given L_t and S_t. If the last two values can be determined, then the remaining unknown value, K, can be found by substitution of known values into the equation.

It is possible to determine the stoichiometric ratio by Job's method as previously described. It is also possible to determine the concentration of the lactose-salt complex. Consider that the observed rotation of a solution is equal to the sum of the individual components' rotation, i.e.,

$$R_{observed} = R_L + R_{LS} + R_S \quad (4)$$

where R is the optical rotation. The optical rotation due to salt is zero since salts are symmetrical. The rotation of a single component is equal to $[\alpha] \times C \times 1$ where $[\alpha]$ is the specific rotation of the component (in degrees-liter/dm-mole), C is the concentration of that component (in moles/liter) and 1 is the length of the polarimeter cell (in dm). Therefore,

$$R_{observed} = ([\alpha]_L \times [L] \times 1) + ([\alpha]_{LS} \times [LS] \times 1) \quad (5)$$

The amount of free lactose is equal to $[L_t] - [LS]$ so

$$R_{observed} = [\alpha]_L \times ([L_t] - [LS]) \times 1 + [\alpha]_{LS} \times [LS] \times 1 \quad (6)$$

or

$$R_{observed} = [\alpha]_L \times [L_t] \times 1 + ([\alpha]_{LS} - [\alpha]_L) \times [LS] \times 1 \quad (7)$$

The unknowns in Eq (7) are the specific rotation and the concentration of the lactose-salt complex. If the specific rotation of the complex could be determined, then the concentration could be deduced.

By the above method, the specific rotation of the lactose-salt complex can be determined. By substitution of $[\alpha]_{LS}$ into Eq (7), the concentration of the complex can be found. By substitution of [LS] into Eq (3), with the appropriate numbers for m,n and p, the equilibrium constant for the lactose-salt reaction can be determined.

After finding K, the change in free energy can be calculated by this formula:

$$\Delta G = -R\,T \ln K \quad (8)$$

where ΔG = change in free energy; R = gas constant; and T = absolute temperature. By comparing the K's and the ΔG's for various salts, trends in the binding abilities of the cations can be deduced.

RESULTS & DISCUSSION

Equilibration of the complexing reaction

By the method described above, all solutions of lactose and salt equilibrated within 3 hr.

Stoichiometric ratio

The observed optical rotations of lactose-salt mixtures were subtracted from values for similar solutions which were salt free to give ΔR. The mean optical rotation was determined from six replicate values for each lactose mole fraction. The 60 data points of ΔR vs mole fraction lactose for each lactose-salt mixture were analyzed using a computer programmed for a parabolic regression. Typical data are presented in Figure 1 which shows the experimental means and the calculated curves for three of the lactose-salt mixtures. Table 1 gives the results of the mathematical analysis. The F value is an indication of how well the data points follow the calculated curve. An F value of 7.17 or higher indicates that the data points are statistically significant at the 0.01 level. The maximum peak values, as defined by the curves, are shown under vertex.

The use of the refractive index was also studied as the measurable parameter to determine the stoichiometric ratio. A plot of observed refractive index vs mole fraction lactose gave a linear relationship; the refractive index increased proportionally to the mole fraction of salt. The salt completely covered any response from lactose or the lactose-salt complex.

Table 1—Mathematical analysis of nonlinear regression

Salt	Equation for curve[a] $y = ax^2 + bx + c$ a	b	c	Standard errors for the coefficients a, b, c Sa	Sb	Sc	F[b]	Vertex
$CaCl_2$	−0.430	0.432	−0.002	0.009	0.009	0.001	1137	0.502
$Ca(NO_3)_2$	−0.421	0.427	−0.005	0.012	0.012	0.001	686	0.507
$SrCl_2$	−0.427	0.423	−0.001	0.010	0.010	0.001	1010	0.495
$BaCl_2$	−0.399	0.404	−0.004	0.010	0.010	0.000	864	0.506
$MgCl_2$	−0.137	0.138	−0.002	0.005	0.005	0.000	352	0.504
$ZnCl_2$	−0.152	0.153	−0.002	0.006	0.006	0.000	376	0.503
$FeCl_2$	−0.174	0.168	+0.001	0.006	0.006	0.000	419	0.483
$MnCl_2$	−0.112	0.111	−0.001	0.005	0.005	0.000	231	0.496
NH_4Cl	−0.019	0.012	+0.001	0.006	0.006	0.000	14	0.792
KCl	−0.017	0.019	−0.003	0.005	0.005	0.000	8	0.559
NaCl	−0.124	0.127	−0.003	0.005	0.006	0.000	258	0.512
LiCl	−0.090	0.092	−0.003	0.006	0.006	0.000	126	0.511

[a] y = expected change in optical rotation; x = mole fraction of lactose standard.
[b] F = rat o of dispersion of the sample means about the grand mean to the dispersion of the varieties within the samples about their respective sample means.

This method was not sufficiently sensitive for determining complex formation in lactose-salt mixtures in the presence of high salt concentrations.

Each salt gave a curve with a different amplitude. Since the change in optical rotation is a function of the specific rotation of the complex as well as the concentration of the complex, a large change in optical rotation does not necessarily indicate more complex formation.

The curves indicate a binding ratio of 1:1 rather than a higher multiple (2:2, 3:3, etc.). The data points closely approximate the theoretical plots which were derived for a ratio of 1:1. For the salts which complex with lactose, the F values given in Table 1 are all significantly greater than 7.17. This indicates the data follow the 1:1 binding curve at the 0.01 level. The curves would be narrower if a higher multiple of complex were formed.

Note that changing the anion of the calcium salt from Cl^- to NO_3^- gave no significant change in observed rotation.

Interestingly enough the data points for lactose with KCl give a straight line defined by linear regression as

$$y = 0.001x + 0.000$$

where y is the expected optical rotation and x is the mole fraction lactose. This is essentially a horizontal line which indicates no complex formation.

It was observed the K^+ had a significantly larger radius/charge ratio than any of the other cations studied (Table 2). A hypothesis was proposed which suggested that K^+ was too large and had too small an attractive force to bind with the lactose. In order to test this hypothesis, NH_4Cl was examined in the same manner as KCl. NH_4^+ and K^+ have similar radius/charge ratios while other physical properties are dissimilar.

NH_4Cl gave identical results to KCl—no binding was indicated. Linear regression of the data points for lactose plus NH_4Cl gave a line defined as

$$y = -0.004x + 0.003$$

where y is the expected optical rotation and x is the mole fraction lactose.

Therefore, it appears that size and charge can determine the extent of lactose binding with cations. It is proposed that NH_4^+ and K^+ exhibit such a weak electronic interaction with lactose that no binding will occur.

Table 2—Ion sizes

Cation	Radius (Å)	Radius/charge
Li^+	0.60	0.60
Mg^{++}	0.65	0.33
Zn^{++}	0.74	0.37
Fe^{++}	0.75	0.38
Mn^{++}	0.80	0.40
Na^+	0.95	0.95
Ca^{++}	0.99	0.50
Sr^{++}	1.13	0.57
Ba^{++}	1.35	0.68
K^+	1.33	1.33
NH_4^+	1.42	1.42

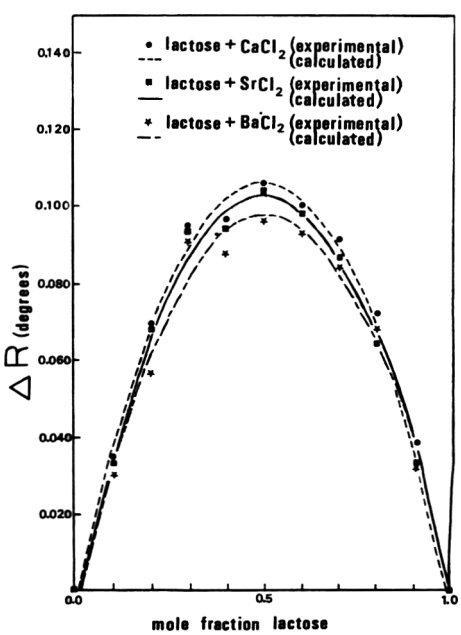

Fig. 1—Change in optical rotation vs mole fraction lactose for $CaCl_2$, $SrCl_2$ and $BaCl_2$.

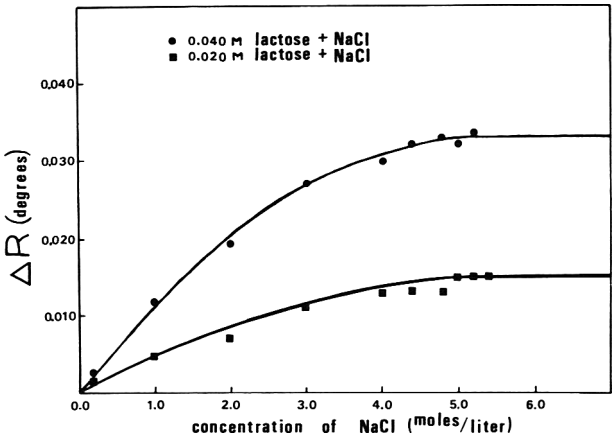

Fig. 2—Change in optical rotation vs concentration NaCl.

Table 3—Specific rotation determinations for NaCl, LiCl and $Ca(NO_3)_2$

Salt	Lactose conc (molar)	Apparent rotation (degrees)	Specific rotation (°L/dm-m)
NaCl	0.040	0.790	19.7
	0.020	0.395	19.7
LiCl	0.040	0.789	19.7
	0.020	0.394	19.7
$Ca(NO_3)_2$	0.020	0.479	24.9
	0.015	0.375	25.0

Table 4—Equilibrium constant values for NaCl, LiCl and $Ca(NO_3)_2$

Conditions		K	K	K
Lactose	Salt	NaCl	LiCl	$Ca(NO_3)_2$
0.30M	0.30M	0.544	0.390	0.204
0.15M	0.45M	0.501	0.358	0.199
0.45M	0.15M	0.531	0.384	0.210
0.10M	0.50M	0.537	0.400	0.210
0.50M	0.10M	0.521	0.364	0.201
Mean value		0.527 ± .015	0.379 ± .016	0.205 ± .005

Table 5—Amount of binding between lactose and various salts

Combination	Amt of lactose bound (M)	Mole %
NaCl + lactose	0.037	12
LiCl + lactose	0.028	9.3
$Ca(NO_3)_2$ + lactose	0.016	5.3

Table 6—Free energy values for lactose-salt interactions

Combination	ΔG (cal/mole)
Lactose + NaCl	379
Lactose + LiCl	574
Lactose + $Ca(NO_3)_2$	938

Specific rotation of the lactose-salt complex

The optical rotation for the lactose solution was subtracted from the observed readings for the lactose-salt solutions to give ΔR. ΔR values were plotted vs concentration of salt. Curves were drawn for best fit by approximation. Typical results are shown in Figure 2.

The specific rotations of the complexes were determined using the following equation:

$$[\alpha]_{LS} = \frac{R_{LS}}{l \times [LS]}$$

where $[\alpha]_{LS}$ is the specific rotation of the lactose-salt complex in degrees-liters/dm-mole, R_{LS} is the observed optical rotation of the pure complex in degrees, l is the length of the polarimeter tube in dm and [LS] is the concentration of the complex in moles/liter. The results are shown in Table 3.

Only NaCl, LiCl and $Ca(NO_3)_2$ were studied with lactose because most of the other salts were not sufficiently soluble to reach the point where no further change in optical rotation could be observed. The ferrous chloride was very soluble, but at high concentrations the solutions were highly colored making it impossible to obtain accurate polarimetric readings.

For the experiments using $Ca(NO_3)_2$, the concentrations of lactose were reduced to permit a higher lactose to $Ca(NO_3)_2$ ratio. This was necessary to observe the plateau. This indicates that the amount of Ca^{++}-lactose complex formed is less than for the case of Li^+ and Na^+ since it takes a higher ratio of salt to lactose to achieve total complexing of lactose.

Equilibrium constant

For each mixture, the mean optical rotation of three replicates was substituted into Eq (7): where $R_{observed}$ is the optical reading of the lactose-salt mixture; $[\alpha]_L \times [L_t] \times l$ is the reading of the lactose-water mixture; $[\alpha]_{LS}$ is the specific rotation of the complex; $[\alpha]_L$ is the specific rotation of lactose; and [LS] is the amount of complex (now the only unknown) formed at a given L_t and S_t. Solving for [LS] and substituting into Eq (3) gives K, the equilibrium constant:

$$K = \frac{[LS]}{([L_t] - [LS])([S_t] - [LS])} \quad (9)$$

Table 4 gives the resulting K values. For each lactose-salt combination, the mean of five values was obtained and the standard deviation determined. For all three salts, the five values obtained for K were quite constant as would be expected for a true equilibrium reaction.

The equilibrium constants indicate that only a small fraction of the lactose is bound. In solutions of 0.3M lactose and 0.3M salt, the amount of binding is shown in Table 5.

NaCl is the most effective chelating salt followed by LiCl and $Ca(NO_3)_2$. No trends in the binding ability of cations can be stated since only these three cations were studied.

It is interesting that lactose and $Ca(NO_3)_2$ do not show a large amount of chelation. Assuming that this model system of lactose and Ca^{++} can be applied to milk, it indicates that only a small amount of the total calcium in milk is complexed. There are approximately 30 μM of calcium in 1 L of milk; of this, only 10 μM of the calcium is in soluble form. Milk also contains 50g of lactose per liter or 0.14M. If these concentrations were duplicated in solution, only 3% of the soluble calcium would be bound (1% of the total calcium). It is questionable whether this small amount of binding could be responsible for an increase in calcium metabolism, as has been hypothesized.

Gibbs free energy

The free energy (Table 6) was calculated by substituting the equilibrium constant, K, into Eq (8).

Table 7—Range of pH values for lactose-salt solutions

Salt	pH Range of lactose-salt solns
$CaCl_2$	4.20–6.21
$Ca(NO_3)_2$	4.01–5.65
$SrCl_2$	4.10–4.94
$BaCl_2$	3.81–4.51
$MgCl_2$	4.03–5.14
$ZnCl_2$	2.75–4.38
$FeCl_2$	2.54–4.60
$MnCl_2$	3.91–4.75
NH_4Cl	4.12–4.55
KCl	4.32–4.78
NaCl	4.02–4.32
LiCl	4.20–6.29

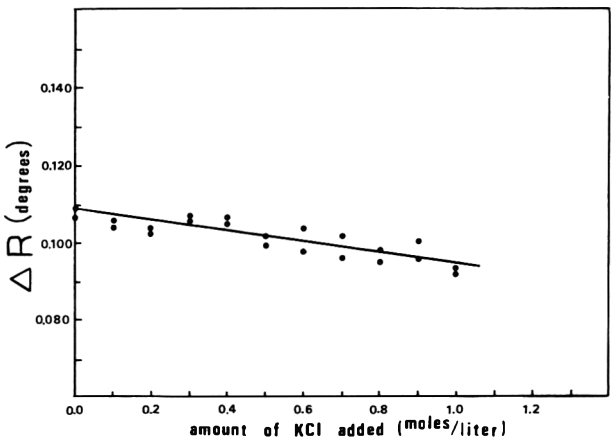

Fig. 3—Change in optical rotation vs concentration of KCl in a lactose-$CaCl_2$ mixture.

Effect of pH on complex formation

Depending on the nature of the added salt and the respective concentrations of salt and lactose, the pH values for each solution prepared generally were in the range of pH 4–6. The pH values of all experimental mixtures are presented in Table 7.

It should be noted that KOH was chosen as the added base because the K^+ did not bind with lactose. Also, the ionic strength was not significantly increased due to the addition of acid or base. The maximum increase in ionic strength was 0.05.

To determine the influence of pH, complexing in $CaCl_2$-lactose solutions was measured where pH ranged from 2.0–6.5. The value of the optical rotation of pure lactose was subtracted from that of each lactose-$CaCl_2$ mixture at the same pH values. The data points (values of ΔR vs pH) were analyzed by linear regression. There is no effect on the amount of complex formed between lactose and $CaCl_2$ in this pH range (slope 0.000, standard error 0.002).

Effect of ionic strength on complex formation

The values of the optical rotations from the lactose-KCl mixtures were subtracted from the lactose-KCl-$CaCl_2$ readings. These experimental values (ΔR vs conc of added KCl) were analyzed by linear regression. The line best representing these data points is:

$$y = -0.014x + 0.109$$

where y is the calculated optical rotation value and x is the amount of KCl added in moles/liter. The standard error was 0.002. These results are shown in Figure 3.

Increasing the concentration of $CaCl_2$ in a lactose solution increases the ionic strength yet does not interfere with the equilibrium relationship in complexing, i.e., increasing the ionic strength by adding a binding salt does not decrease complex formation. This is confirmed by the stability of equilibrium constant values at high and low salt concentrations (Table 4).

The addition of ions which do not bind appears to interfere with the chelation of salt and lactose. For example, the addition of 1.0M KCl to the lactose-$CaCl_2$ solution increased the ionic strength by 1.0 and decreased complex formation by 13%.

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TERUO MIYAZAWA and YASUHIKO FUJINO
Dept. of Agricultural Chemistry, Obihiro University, Obihiro, Japan

CHEMICAL CONSTITUTION OF N-ACYLETHANOLAMINEPHOSPHATIDES IN PEA SEED

ABSTRACT

N-Acylphosphatidylethanolamine (APE) and N-acyllysophosphatidylethanolamine (ALPE) were isolated from pea seed and studied on the chemical constitution. The major component fatty acids of APE and ALPE were palmitic, oleic and linoleic acids. In both lipids the N-acyl groups contained the same component acids, being more saturated than the O-acyl groups. The 2-C position of APE contained almost entirely unsaturated acids, and the 1-C position mostly saturated acids. The 1-acyl isomer of ALPE was richer in saturated fatty acids than the 2-acyl isomer. The main molecular species of APE were 1-palmitoyl-2-linoleoyl, 1-palmitoyl-2-oleoyl, 1-oleoyl-2-oleoyl, 1-oleoyl-2-linoleoyl and 1-linoleoyl-2-linoleoyl types. N-Palmitoyl (or oleoyl and linoleoyl) glycerophosphorylethanolamine, which was the skeletal component of APE and ALPE, was confirmed as their TMS ether derivatives by GC-MS.

INTRODUCTION

IN THE LAST SEVERAL YEARS, studies on minor constituents in foodstuffs have become active, and the information has accumulated gradually as to whether the compounds have a role and/or function in food. It is known that some glycerolipids and glycerophospholipids give significant effects on the nature of foods in manufacturing, processing and storage steps. In our investigation, reported in this paper, acylethanolaminephosphatides, minor components in foodstuffs, were isolated from pea seed and characterized in detail for the structure, the molecular species and their componential relationship. Since the first demonstration of N-acylphosphatidylethanolamine (APE) and N-acyllysophosphatidylethanolamine (ALPE) in soft wheat flour (Bomstein, 1965), this class of lipids has been found to be distributed in plant seeds such as soy bean (Aneja et al., 1969), pea seed (Quarless et al., 1968), spring bean and oat (Dawson et al., 1969). However, the structural characteristics of those lipids are not always clarified (Aneja et al., 1969). Further, APE has been isolated from pea seed (Dawson et al., 1969), but so far no ALPE. Up to now, the natural existence of ALPE has been confirmed only in soft wheat flour (Bomstein, 1965; Clayton and Morrison, 1972).

EXPERIMENTAL

Isolation of APE and ALPE

Total lipid (49.2g), extracted from mature pea seed (*Pisum sativum* L., 2kg) as indicated in our previous paper (Miyazawa et al., 1974), was chromatographed on a silica gel column (Rouser et al., 1967) to obtain a crude phospholipid fraction (21.2g). This fraction was applied to a silica gel column and eluted first with chloroform to remove pigments and nonphosphatides, and with chloroform-methanol (90:10, v/v) to obtain the N-acylethanolaminephosphatide fraction (2.3g). Rechromatography of the fraction on a silicic acid column by stepwise elution with each 100 ml portion of chloroform-methanol (99:1, 98:2, 97:3, 96:4 and 95:5, v/v), followed by preparative silica gel TLC with chloroform-methanol-water-conc NH_4OH (130:60:6:3.4, v/v/v/v), yielded purified APE (Rf 0.79, 89 mg) and ALPE (Rf 0.68, 31 mg). N-Palmitoylphosphatidylethanolamine used as a standard was prepared from pea phosphatidylethanolamine (Miyazawa and Fujino, 1976) and palmitoylchloride by a chemical condensation (Dawson et al., 1969).

Mild alkaline hydrolysis

About 15 mg each of APE and ALPE were incubated for 20 min at 37°C in 12 ml of methanolic 0.2N KOH. The mixture was added to 8 ml of ethanol-water (1:1, v/v) acidified with conc HCl and extracted twice with 10 ml each of hexane and three times with 15 ml each of chloroform. Fatty acids in hexane extracts were converted to methylesters and analyzed by GC. The chloroform extracts were evaporated to dryness and applied to a silica gel column to obtain the partially deacylated product (N-acylglycerophosphorylethanolamine), which was eluted with chloroform-methanol (1:1, v/v).

Acetolysis

About 10 mg each of APE and ALPE were applied to the acetolysis (Renkonen, 1965) to obtain monoacetyldiglyceride and diacetylmonoglyceride, respectively.

Spectrometric analysis

IR spectra were taken with a Nippon Bunko Kogyo IR-G infrared recording spectrophotometer, using KBr pellets. NMR spectrum was recorded in a Hitachi R-24A high resolution NMR spectrometer (60 MHz) in $CDCl_3$.

GC-MS of TMS ethers of N-acylglycerophosphorylethanolamine

N-Acylglycerophosphorylethanolamine (3 mg) was silylated with 0.3 ml of pyridine and 0.4 ml of bis(trimethylsilyl)acetoamide containing 10% trimethylchlorosilane at room temperature for 1 hr. The GC-MS was carried out by a Hitachi RMU-6MG gas chromatograph-mass spectrometer on a glass column (50 × 0.3 cm) packed with Diasolid ZT. Carrier gas was He (1.2 kg/cm²) and column temperature was programmed from 200–280°C at a rate of 5°C/min; molecular separator and ion source were maintained at 290°C and 210°C, respectively. Ionizing voltage was 20 eV and trap current was 70μA.

Analysis of component fatty acids

Composition. The fatty acid methylesters were analyzed by a Hitachi 063 gas chromatograph on a glass column (200 × 0.3 cm) packed with 10% DEGS-Chromosorb WAW at 180°C as column temperature. The compositions of fatty acids at the glycerol (0-acyl) and ethanolamine (N-acyl) moieties in N-acylethanolaminephosphatides were determined by GC of the fatty acid methylesters (Stoffel et al., 1959) obtained from the mild alkaline hydrolysates described above.

Positional distribution. Monoacetyldiglycerides and diacetylmonoglycerides respectively obtained from the acetolysis of APE and ALPE were suspended in 1M tris-HCl buffer (pH 8.0). Being added to $CaCl_2$, sodium cholate and pancreatic lipase (Privett and Nutter, 1966), the mixture was incubated at 40°C for 15 min. After stopping the reaction, the lipids extracted with ether were chromatographed on a florisil column (Carroll, 1961) to separate free fatty acid from monoglyceride. The positional fatty acid composition at 1-C and 2-C of the glycerol in N-acylethanolaminephosphatides was determined by GC of the fatty acid methylesters of the free fatty acid and monoglyceride fractions, respectively.

Analysis of molecular species

APE was derived to monoacetyldiglycerides, which were subfractionated by 12% $AgNO_3$-silica gel TLC with hexane-benzene (4:1, v/v) and applied to GC-MS (Hasegawa and Suzuki, 1973) under the same conditions as mentioned above, except the column temperature was 300°C.

RESULTS

Identification of APE and ALPE

TLC. Silica gel thin-layer chromatogram of N-acylethanolaminephosphatide fractions (Fig. 1) showed the major spots corresponding to APE (fraction 2 ∿ 11) and ALPE (fraction 7

~ 12). The fractions also contained phosphatidic acid and the minor contaminant lipids and pigments.

IR spectrum. IR spectrum of pea seed APE (Fig. 2) was identical with that of N-palmitoylphosphatidylethanolamine, showing characteristic absorption bands due to NH (3380, 3270 cm^{-1}), ester C=O (1735 cm^{-1}), NH-CO (1645, 1535 cm^{-1}), P=O (1240 cm^{-1}) and P-O-C (1070 cm^{-1}). IR spectrum of pea seed ALPE (Fig. 2) was similar to that of APE in the approximate pattern of absorptions, except for the ester C=O absorption which was weaker than that of APE compared with its corresponding P-O-C absorption. The ALPE spectrum showed strong absorption band at 3350 cm^{-1} due to the OH group as expected for lysophospholipid.

NMR spectrum. NMR spectrum of pea seed APE (Fig. 3) was similar to that of N-palmitoylphosphatidylethanolamine. The signal due to the CH_2-N^+, which occurred at τ 6.70 in phosphatidylethanolamine (Chapman and Morrison, 1966), shifted upfield to τ 6.85 and the signal due to the NH_3^+proton which occurred also in phosphatidylethanolamine (Chapman and Morrison, 1966) was absent.

Hydrolytic product. The mild alkaline hydrolysate, N-acylglycerophosphorylethanolamine from APE and ALPE, gave the same spot (Rf 0.20) on silica gel TLC with an ammoniacal solvent. The IR spectra were identical with that of N-palmitoylglycerophosphorylethanolamine, showing absorptions for NH-CO and OH group, but not ester C=O (Bomstein, 1965).

Acetolytic product. The acetolytic products of APE and ALPE gave the spots identical with monoacetyldiglyceride (Rf 0.33) and diacetylmonoglyceride (Rf 0.15) respectively on silica gel TLC with petroleum ether-ether (4:1, v/v) (Morley et al., 1975). IR spectra of these acetolysates showed the characteristic absorption band due to CH_3-$\overset{O}{\overset{\|}{C}}$-O (1230 cm^{-1}).

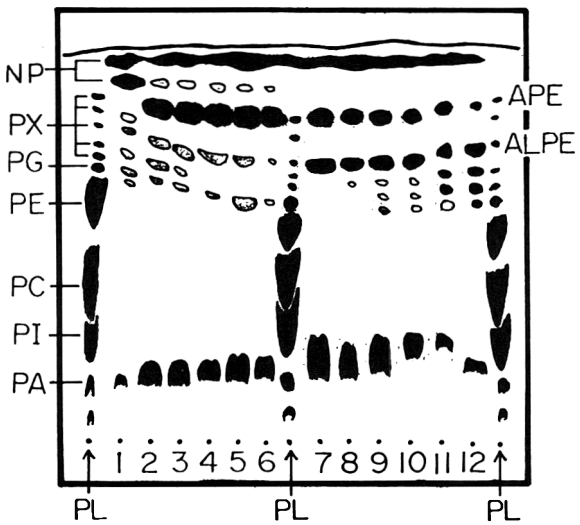

Fig. 1—Silica gel thin-layer chromatogram of N-acylethanolamine-phosphatides fractionated by column chromatography. Fraction, chloroform methanol (90:10, v/v) eluate from pea seed total phospholipids by column chromatography, was rechromatographed with chloroform for No. 1, chloroform-methanol (C-M) (99:1, v/v) for No. 2–3, C-M (98:2, v/v) for No. 4–6, C-M (97:3, v/v) for No. 7–8, C-M (96:4, v/v) for No. 9–10 and C-M (95:5, v/v) for No. 11–12. Plate was developed by chloroform-methanol-6.5N NH_4OH (130:30:4.7, v/v/v) and detected by 50% H_2SO_4. PX, Unidentified phospholipid and pigments; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PA, phosphatidic acid; PL, total phospholipid fraction of pea seed; NP, nonphosphatides.

Composition and positional distribution of fatty acids in APE and ALPE

As shown in Table 1, the major component fatty acids of APE and ALPE were palmitic, oleic and linoleic acids. The 1-C of APE was more unsaturated than the 1-C. Fatty acid pattern of 1-C and 2-C in APE was quite similar to that in pea phosphatidylethanolamine (Miyazawa and Fujino, 1976). The 1-isomer of ALPE was also more unsaturated than the 1-isomer. N-Acyl in APE and ALPE were more saturated than O-acyl, and had almost the same composition with each other.

Mass spectra of TMS ethers of N-acylglycerophosphorylethanolamine

TMS derivatives of partially deacylated products (N-acylglycerophosphorylethanolamine) of APE and ALPE were analyzed by GC-MS to confirm their structures. The chromatograms obtained by the total ion monitor (Fig. 4) showed two major peaks, respectively, b-1 and b-2 in case of APE and C-1 and C-2 in ALPE.

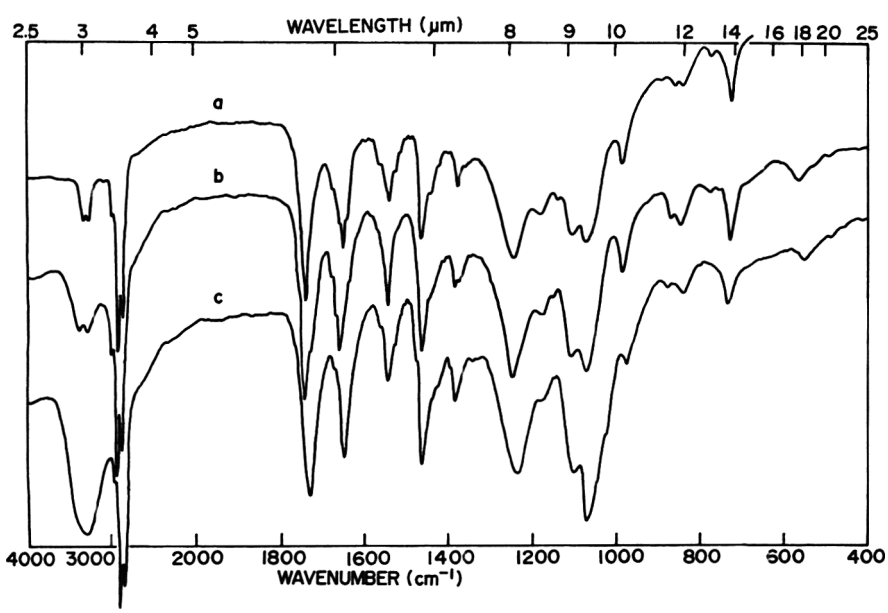

Fig. 2—Infrared spectra of acylethanolamine-phosphatides: (a) N-palmitoylphosphatidylethanolamine, standard; (b) APE from pea seed; (c) ALPE from pea seed.

Table 1—Fatty acid composition of APE and ALPE from pea seed (%)

Fatty acid[a]	APE					ALPE				
	Total	O-Acyl	1-C	2-C	N-Acyl	Total	O-Acyl	1-Isomer	2-Isomer	N-Acyl
14:0	1.0	0.5	0.8	–	3.0	1.4	0.6	2.2	1.2	2.1
15:0	0.3	–	0.2	–	0.7	0.9	–	0.3	0.2	0.7
16:0	21.7	17.5	43.6	3.8	28.1	25.2	20.3	26.1	18.1	29.5
16:1	0.9	1.0	0.3	0.5	1.1	2.1	2.3	1.8	1.6	2.0
17:0	0.5	0.7	1.1	–	0.2	0.9	1.2	1.3	0.8	0.6
UC[b]	0.4	0.1	–	–	–	2.5	3.2	1.6	1.3	0.3
18:0	5.3	3.0	4.6	2.4	6.6	6.6	9.3	11.4	6.7	5.3
18:1	38.1	41.3	28.3	48.3	33.1	28.1	28.4	26.8	32.9	31.7
18:2	29.6	32.8	18.5	42.1	26.1	28.5	31.5	26.5	34.5	24.9
18:3	2.2	3.1	2.6	2.9	1.1	3.8	3.2	2.0	2.7	2.9
Saturated	28.8	21.7	50.3	6.2	38.6	35.0	33.7	41.3	27.0	38.2
Unsaturated	70.8	78.2	49.7	93.8	61.4	62.5	63.1	57.1	71.7	61.5

[a] Carbon number : number of double bond
[b] Unconfirmed

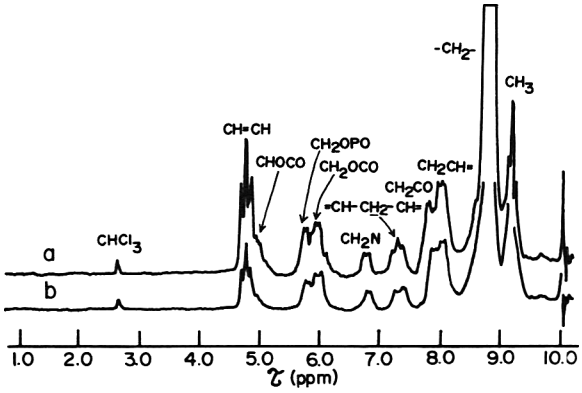

Fig. 3—NMR spectra of acylethanolaminephosphatides: (a) APE from pea seed; (b) N-palmitoylphosphatidylethanolamine, standard.

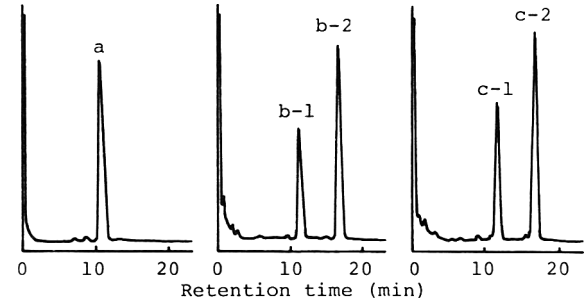

Fig. 4—Chromatogram of total ions of TMS ethers of (a) N-palmitoylglycerophosphorylethanolamine, standard; (b) N-acylglycerophosphorylethanolamine from APE; and (c) N-acylglycerophosphorylethanolamine from ALPE.

N-Palmitoyl-glycerophosphorylethanolamine. The mass spectrum of peak b-1, which was the same pattern in case of peak C-1, is shown in Figure 5. The molecular weight of this compound was indicated by the ions, m/e 669 (M^+), 654 (M-15:CH_3), 596 (M-73:TMS), 579 (M-90:TMSOH) and 566 (M-103:$TMSOCH_2$). Ions due to the glycerophosphate moiety were m/e 387, 389 (387+2H) and 452 (M-217). The ions, m/e 389 and 452 were due to intermolecular rearrangements of hydrogen atoms (Duncan et al., 1971). The ions, m/e 211 and 239 indicated that the N-acyl moiety was only the palmitoyl. Ions, m/e 282 (M-387) and 268 (M-401) characteristic for ethanolamineglycerophosphate (Duncan et al., 1971) were also detected. The mass spectrometric data, identical with those of the standard (peak a) indicate that both peak b-1 and C-1 were tris (TMS)-N-palmitoylglycerophosphorylethanolamine.

N-Oleoyl and N-linoleoyl glycerophosphorylethanolamine. The mass spectrum of peak b-2 (Fig. 6) showed the same fragmentation patterns with those of peak C-2. They had m/e 693 (M^+) and 695 (M^+) for molecular weight and m/e 265 and 237 for N-oleoyl, and m/e 263 and 235 for N-linoleoyl. Thus the compounds of both peak b-2 and C-2 were found to be a mixture of tris(TMS)-N-oleoylglycerophosphorylethanolamine and tris(TMS)-N-linoleoylglycerophosphorylethanolamine.

Molecular species of APE

The monoacetyldiglycerides derived from pea seed APE were separated into five bands: saturated, monoenic, dienic, trienic and tetraenic subfractions, on $AgNO_3$-silica gel TLC. The major subfraction of dienic was identified as 1-palmitoyl-2-linoleoyl-3-acetyl-sn-glycerol (Fig. 7) by the mass spectrometry. Diglyceride moiety of pea seed APE (Table 2), analyzed likewise, demonstrated that the principal molecular species were 1-palmitoyl-2-linoleoyl, 1-palmitoyl-2-oleoyl and 1-oleoyl-2-oleoyl types.

DISCUSSION

IN THIS STUDY, the chemical structure of APE and ALPE was confirmed to be 1,2-di-O-acyl-glycerophosphoryl-N-acyl-ethanolamine and 1(or 2)-mono-O-acyl-glycerophosphoryl-N-acyl-ethanolamine, respectively. Glycerophosphoryl-N-acyl-ethanolamine, which was confirmed as their TMS ether derivatives by GC-MS analysis. The structures established here supported the general structure of acylethanolaminephosphatides previously proposed (Bomstein, 1965; Quarless et al., 1968; Aneja et al., 1969).

The resemblance of the positional fatty acid composition and the molecular species of diglyceride moiety in pea seed APE and phosphatidylethanolamine (Miyazawa and Fujino, 1976) suggests that these were closely related in their metabolism. The similar N-acyl composition between APE and ALPE confirms that both lipids are in a close relationship in some ways (Hazlewood and Dawson, 1975). It is assumed in general

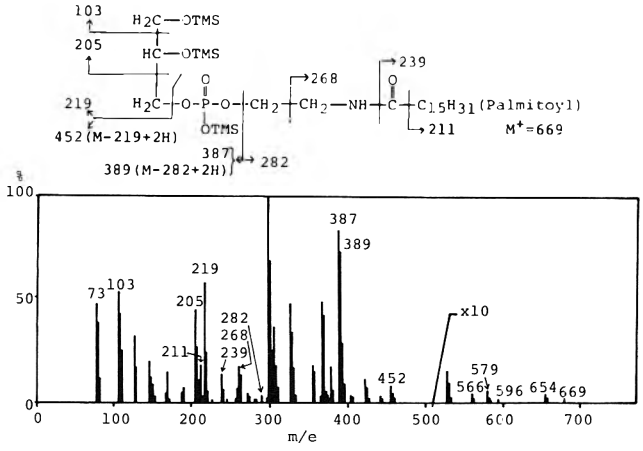

Fig. 5—Mass spectrum of peak b-1 (in Fig. 4).

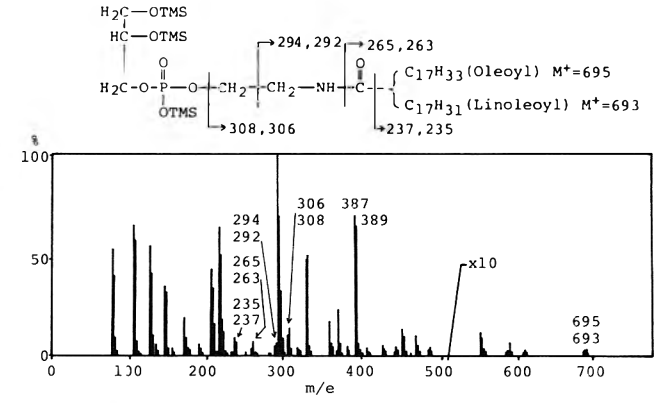

Fig. 6—Mass spectrum of peak b-2 (in Fig. 4).

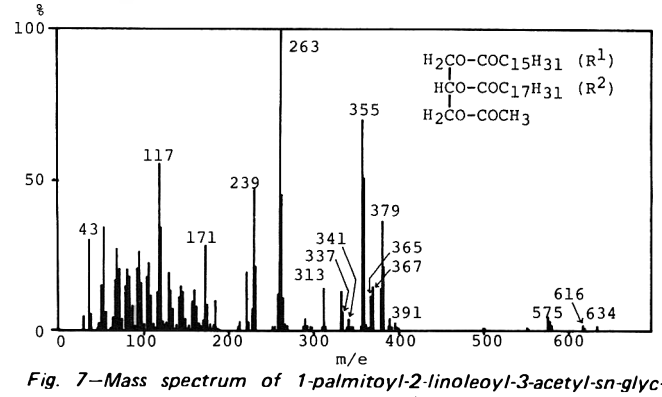

Fig. 7—Mass spectrum of 1-palmitoyl-2-linoleoyl-3-acetyl-sn-glycerol. Fragment ions (m/e): 43 $[CH_3CO]^+$, 117 $[CH_3CO+74]^+$, 171 $[CH_3CO+128]^+$, 239 $[R^1CO]^+$, 263 $[R^2CO]^+$, 313 $[R^1CO+74]^+$, 337 $[R^2CO+74]^+$, 341 $[M-R^2COOCH_2]^+$, 355 $[M-R^2COO]^+$, 365 $[M-R^1COOCH_2]^+$, 367 $[R^1CO+128]^+$, 379 $[M-R^1COO]^+$, 391 $[R^2CO+128]^+$, 575 $[M-CH_3COO]^+$, 616 $[M-18]^+$ and 634 $[M]^+$.

Table 2—Molecular species of diglyceride moiety in pea seed APE

Fatty acid		
1-C	2-C	APE (%)
16:0	16:0	0.4
16:0	18:0	0.4
18:0	18:0	0.1
16:0	18:1	24.7
18:0	18:1	5.9
16:0	18:2	25.3
18:1	18:1	16.1
18:1	18:2	13.9
18:2	18:2	13.2

that APE, ALPE, phosphatidylethanolamine and lysophosphatidylethanolamine are in the common metabolic pool in the plant tissues.

The possibility that APE and ALPE might have been produced artifactually during the preparative process by an acyltransferase activity was eliminated by the fact that these compounds were obtained in like amounts after treatment of the seeds with steam prior to the extraction procedure. This confirms that the APE and ALPE, especially the latter which has been isolated only from soft wheat flour (Bomstein, 1965; Clayton and Morrison, 1972), exist naturally as they do. Recently, APE was detected also in bovine erythrocytes (Matsumoto and Miwa, 1973) and pig epidermis (Gray, 1976).

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S. D. CUNNINGHAM,[1] C. M. CATER and K. F. MATTIL
Food Protein R & D Center, Oilseed Products Div.
Texas A & M University, College Station, TX 77843

EFFECTS OF GERMINATION ON COTTONSEED PROTEIN

ABSTRACT

Mechanically dehulled glandless cottonseeds were germinated for up to 5 days in moist paper towels in a seed germinator. Samples were lyophilized and subjected to proximate analyses. The dry matter content decreases with germination time, while seed moisture increases from 6% to 65%. There is a threefold increase in free fatty acids. Gel electrophoresis of the germinated seed protein on acrylamide revealed five major banding areas. The clearly defined bands on day 0 become very diffuse by day 5. On day 2, a new brand appears and remains through day 5. Amino acid analyses of the germinated seeds reveal a substantial increase in ammonia and aspartate while arginine and glutamate decrease with germination time. Protein solubility data indicated that while TCA soluble protein increases with germination time, nonprotein nitrogen remains constant. Ultracentrifuge data revealed no apparent difference in relative concentration of the 2S, 7S or 12S peaks at different germination times.

INTRODUCTION

ALTHOUGH GLANDLESS COTTONSEED protein products are relatively new on the commercial market, the purpose of this paper is to provide information concerning an even greater range of utilization for these materials. The utilization of cottonseed protein products in food systems will depend upon their behavior in these systems. Modification of existing properties of proteins has been accomplished in the past by chemical (Groninger, 1973; Paton, 1974) and enzymatic (Fujimaki et al., 1970; Childs, 1975; Arzu et al., 1972) methods. Fordham et al. (1975) studied changes in nutrient composition of various peas and beans caused by sprouting.

In the germination of fat-containing seeds, a conversion of fat to carbohydrate has been shown by Kornberg and Beevers (1957). Cavin and Beevers (1961) demonstrated that this conversion of fat to carbohydrate proceeds via a variant of the tricarboxylic acid cycle, the glyoxylate cycle. Fat utilization by germination of cottonseed was described by White (1958). A study by Yatsu (1965) noted that seed hydration causes rapid changes in seed ultrastructure. This paper reports an investigation of the effects of germination on cottonseed and explores possibilities for the use of partially germinated cottonseed kernels as a raw product for industrial food use.

MATERIALS & METHODS

Cottonseed germination and sample preparation

Glandless GL-6 cottonseed was obtained from Rogers Seed Company in Waco, Texas. Small quantities were dehulled mechanically and the kernels were separated from the hulls. Kernels were screened with a 1/8 inch vibrating screen to select only the larger kernels and these were used in germination experiments. Several treatments such as the use of Captan-75, surface cleaning with sodium hypochlorite followed by multiple washes and use of sterile equipment were employed to delay microbiological contamination. Small quantities of kernels were germinated by rolling them in moist paper towels and placing them in plastic bags. Larger quantities were placed between layers of moist paper towels in plastic pans which were wrapped in plastic. Kernels were then placed in a commercial seed germinator (38°C for 8 hours and 32°C for 16 hours). Samples were taken at 12 hr intervals and placed in a freezer to stop the germination process. Samples were lyophilized, ground with a mortar and pestle and stored in a desiccator.

Analyses

Nitrogen, moisture, oil, ash, crude fiber and free fatty acids were determined according to standard AOCS methods (AOCS, 1957). All nitrogen determinations on solid samples were accomplished by the Kjeldahl method. The percent protein was calculated by multiplying the percent nitrogen by 6.25. Liquid samples were determined by Lowry's modification of the Folin-Ciocalteau method for protein analysis (Lowry et al., 1951). Solubility of protein was determined by a method similar to Cunningham et al. (1975).

Electrophoresis

A Canalco disc electrophoresis apparatus (model 1200) and a Canalco (model 300B) power source were used for electrophoretic separation. One procedure for electrophoresis was accomplished by the method of Davis (1964) except that no stacking gel was used. Samples were run 12 at a time in cylindrical glass tubes (0.5 cm × 17.5 cm). Gels were prepared by placing cleaned tubes in a tube rack with a thin strip of parafilm across the bottom to act as a stopper. A 40% sucrose solution was added to all tubes to about 1 cm in height. The running gel was produced by dissolving 2.88g Cyanogum 41 (E-C Apparatus Corp.) in 20 ml of distilled water. Ten milliters of 0.48% ammonium persulfate, 10 ml of stock 0.72M tris-borate-EDTA buffer (pH 8.3) and 0.05 ml TEMED (N, N, N', N'–Tetramethylenediamine) were then added. This mixture was rapidly layered onto the sucrose solution to the top of the tube. A drop of water was used to cover the gel and the gels were allowed to photopolymerize for at least 35 min. The tubes were placed in position in the apparatus and the buffer chambers were filled with a 1:4 dilution of the stock buffer.

Samples were obtained by extraction of 0.5g lyophilized cottonseed with 5 ml of 0.1M sodium borate buffer (pH 9.8). Samples were stirred in the cold (4°C) for 30 min and then centrifuged at 10,000 × G for 10 min. The pellet was discarded and the supernate was recentrifuged at 15,000 × G for 20 min. One ml of the supernate was mixed with 1 ml of 40% sucrose and three drops of 0.005% bromophenol blue and 0.1 ml of this solution was loaded onto the gel surface. Electrophoresis runs were begun at 0.75 ma/tube for 30 min and continued at 2 ma/tube for 5 hr. Gels were stained with 1% Amido Black in 7% acetic acid and destained in 7% acetic acid for 30 min with an electrophoretic destainer.

A second electrophoresis technique was also used. The procedure was the sodium dodecyl sulfate (SDS)-acrylamide gel electrophoresis procedure of Weber et al. (1972), for molecular weight determination. Gel tubes (0.5 × 10.0 cm) were filled with 10% acrylamide. The lyophilized, germinated cottonseed kernels were ground with a mortar and pestle and the oil extracted by petroleum ether reflux. Air desolventized samples were then extracted for electrophoresis with a 0.01M phosphate buffer (pH 7.0). Gels were run at 6 ma/tube for 5 hr. Molecular weight of polypeptides were calculated from a plot of log molecular weight vs relative mobility of BSA, ovalbumin, pepsin, trypsin, and cytochrome C (Sigma Chemical Co., St. Louis, MO).

Amino acid analysis and analytical ultracentrifugation

Amino acid analyses were perfomed with a Beckman (model 121) analyzer. Kernels were germinated, lyophilized, ground with mortar and pestle and the oil extracted before being hydrolyzed in constant boiling 6N HCl for 24 hr under N_2. No corrections were made for losses due to hydrolysis.

Sedimentation experiments were performed in a Beckman (model E) analytical ultracentrifuge equipped with schliern optics. Electronic speed control was used at a calculated rotor speed of 51,844 rpm. The lyophilized, germinated kernels were ground and extracted (10% w/v) with 0.2M sodium carbonate buffer (pH 10.5). After centrifugation of the slurry at 10,000 × G for 10 min, 1 ml of the supernate was diluted with 1 ml buffer and 0.6 ml of this solution was used to fill the ultracentrifuge cell. The cell was aligned in the rotor, the rotor was

[1] Present address: Food Technology & Science Dept., Univ. of Tennessee, P.O. Box 1071, Knoxville, TN 37901

counterbalanced and locked onto the drive shaft. Ultracentrifuge vault temperature was maintained at 20°C and photographs were taken at 8 min intervals with a 75 degree phase angle.

RESULTS & DISCUSSIONS

Germination and proximate analysis

Germinating GL-6 glandless cottonseed kernels were evaluated at 24-hr intervals during a 5-day germination period. Mold growth and off-odors were detectable on untreated seeds at 2½ days. Kernels with obvious mold growth were discarded. All treatments used delayed mold growth only until about the 6th day. Microbiological contamination is viewed as a serious problem, particularly with partially damaged kernels with exposed endosperm. Rigid microbiological and time controls are essential to successful industrial germination of cottonseed kernels.

Table 1 contains the proximate analysis of cottonseed kernels at various stages of germination. The protein and oil content both increased slightly as germination proceeds, probably because of utilization of carbohydrate as an energy source. White (1958) showed that a large weight loss is evident through 1.5 days. At 6 days of germination the weight loss was over 12%. There is a threefold increase in free fatty acids during the first five days of germination and this probably accounts for some of the off-odors present in the later stages of germination.

Table 1—Proximate analysis of glandless cottonseed kernels germinated for various periods of time[a]

Days	% Moisture	% Oil	% N	% N X 6.25	% Ash	% Crude fiber	% FFA	% Wt loss
0	6.58	36.54	6.36	39.72	4.65	1.43	.18	0
1	53.56	37.26	6.47	40.46	4.46	1.74	.23	1.8
2	55.79	38.50	6.65	41.63	4.59	1.56	.45	3.1
3	61.59	41.42	6.6	41.26	4.49	1.73	.40	3.2
4	65.80	38.08	6.91	43.20	4.81	1.68	.51	3.5
5	66.93	37.82	6.75	42.21	4.77	1.71	.60	7.5

[a] Values expressed on moisture-free basis

Electrophoresis

Figure 1 contains the acrylamide gels loaded with the protein samples taken at 12-hr intervals through germination. At 0 days there are five major banding areas and these appear quite sharp. As germination proceeds, these bands become diffuse and spread out into the clear area between the bands. By the 5th day, the bands have become very diffuse and the major banding areas are difficult to distinguish. The blurring of these major banding areas of cottonseed protein may be caused by the action of proteolytic enzymes produced in vivo during germination. Rossi-Fanelli et al. (1965) noted evidence of proteolytic activity in incubating cottonseeds. Biosynthesis of a cottonseed protease during germination was shown to be directed by mRNA synthesized during embryogenesis (Ihle and Dure, 1969), and the proteolytic enzyme was later identified as a carboxypeptidase (Ihle and Dure, 1972). Whether this band blurring is caused by proteolytic enzymes produced in vivo or by mold growth is unknown, but blurring begins very early in the germination process before mold growth is evident. On day 2 (gel E) a new band can be seen and this

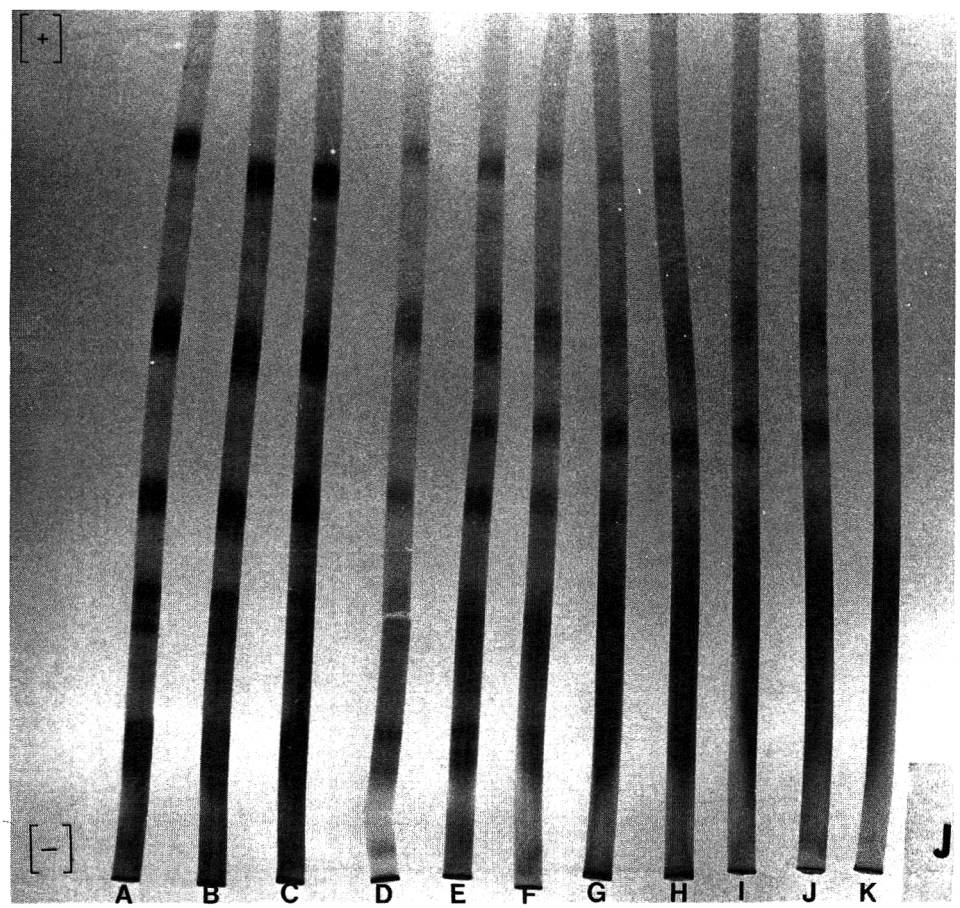

Fig. 1—Acrylamide gel electrophoresis of cottonseed protein extract: Gel A (0 days), B (1/2), C (1), D (1½), E (2), F (2½), G (3), H (3½), I (4), J (4½), K (5). 0.1 ml extract/tube.

remains through the 5th day (gel K). On days 2–3 (gels E, F and G) six major banding areas are observed rather than the five areas of the other gels. The bands are not identified.

Figure 2 shows SDS-acrylamide gels at 24-hr intervals during germination for 5 days. Molecular weights (mol wt) of polypeptides were calculated from a plot of log mol wt vs relative mobility of the five standard proteins (Linear correlation coefficient 0.998). A number of very narrow bands exist with a mol wt too high to accurately measure. The two major bands, bands a and b, correspond to molecular weights of 54,000 and 48,000 daltons respectively, and can be seen in all gels. Band b decreases slightly in density as germination time increases. Band c appears in gel #0 at a very low concentration but as germination proceeds its density increases. Relative density of band c was greatest in gel #3. Band c has an estimated mol wt of 30,500 daltons. All bands are unidentified.

Amino acid analysis

Table 2 contains the amino acid analyses of germinated cottonseed kernels. Ammonia and aspartate increase while arginine and glutamate decrease with germination time. Fasella et al. (1965) has isolated Glutamic Oxaloacetic Transaminase (GOT) 2.6.1.1 from glandless cottonseeds and it may be that this enzyme is responsible for the rise in aspartate and the

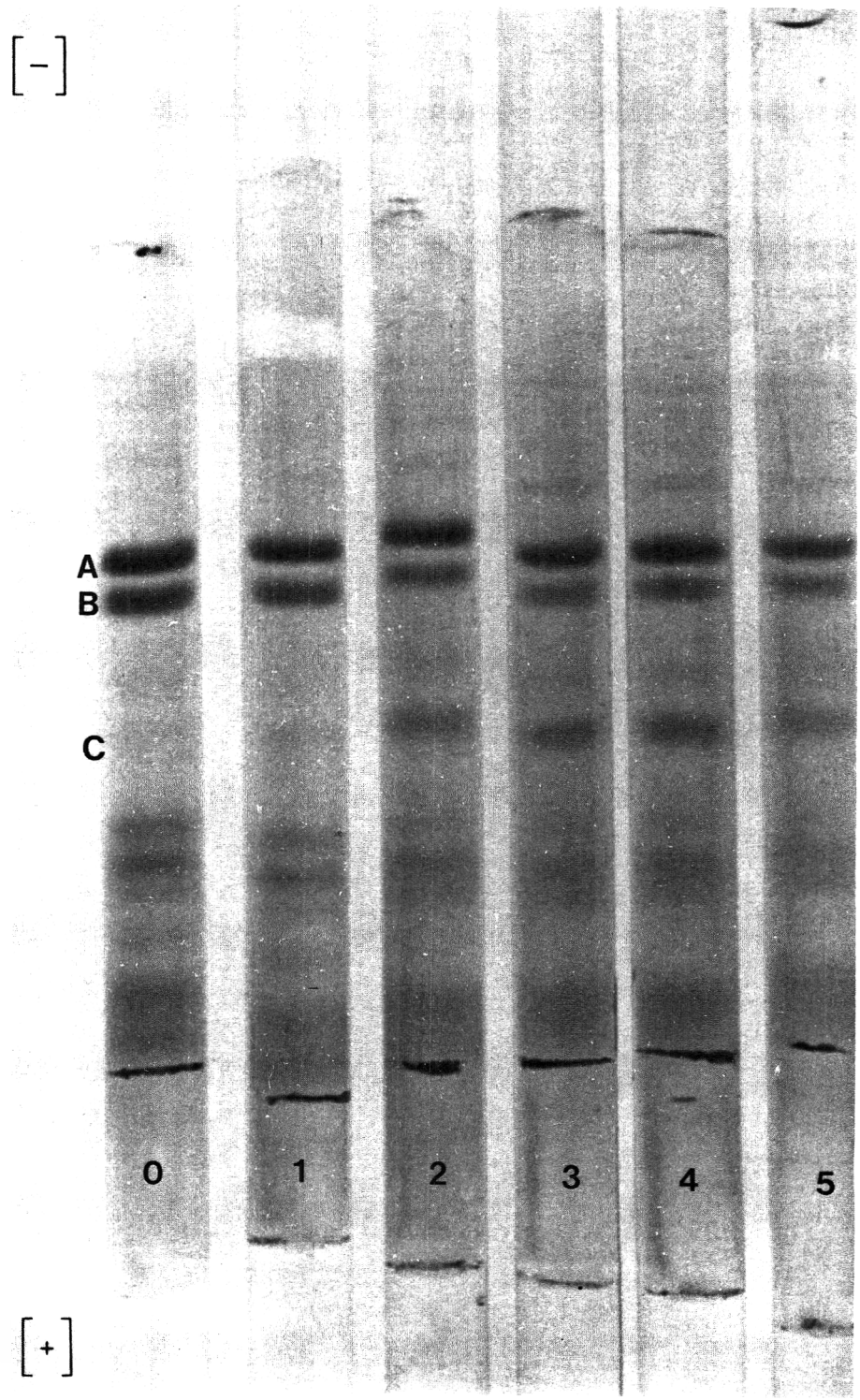

Fig. 2—SDS gels (10% acrylamide) of cottonseed proteins, 0–5 days of germination: Band A—54,000 daltons; B—48,000 daltons; and C—30,500 daltons. Each gel had been loaded with 75 μl of sample.

Table 2—Amino acid analysis of glandless cottonseed kernels germinated for various periods of time

	Day						
	0[a]	1[a]	2[a]	3[a]	4	5	6
Amino acid	g/16g N						
Lysine	4.2	4.0	4.2	4.1	4.1	4.1	3.9
Histidine	2.6	2.6	2.6	2.7	2.6	2.6	2.5
Ammonia	1.9	2.0	2.1	2.4	2.7	2.7	2.7
Arginine	11.6	11.7	10.4	9.9	9.2	10.0	9.7
Aspartate	8.6	8.8	9.7	11.9	14.0	13.3	13.3
Threonine	3.2	3.4	3.1	3.1	3.3	3.3	3.0
Serine	3.9	3.9	3.9	3.9	3.8	4.0	3.9
Glutamate	20.4	19.9	19.0	17.7	17.5	17.7	17.0
Proline	3.3	3.5	2.8	2.7	3.5	4.1	4.1
Glycine	3.9	3.8	3.6	3.5	3.4	3.6	3.4
Alanine	3.5	3.5	3.6	3.8	3.5	4.0	3.8
Valine	4.3	4.2	4.3	4.3	4.1	4.3	4.0
Methionine	1.2	1.2	1.1	1.1	1.2	1.3	1.1
Isoleucine	2.8	2.9	2.9	2.9	2.8	2.9	2.7
Leucine	5.3	5.4	5.3	5.2	4.9	5.1	4.7
Tyrosine	3.1	3.1	3.0	3.0	2.7	2.9	2.7
Phenylalanine	5.3	5.3	5.1	4.8	4.4	4.8	4.6

[a] Duplicate analysis.

Table 3—Protein solubility of cottonseed proteins in 10% TCA at various stages of germination

Day	% Soluble Kjeldahl	% Soluble Lowry	Nonprotein nitrogen
0	10.2	3.6	6.6
1	11.6	4.9	6.7
2	13.4	5.0	8.3
3	12.9	6.1	6.8
4	15.8	9.1	6.7

Table 4—Sedimentation constants for proteins of germinating cottonseed in alkaline buffer

Day	2S	7S	12S
0	1.2S	7.6S	12.9S
1	1.2S	7.9S	13.9S
2	1.2S	7.8S	13.7S
3	1.5S	8.2S	13.8S
4	1.5S	7.1S	13.1S

decrease in glutamate. Ammonia liberation was also found in incubated cottonseed, presumably as a result of the presence of Glutamate Dehydrogenase (Rossi-Fanelli et al., 1965).

Protein solubility

Table 3 contains protein solubility data at 24-hr intervals during germination. The percent protein soluble in 10% trichloroacetic acid (TCA), as determined by Kjeldahl and Lowry, increases during germination. The percent TCA soluble protein by the Kjeldahl method reflects the amount of total soluble nitrogen X 6.25. This includes nitrogen from soluble protein, peptides, amino acids and nucleic acids. The percent TCA soluble protein as determined by the Lowry method should indicate only protein, peptides and free amino acids and this value is less than the Kjeldahl level. The difference between these values which remains relatively constant in this case, is nonprotein nitrogen.

The protein extracted from the germinated cottonseed was subjected to ultracentrifugation. Table 4 contains the calculated sedimentation coefficients from peaks produced by proteins extracted from germinating cottonseeds at 24 hr intervals. Since constant protein extraction conditions and a constant phase angle were used, the area under the 2S, 7S and 12S peaks was a function of relative protein concentration. No changes in the relative protein concentrations were detected.

CONCLUSIONS

GERMINATION of cottonseed kernels was investigated as a potential process for conditioning of kernels prior to protein extraction. Changes in electrophoretic mobility of the protein is evident and certain amino acid levels also change. The increase in solubility may increase protein extraction but may, at the same time, complicate protein recovery, at least when approached by isoelectric precipitation. Proteins soluble at their isoelectric points may be difficult to recover by conventional protein processing. Minimum germination times are desirable to avoid mold growth.

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H.M. BAU, B. POULLAIN, M. J. BEAUFRAND and G. DEBRY
Dept. de Nutrition et des Maladies Metaboliques de L'Universite de Nancy I
and Groupe de Recherches de Nutrition et Dietetique de L'Inserm U.59
40, rue Lionnois, 54 000, Nancy, France

COMPARISON OF COLD-, ACID- AND SALT-PRECIPITATED SOY PROTEINS

ABSTRACT

A comparison was made for three soybean protein fractions, cool-precipitated fraction (cool F), acid-precipitated fraction (acid F) and salt-precipitated fraction (salt F) on yield, physical and chemical characteristics, protein efficiency ratio, amino acid composition and oligosaccharide content. The starch-gel electrophoresis and ultracentrifugal patterns of the three fractions were very different. Salt F had a low galacto-oligosaccharide content and was superior in yield to both acid F and cool F. The solubility and the viscosity of salt F were the least sensitive to variations in pH and heat treatment. There were only small differences in amino acid content and protein efficiency ratio between the three fractions.

INTRODUCTION

A NUMBER of technological processes have been developed with a view to improving the quality and lowering the cost of soybean products. Industrial treatment however may influence nutritional value and affect other biological factors (Rackis et al., 1975).

Many new soy protein products are being developed which differ in enzyme activity, protein dispersibility, flavor, nutritive value and functional properties. Most products on the market at present have been developed from the acid precipitated fraction (acid F) (pH 4.5 insoluble protein); this fraction contains the major portion of the reserve proteins of the soybean cotyledons (Smith and Wolf, 1961) and is currently used in the food industry to prepare protein isolates and spun fibers. The salt-precipitated fraction of soybean protein (salt F) (curd prepared by coagulation with calcium salt) has been used for many centuries in oriental countries and it has been developed into a great variety of soybean products (Watanabe, 1968). The cool precipitated fraction (cool F) consists primarily of the 11S ultracentrifugal component which is the major portion of soybean globulin. This fraction has been well investigated by Briggs and Wolf (1957), Catsimpoolas et al. (1969), Wolf and Sly (1967), Catsimpoolas and Meyer (1968), Catsimpoolas et al. (1967) and it appears to possess a great potential as a functional and nutritional ingredient in foods. All three crude protein fractions have considerable advantages over soy flours in relation to human consumption, as they have an almost bland taste, white color, and good keeping qualities. They are therefore worth further investigation to ascertain their usefulness in food products.

In this paper, the yields, sugar contents and the availability of amino acids in the three fractions were evaluated. The physical characteristics and nutritional value were also studied.

MATERIALS & METHODS

Preparation of protein fractions

The three fractions were extracted from hexane-defatted nontoasted soybean flakes (Société Soja-France) containing approximately 50% protein (N × 6.25). They were the cool precipitated fraction (cool F), the acid precipitated fraction (acid F) and the salt precipitated fraction (salt F). The extraction procedures were adapted from the methods of Briggs and Wolf (1957), and Saio et al. (1973).

Cool F curd was prepared by cooling soymilk at 3–4°C for 72 hr (Fig. 1).

Acid F curd was obtained by precipitating soymilk in diluted HCl at pH 4.5 (isoelectric point) (Fig. 2).

Salt F curd was prepared by precipitation with 0.03M calcium chloride final concentration (Fig. 3).

All protein curds were washed and neutralized, then freeze dried for chemical analysis or moist toasted for the PER determination.

Chemical analysis in protein fractions

Nitrogen was measured in duplicate by the micro-Kjeldahl procedure (AOAC, 1970).

Isolation of sugars from protein fractions

The procedure is summarized in Figure 4.

Soybean fractions were diluted in 70 ml 80% ethanol and heated in a water bath (70°C) with stirring for 4 hr. The extract was decanted and the procedure was repeated with 30 ml 80% ethanol. The two extracts were combined and filtered. A tertiary solvent system was used to ensure the elimination of residual lipid and liposoluble substances from the filtrate. Barium hydroxide (0.3N) and zinc sulfate (0.3N) solutions were used to remove the contaminating proteins. Desalting was achieved by passing the extracts through a 2 × 10 cm column of ion exchange resin AG 501 -x 8 (D). The neutralized eluate was concentrated and freeze dried prior to thin-layer and gas-liquid chromatography analysis.

Isolation of verbascose

As pure verbascose was not commercially available, we isolated it from the roots of the mullein, verbascum thapsus, on a column of sepahdex G-15 (Speck and Rynbrandt, 1967) with a phosphate buffer at pH 7. It was purified by paper chromatography.

Qualitative determination of oligosaccharides by thin-layer chromatography

The purified sugar solutions were applied to a thin-layer chromatoplate (Kieselgel type 60 Merck) and developed by ascending partition chromatography using the solvent system isoamyl alcohol/acetic acid/water (6:4:3). Diphenylamine-aniline-phosphate was used to detect both reducing and nonreducing sugars (Bailey and Bourne, 1960).

Quantitative determination of oligosaccharides by gas-liquid chromatography

This analysis was made using a Girdel model 75 CD gas chromatograph (Girdel 75 RS1 Recorder). The column consisted of 2m of stainless tubing (2.17 mm i.d. × 3.18 mm o.d.) packed with 3% OV-1 (stationary phase) on chromosorb W (80–100 mesh). The peaks were identified and quantified as described by Jouany (1972) and Delente and Ladenburg (1972).

Viscosity measurements

In order to determine viscosity as a function of the length of heating, aqueous dispersions (conc = 10% w/v; pH = 6.5) of the soy protein fractions were heated for 5, 10, 20 and 30 min, at 100°C and their viscosities were determined using the model UT 180 Gebrüder Haake viscometer. Viscosity as a function of pH was also measured with the pH of the samples being adjusted to 1.5, 2.5, . . ., 9.5. With suitable calibration and thermal control system, the readings were readily converted into apparent viscosities in centipoises.

Solubility measurements

A 5-g protein sample was placed into a 250 ml graduated flask made up to volume with distilled water, shaken frequently and allowed to stand overnight. It was then centrifuged at 2000 rpm for 15 min and filtered through a Whatman number 54 filter paper. Total nitrogen was determined by micro-Kjeldahl analysis on 2 ml of the clear liquor.

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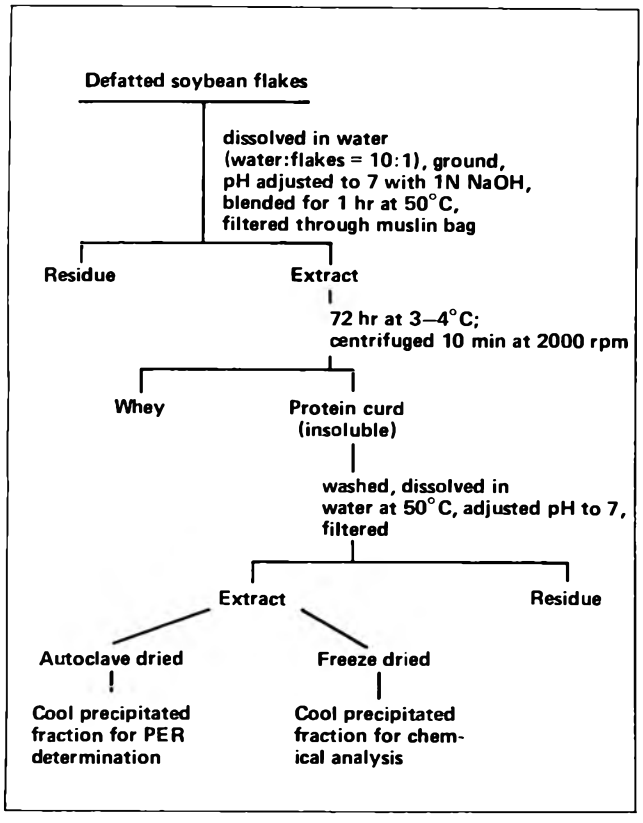


Fig. 1—Preparation of cool precipitated fraction (cool F).

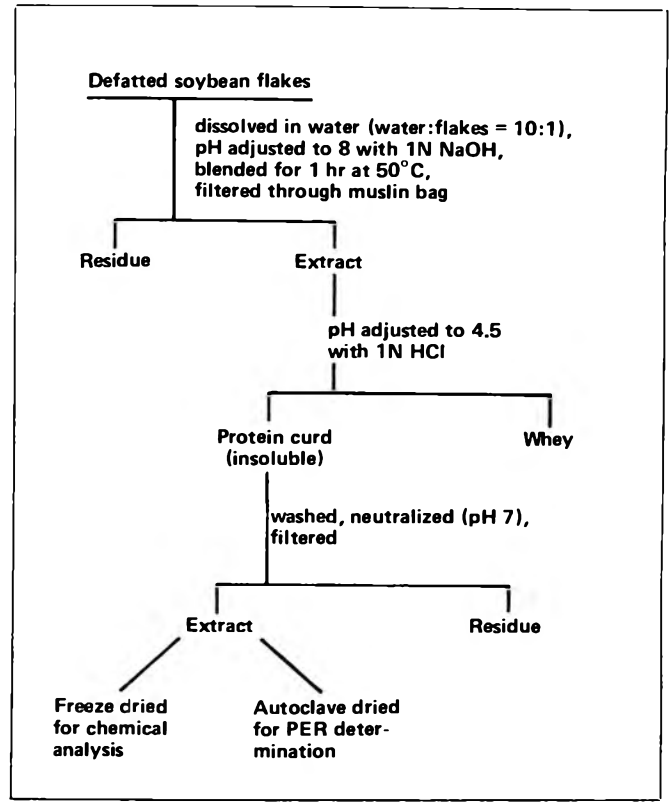


Fig. 2—Preparation of acid precipitated fraction (acid F).

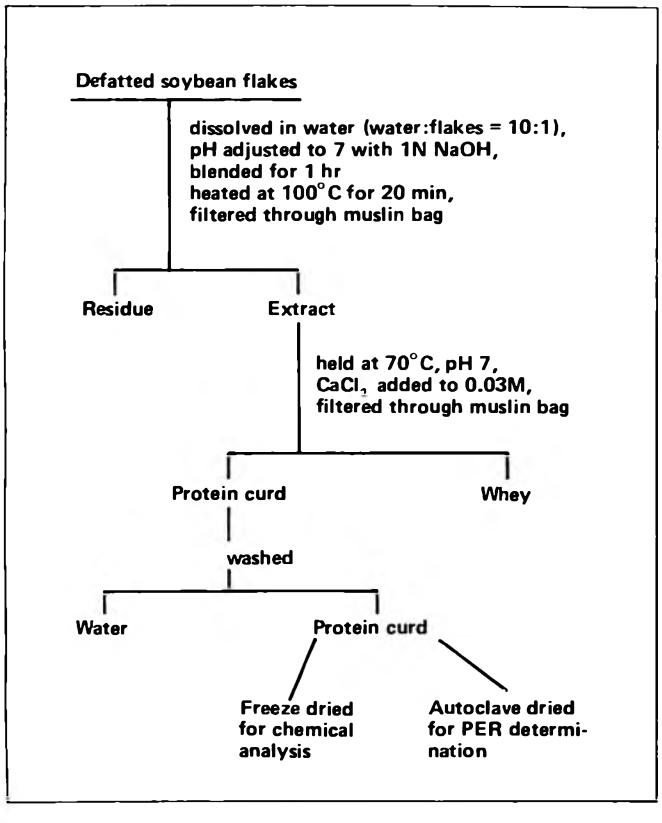


Fig. 3—Preparation of salt precipitated fraction (salt F).

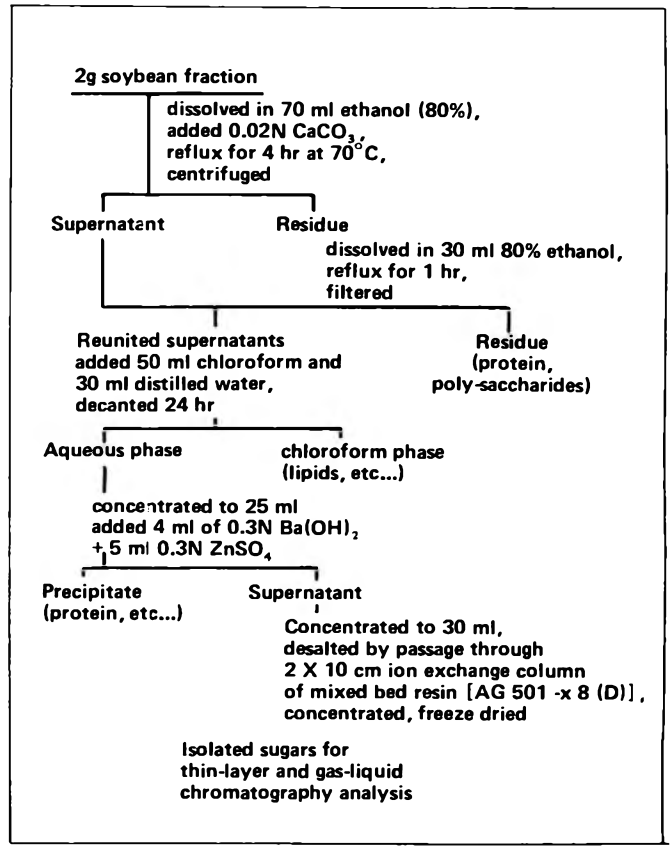


Fig. 4—Isolation of oligosaccharides from prepared fractions.

Starch gel electrophoresis

Samples for electrophoresis analysis were dialyzed for 3 days at 3–4°C against distilled water and then freeze dried. Starch gel analysis was carried out as described by Wake and Baldwin (1961). The starch gel was composed of hydrolyzed starch (Connaught, Sigma), 0.02M 2-mercaptoethanol and 7M urea in 0.076M tris-citrate buffer containing 0.076M tris(hydroxymethyl)-aminomethane; the pH was adjusted to 8.6 with solid citric acid.

Ultracentrifuge analysis

The soluble fractions, containing 1% protein, were examined at 20°C in a Spinco (Beckman) Model E analytical ultracentrifuge. In all cases a 12 mm Single-Sector cell was used at a speed of 59,780 rpm. All three fractions (A, B and C) were prepared for ultracentrifuging by overnight dialysis at room temperature against a phosphate buffer containing 0.0325M dipotassium monohydrogen phosphate, 0.0026M monopotassium dihydrogen phosphate, 0.40M sodium chloride, 0.01M 2-mercaptoethanol and 1.5M urea. In addition the acid F (sample D) was prepared in the same buffer but without 2-mercaptoethanol and urea.

Amino acid analysis

Amino acid analysis was carried out in duplicate according to the method described by Moore et al. (1958), using a Technicon TSM auto-analyzer, Model II. The samples were sealed in tubes in a nitrogen atmosphere and hydrolyzed in 6N HCl at 110°C for 24 hr. Cystine and methionine were analyzed following their oxidation to cysteic acid and methionine sulfone respectively (Hirs, 1967).

PER determinations

Samples for the PER determinations were moist heated at 100°C for 20 min, then dried by warm air at 60°C using a preheated autoclave system and finally ground to powder (100 mesh).

Protein efficiency ratio (PER) was measured by the method of AOAC (1970). A 28-day feeding trial was made with male weanling rats (same strain, 28 days old, initial weight 55–65g), separated into groups of ten, housed in individual cages and fed the diets ad libitum.

RESULTS AND DISCUSSION

Composition of fractions

Nitrogen and ash content of the fractions are given in Table 1. Cool F contains slightly more protein (90%) than the other two fractions, while salt F contains the most ash (6.8%).

Sugar content is shown in Table 2. The total sugar content of the extracted fractions varied from 1–7%. Thin-layer (Fig. 5) and gas-liquid chromatography analysis (Table 2) show that acid F and salt F contain more sugar than cool F which contains almost no sugar at all, over 90% of the sugar has been removed in the whey.

The total sugar content of salt F is only 4% (dry basis) of which only 0.53% is raffinose + stachyose and about 3% mono and disaccharides. The whey from this fraction contains about 10% total sugar, 3.56% raffinose + stachyose and over 5.7% mono and disaccharides. As the salt F fraction has a low oligosaccharide content, it would appear to be suitable for human consumption. The sugar content of salt F can probably be attributed to thermal processing in excess water at pH 7, 100°C for 20 min prior to coagulation, causing a part of the oligosaccharides to be degraded into mono and disaccharides. We would also suggest that $CaCl_2$ precipitation causes a greater separation of proteins from sugar than does acid precipitation.

Acid F contains about 7% total sugar of which 2.37% is stachyose + raffinose (Table 2). The total sugar content is almost twice that of salt F. This might suggest that the formation of cross-links between ionized carboxyl in acid F occurs more readily and is more stable than that in salt F and that the cross-links might retain a certain proportion of the sugar.

Fractionation yields

In order to obtain maximum yields, we used an extraction period of 1 hr, as reported by Cogan et al. (1967) and Smith et al. (1952). Fractionation yields for the three fractions are given in Table 1.

Protein preparation was carried out at pH 8 for acid F and pH 7 for salt F and cool F. The higher pH used (over pH 7) decreases the rate of extraction for salt F and cool F. The results refer to laboratory assay runs with batch size of 10 liters. The maximum recovery of dry weight, 36% (58.7% nitrogen recovery), was obtained with salt F, while minimum recovery (dry basis) was 21% (36.1% nitrogen recovery), obtained with cool F. Nitrogen recovery for acid F was almost as good as for salt F, reaching 33% (53% nitrogen recovery). From the viewpoint of nitrogen recovery, salt F and acid F are the best suited for industrial production.

Table 1—Chemical analyses and % nitrogen recovered in extracted fractions[a]

Sample	Nitrogen (g/100g fraction	Ash (g/100g fraction	Dry wt recovery %	Nitrogen recovery %
Defatted soy flakes[b]	8.43	6.3	–	–
Cool ppt fraction[c]				
Cool F	14.49	3.7	21	36.1
Salt opt fraction[c]				
Salt F	13.76	6.8	36	58.7
Acid ppt fraction[c]				
Acid F	13.44	3.0	33	53

[a] Dry-basis
[b] Hexane extracted from "Société Soja-France"
[c] See text for details

Table 2—Sugar content of soybean flakes, protein fractions and whey fractions

Sugars	Defatted soybean flakes (%)	Protein fractions Cool F (%)	Salt F (%)	Acid F (%)	Whey of protein fractions Cool F (%)	Salt F (%)	Acid F (%)
Stachyose	4.8		0.15	1.72	3.12	2.51	2.72
Raffinose	2.4		0.38	0.65	1.4	1.04	0.82
Disaccharide	8.14	0.51	0.67	2.91	6.57	3.04	4.41
Monosaccharide	1.3	0.2	2.22	1.8	1.6	2.68	2
Total sugars	17	1	4	7	12	10	10

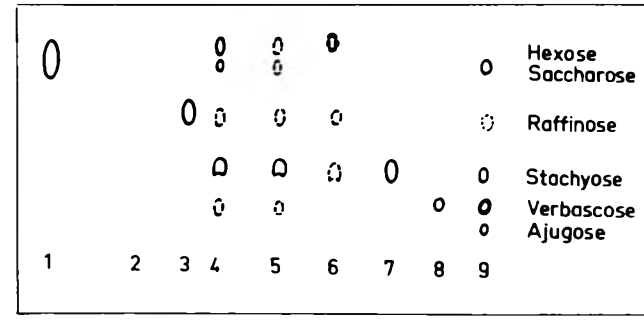


Fig. 5—Chromatogram of saccharide content of extracted fractions and whey of fractions: (1) sucrose; (2) sugar content of cool F; (3) raffinose; (4) sugar content of acid F; (5) sugar content of whey of acid F; (6) sugar content of salt F; (7) stachyose; (8) verbascose; and (9) sugar content of the roots of the mullein Verbascum thapsus.

Solubility and viscosity of soybean protein fractions as a function of pH

The pH of the soy fraction dispersions (10% w/v) was varied from 1.5–9.5 and their solubilities determined. Solubility results are shown in Figure 6. The minimum solubility obtained was situated at pH 4.4–4.6 for acid F and 5–6 for cool F and salt F, the isoelectric region. The addition of alkali (to pH 9.5) increased extraction of nitrogenous material by 32–34% for cool F and acid F and by only 14% for salt F. The

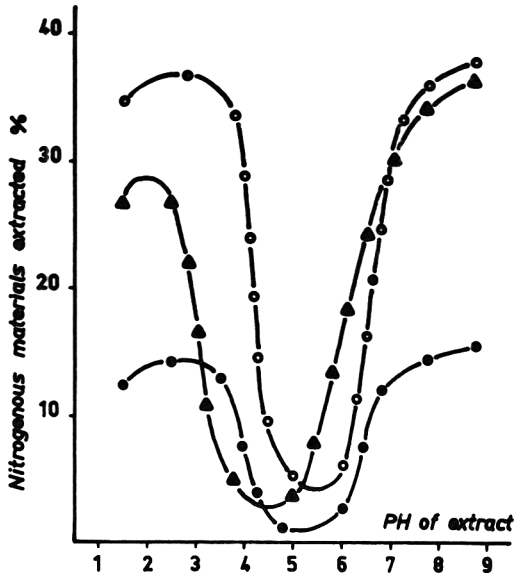


Fig. 6—Solubility of soybean protein fractions as a function of pH. [Temp 20°C; conc of protein 10%; ○—○ cool F; ▲—▲ acid F; ●—● salt F]

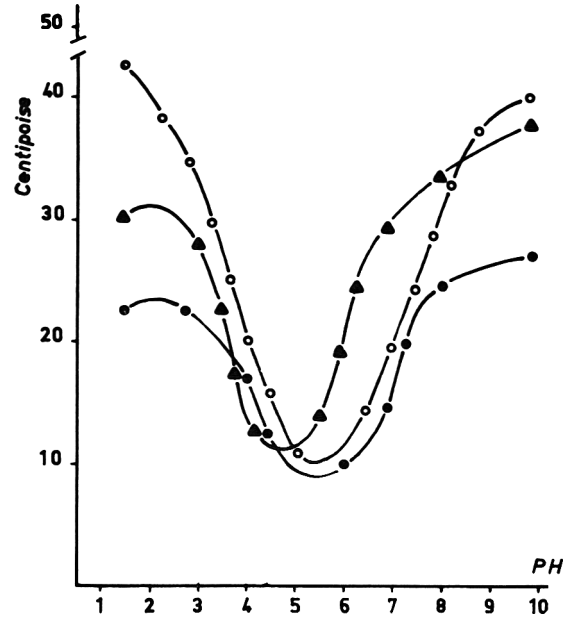


Fig. 7—Viscosity behavior of soybean protein fractions as a function of pH. [Temp 20°C; conc of protein 10%; ○—○ cool F; ▲—▲ acid F; ●—● salt F]

addition of acid (to pH 2–2.5) increased protein solubility by 30% for cool F, 25% for acid F and 12% for salt F. The results of this experiment show that the solubility of cool F and acid F is more sensitive to pH changes than that of salt F. Solubility of cool F and acid F is almost identical in an alkaline environment (pH 9.5) but differs by some 8% in the presence of acid (pH 2–2.5).

The effect of pH on the viscosity of the fractions, illustrated in Figure 7, is similar to its effect on solubility. In the vicinity of their isoelectric points, a minimum viscosity of some 12 centipoises is obtained for cool F and acid F and 9 centipoises for salt F. Viscosity increases as the pH moves away from the isoelectric point in either direction. Salt F is less viscous than the other two fractions.

Solubility and viscosity of soybean protein fractions as a function of heating

The effect of heating on the solubility of the fractions is illustrated in Figure 8. The pH of a suspension (10% w/v) of each fraction was adjusted to 6.5. Heating acid F and cool F at 100°C decreased solubility: in 7 min the initial value of 25% nitrogenous materials extracted for acid F decreased to 14.2% and for cool F from 20.4% to 10%. Continued heating had no significant effect on the rate of insolubilization for both fractions; there was instead a slight increase in solubility, probably due to dissociation of protein subunits. In the case of salt F, heating had no significant effect on solubility, perhaps on account of prior denaturation of the protein during the extraction process.

The viscosity results obtained as a function of heating are shown in Figure 9. Moist heating at 100°C for a protein concentration of 10% (w/v) increased the viscosity of all fractions in the first 5 min. Viscosities then decreased sharply, reaching a range of some 13–15 centipoises for all the fractions after 10 min heating. This phenomenon might be accounted for by the findings of Catsimpoolas and Meyer (1970). They reported that moist heating initially increases viscosity on account of aggregation and progel formation, but that continued heating at high temperatures decreases viscosity as a result of irreversible conversion to a metasol state. In our experiments, when heating was continued beyond 20 min, the viscosity increased slightly perhaps due to dissociation of the protein into

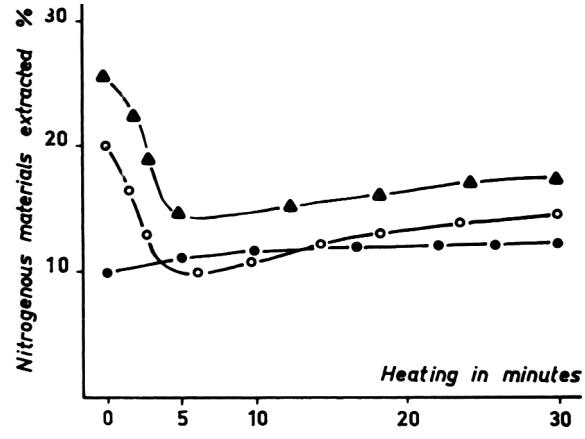


Fig. 8—Solubility of soybean protein fractions as a function of heating. [At 100°C; pH 6.5; conc of protein 10%; ○—○ cool F; ▲—▲ acid F; ●—● salt F]

subunits. Changes in the number of SH groups and the number of hydrogen bonds, as a result of heating and different pH, play an important role in all these phenomena (Saio et al., 1971).

Ultracentrifuge analysis

Figure 10 shows the ultracentrifuge patterns for samples A (salt F), B and D (acid F) and C (cool F). Ultracentrifuge analysis was conducted in a standard phosphate buffer containing 1.5M urea and 0.01M mercaptoethanol for all the samples except D, in order to compare the results with starch gel electrophoresis analysis which was carried out in a tris-citrate buffer also containing urea and 2-mercaptoethanol (Fig. 11).

The data indicate that in cool F and salt F the predominant component is the 11S fraction and cool F contains some 7S and 15S but salt F contains only small amounts of these components. Acid F on the other hand contains large proportion of both 7S and 11S, but less 15S.

Figure 10D shows the pattern of acid F in phosphate buffer without urea and mercaptoethanol. The addition of 0.01M mercaptoethanol, a disulfide bond cleaving agent, and 1.5M

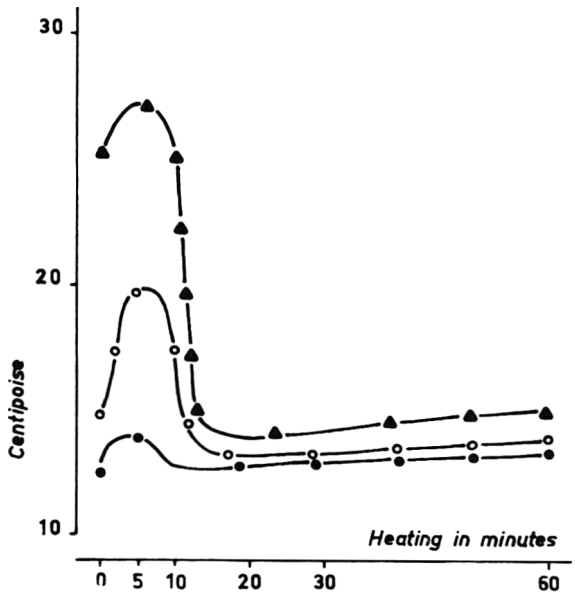
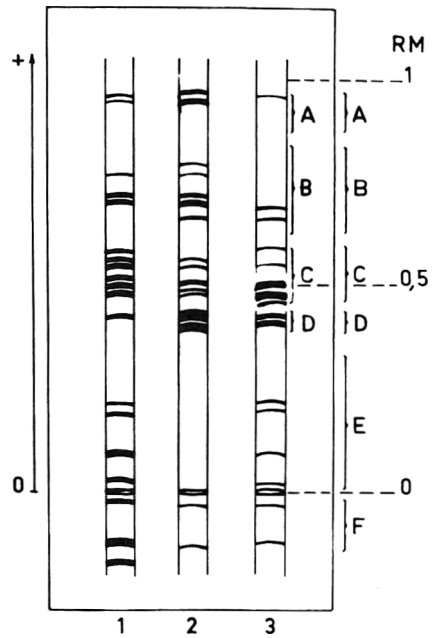


Fig. 9—Viscosity behavior of soybean protein fractions as a function of heating. [At 100°C; pH 6.5; conc of protein 10%; ○—○ cool F; ▲—▲ acid F; ●—● salt F]



Protein bonds were stained with amido schwartz dye
1—Cool precipitated fraction (Cool F)
2—Acid precipitated fraction (Acid F)
3—Salt precipitated fraction (Salt F)

Fig. 11—Starch gel electrophoresis.

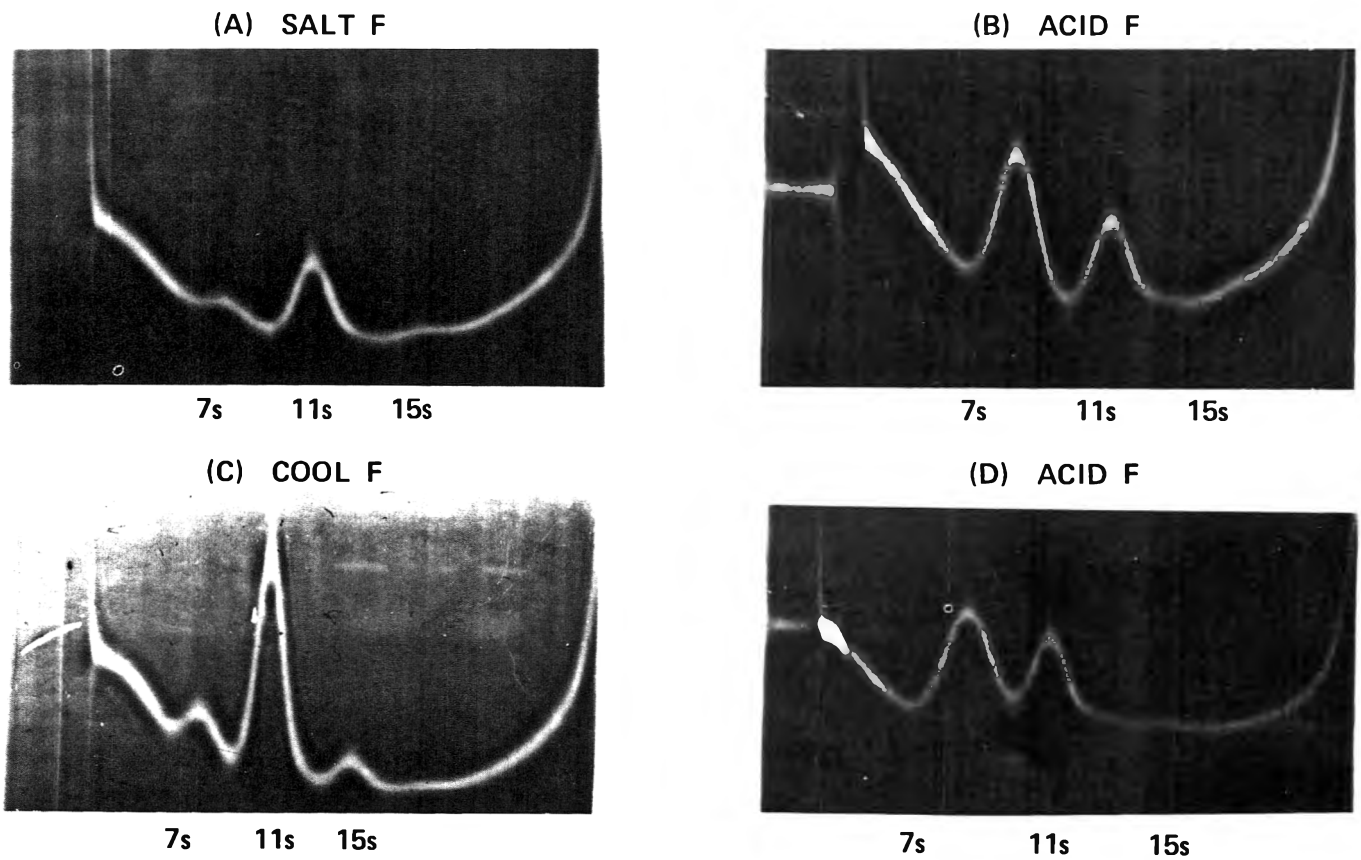


Fig. 10—Effect of mercaptoethanol and urea on ultracentrifugal patterns. [The ultracentrifugal analyses were conducted in the following buffer systems: (A), (B), (C) phosphate buffer, pH 7.6 ionic strength 0.5, phosphate buffer containing 0.01M mercaptoethanol and 1.5M urea; (D) phosphate buffer without urea and mercaptoethanol.]

urea, a hydrogen bond cleaving agent, to the buffer prior to dialysis comparatively increases the relative concentration of the 7S material and to some extent the 11S component (Fig. 10B). Unfortunately, our analyses did not have a solvent baseline and we have not therefore calculated precisely the area of each peak. All the freeze-dried samples except salt F were well dissolved. This had a solubility of only 78.2% in the standard phosphate buffer containing 1.5M urea and 0.01M mercaptoethanol. Acid F and Cool F had 97.2% and 90.1% solubilities, respectively. Solubilities were expressed as percentages of the total nitrogen (method described above). The ultracentrifuge patterns were obtained from the soluble portions of the samples. All the fractions contain little of the 2S component.

Starch gel electrophoresis

Figure 11 shows the results obtained by starch gel [containing the cleaving reagents urea (7M) and 2-mercaptoethanol (0.02M)] electrophoresis of the three soybean protein fractions. Cool F contains 19 components, acid F and salt F contains 16 components. To compare the components in each fraction, the three columns (Fig. 11) have been arbitrarily divided into six regions, labelled A to F.

The data indicate that the predominant components of the three fractions are situated in region C (RM 0.45–0.6). Each fraction has five bands in this region and the concentration of these components is in the order cool F > salt F > acid F. There are also five bands in region B (RM 0.88–0.63) for acid F, three bands for cool F and two bands for salt F. The concentration of protein in region D (RM 0.38–0.41) is in the order acid F > salt F > cool F. The highest concentration of protein in region E and F was found in cool F. Region E is the basic zone. The fastest-moving bands were observed to be most concentrated in acid F.

Amino acid analysis

The essential amino acid values are given in Table 3. There are only small differences between the three protein fractions and defatted soya flakes. Considering the nutritionally important amino acids, methionine + cystine and lysine, it would appear that salt F has a slightly lower sulfur containing amino acid content than the other samples but that it has a slightly higher lysine value. Cool F has the highest sulfur containing amino acid level; however, it did not appear to have a superior protein value as measured by the PER test (Table 4). Tryptophan was not determined.

PER determinations

The results are shown in Table 4. There appeared to be a little difference in the protein quality of the different fractions. The PER value of toasted defatted soy flakes is 1.9. PER of salt F is slightly better than both acid F and cool F while there would be no significant difference between the PER values of acid F and cool F.

CONCLUSION

THE SALT-PRECIPITATED FRACTION (salt F) appears to be the most suitable fraction for use in the food industry as an extender to ground meat and fish products. It has a low oligosaccharide content and is superior in yield to both acid F and cool F. The solubility and viscosity of this fraction are also the least sensitive to variations in pH and heat treatment. Cool F is almost free of oligosaccharides but has the lowest yield. Salt F and cool F therefore have considerable potential in new food used both functionally and nutritionally and could take over the role currently held by acid F.

Table 3—Amino acid content of extracted fractions (mg/16 mg nitrogen)

	Defatted soy flakes	Acid F	Cool F	Salt F
Methionine	1.38	1.36	1.70	1.20
Cystine	1.00	1.21	1.18	1.12
Lysine	5.87	5.46	5.73	6.19
Threonine	2.92	3.25	3.09	2.95
Valine	3.56	4.05	4.14	4.24
Isoleucine	4.09	3.93	6.17	5.45
Leucine	8.29	7.17	8.77	9.82
Phenylalanine	4.49	4.88	5.03	5.42

Table 4—Nutritive value of soybean protein fractions

Diet	PER[a]	Relative value to casein std (in %)
Casein	2.5	100
Toasted flake	1.9	75.4
Cool F	1.64	65.1
Salt F	1.92	76.2
Acid F	1.56	62.0

[a] PER obtained directly from rat experiment

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A BRADFORD HOLMES and CHOKYUN PHA
Dept. of Nutrition and Food Science
Massachusetts Institute of Technology, Cambridge, MA 02139

STRUCTURE AND CHEMICAL COMPOSITION OF CRANBERRY CELL WALL MATERIAL

ABSTRACT

The microscopic examination of cell wall material recovered from pressed cranberries indicated that the tissue particles were composed primarily of parenchyma cell walls that had reassumed a three-dimensional configuration similar to their structure in cranberry parenchyma tissue. Settling experiments indicated that large volumes of water were associated with cell wall material, correctly predicting when suspension yield stress would increase. The primary plant cell wall polysaccharides, cellulose, pectin and hemicellulose, were found to be the predominant constituents of cell wall material. Protein, fat, starch and ash were present in only minor amounts suggesting that cell wall material food functionality will be primarily dependent on the polysaccharide constituents.

INTRODUCTION

A PREVIOUS PAPER (Holmes et al., 1977) described the rheological properties of cell wall material recovered from pressed cranberries. Large yield stresses at low cell wall material concentrations (1–2%) suggested potential use of this material as a food thickener. This study examined the structure of cell wall material more closely in an attempt to elucidate the thickening mechanism of cell wall material suspensions. The chemical composition of cell wall material was also examined in order to determine the amounts of potentially functional constituents.

EXPERIMENTAL

Microscopic examination

Cell wall material was recovered from pressed cranberries as described previously (Holmes et al., 1977) and a portion sufficiently diluted with distilled water to form a disperse single layer of particles in the well of a hanging drop microscope slide. Photographs of particles were taken through an Olympus model EH microscope at 40× magnification using a Polaroid ED-10 camera and Type 107 black and white film. Each particle was denoted with a number on the photographs then its thickness determined at a higher magnification (usually 400×) by means of the microscope fine focus micrometer. Particle transparency allowed the thickness to be determined by noting the micrometer readings where the first upper and lower outer surfaces of the particles came into focus. Particle projected area diameter (Allen, 1968) was determined from the photographs using a template containing circles of varying diameters. Particle length and width were also determined from the photographs.

Sedimentation

A portion of the undiluted cell wall material used for microscopic examination had its total and soluble solids content determined in duplicate (Holmes et al., 1977) and six suspensions ranging in insoluble solids content from 0.30–0.55% (by weight) prepared by dilution with distilled water. Any air bubbles were removed by pulling a vacuum for 1.5 min and the suspensions were then carefully poured into 100 cm graduated cylinders to an 18 cm height, covered with Parafilm and allowed to set for 1 hr at room temperature (23–25°C). The suspensions were then mixed by inverting 6–8 times and the heights of the interface between the settling cell wall material and supernatant were recorded as a function of time for 3 hr at 6–min intervals. Settled bed height and volume were also noted after 14 hr of settling time.

The density at 25°C of an approximately 0.9% cell wall material suspension was determined in triplicate using water calibrated 50 ml pycnometers. The contents of the three pycnometers were pooled and triplicate portions taken for total and soluble solids determination (Holmes et al., 1977). The density of the calculated amount of insoluble solids in each pycnometer was determined by utilizing values for the amount and density of water and soluble solids present.

Chemical composition

Sample preparation. Pressed cranberry puree (15 kg) prepared as described previously (Holmes et al., 1977) was centrifuged in 250 ml jars at 890 × G for 10 min at 4°C, the supernatant removed with suction and replaced with an equal weight of water, the centrifuged cell wall material resuspended in the water by swirling the jar for 30 sec and the mixture recentrifuged. Samples of cell wall material (1.1–1.3 kg) that had experienced 1, 2, 3 or 4 water washings were recovered from puree. Soluble solids solutions were recovered from 150g of puree and 550–600g of each cell wall material sample by centrifugation at 27,000 × G for 15 min at 4°C followed by filtration through Schleicher and Schuell No. 595 paper. Solids content of the puree, cell wall material and soluble solids samples were determined in triplicate by vacuum oven drying at 70°C (AOAC, 1970). Duplicate 20-g portions of puree and cell wall material had anthocyanin pigments extracted by an acid-alcohol procedure (Fuleki and Francis, 1968a), the extract alcohol removed under vacuum at 30°C and pigment content determined by a pH differential method (Fuleki and Francis, 1968b). The remaining portions of the puree (1300g), cell wall material (570–630g) and soluble solids samples (130–430g) were freeze dried, left exposed to room temperature and humidity for 5 days and transferred to hermetic glass jars.

The freeze-dried soluble solids samples (1.5–0.2g) were fractionated into alcohol insoluble solids (AIS) and alcohol soluble solids (ASS) by blending for 30 sec with 200 ml of 70% methanol followed by washing of the AIS on a cotton cloth with 150 ml of 70% methanol and 50 ml of 100% acetone. The recovered AIS was dried overnight at 70°C in a vacuum oven and weighed. The water-methanol-acetone filtrates containing the ASS fraction were concentrated under vacuum at 30–50°C, diluted to 100 ml with water and kept at 4°C until used. A solvent blank was prepared to correct for any sugars from the cotton cloth.

AIS was recovered from portions of freeze-dried puree (9.0g) and cell wall material (5.0g) in order to remove low molecular weight substances that could interfere in the starch and pectin assays. The samples were blended for 3 min with 500 ml of 85:15 95% ethanol: 1N HCl, the slurry poured into Schleicher and Schuell No. 595 paper and washed with four 100-ml portions of 75% ethanol and two 100-ml portions of acetone. AIS material was allowed to dry at room temperature and humidity for 2 days, weighed and placed in hermetic glass jars.

Assay methods. The freeze-dried and rehumidified puree and cell wall material samples were assayed for moisture, fat, ash and protein according to AOAC (1970) methods. The acid hydrolysis method was used for fat determination. Crude protein was determined by multiplying micro-Kjeldahl nitrogen by 6.25. Cellulose content was determined by the semimicro method of Updegraff (1969). AIS of known moisture content recovered from puree and cell wall material had starch content determined by the method of Thivend et al. (1972) while pectin was extracted and hydrolyzed by the method of McCready and McComb (1952) and uronic acid determined by the procedure of Eitter and Muir (1962).

The ASS solutions prepared from freeze-dried soluble solids samples were assayed for organic acids (expressed as citric acid) by titration to pH 8.1 with 0.1N NaOH. The pH of sufficiently diluted samples was adjusted to 7.0 with 0.1N NaOH and glucose content determined using a glucose oxidase preparation (Glucostat Special reagent set, Worthington Biochemical Corp., Freehold, NJ). The phenol-sulfuric acid procedure of Dubois et al. (1956) was used to determine the content of other sugars (expressed as arabinose). Glucose standards allowed sample absorbances to be corrected for glucose present.

The AIS samples prepared from freeze-dried soluble solids samples

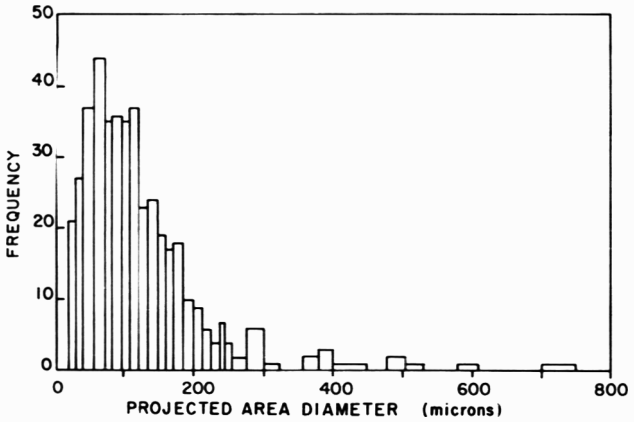

Fig. 1—Frequency histogram of the projected area diameter of 435 cranberry cell wall material particles.

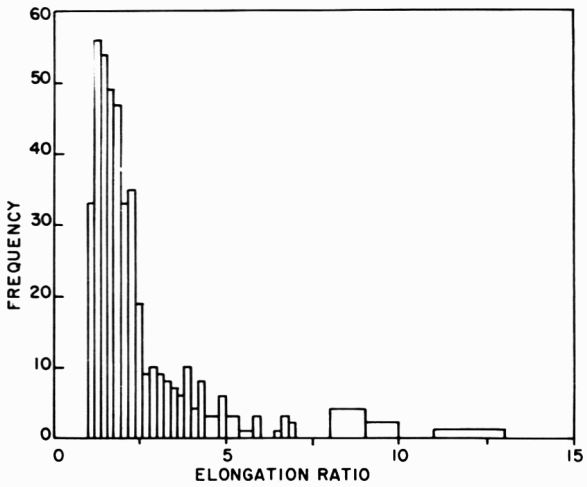

Fig. 2—Frequency histogram of the elongation ratio of 435 cranberry cell wall material particles.

were allowed to dissolve in water at room temperature and sufficiently diluted samples assayed for pectin by the method of Bitter and Muir (1962) and other polysaccharides (expressed as arabans) by the method of Dubois et al. (1956). Galacturonic acid standards allowed the sample absorbances of the araban assay to be corrected for pectin present.

All assays were made in triplicate using standards, blanks and controls where appropriate for each method. Results were expressed as weight-% total or soluble solids and also for the cell wall material samples, as percentage retention of puree constituent.

RESULTS & DISCUSSION

Structure of cell wall material

The determination of projected area diameter, length, width and thickness of over 400 cell wall material particles was conducted in order to derive data concerning the range and frequency of various particle dimensions. Projected area diameter ranged from less than 50 microns for particles consisting of small single parenchyma cells to 400–750 microns for large clumps of attached cells (Fig. 1). Most particle diameters (86%) were in the range of 20–180 microns where particles consisted of one to four attached cells. The elongation ratio, equal to length divided by width, was observed to vary from 1 to 13 (Fig. 2). Most values (75%) were from 1.0–2.6 suggesting near circular or elliptical shape. The flatness ratio, equal to width divided by thickness, ranged from 0.2–6 (Fig. 3). Most values (81%) were from 0.4–1.8 with the histogram mode very close to one; i.e., the thickness of most cell wall material particles was comparable to their width. The similarity of particle thickness to width indicated that many cell wall material particles were close to spherical or ellipsoidal in structure and appear to assume the same three-dimensional shapes as when part of the unpressed fruit parenchymal tissue. Occlusion of significant amounts of water in the cytoplasmic cavities of the cell wall material parenchyma cells would be expected to occur.

Cell wall material was observed to settle in 100 ml graduated cylinders such that the lowering of the cell wall material: water interface height was linear with time (Fig. 4). The cell wall material was assumed to sediment as aggregates composed of tissue particles with occluded and associated water. The modified Stokes law equation of Michaels and Bolger (1962),

$$Q_o = \frac{g(\rho_{cwm} - \rho_w)\overline{d}_A^2}{18\mu_w C_{A/cwm}}(1 - C_{A/cwm}\cdot\phi_{cwm})^{4.65} \quad (1)$$

where Q_o = settling rate, g = gravitational acceleration, ρ_{cwm} = cell wall material density, ρ_w = water density, $\overline{d}_A^2$ = mean aggregate diameter, μ_w = water viscosity, and $C_{A/cwm}$ =

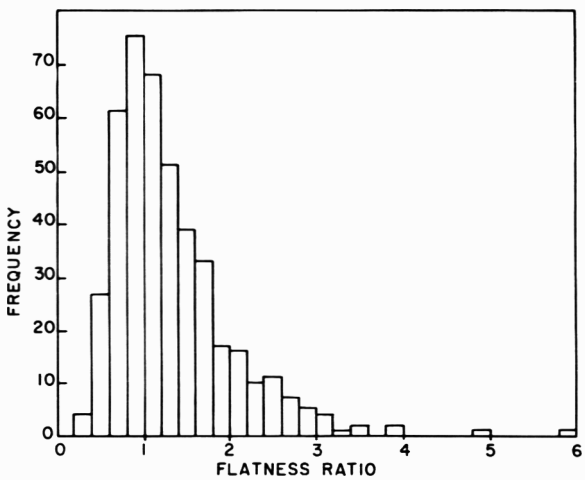

Fig. 3—Frequency histogram of the flatness ratio of 435 cranberry cell wall material particles.

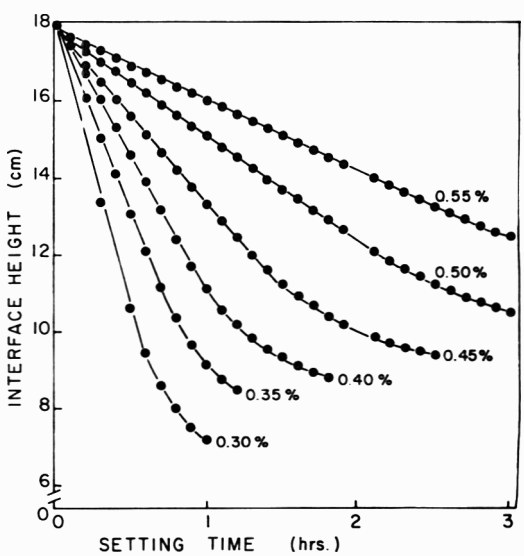

Fig. 4—Sedimentation of cranberry cell wall material suspensions of various concentrations.

Table 1—Composition of pressed cranberry puree and centrifugally recovered cell wall material[a]

Contituent	Puree	Cell wall material 1[b]	2	3	4
Water	98.45 ± 0.01	97.61 ± 0.02	97.71 ± 0.02	97.77 ± 0.01	97.75 ± 0.01
Insoluble solids	0.806 ± 0.003	2.000 ± 0.001	2.117 ± 0.001	2.137 ± 0.002	2.197 ± 0.001
Soluble solids	0.741 ± 0.003	0.386 ± 0.001	0.174 ± 0.001	0.088 ± 0.002	0.053 ± 0.001
Total solids					
Cellulose	18.1 ± 0.1	31.7 ± 1.1	35.2 ± 0.6	37.5 ± 0.8	37.9 ± 1.1
Pectin	15.8 ± 0.3	14.9 ± 0.3	15.4 ± 0.3	14.6 ± 0.4	15.4 ± 0.6
Protein	2.17 ± 0.04	2.98 ± 0.02	3.23 ± 0.06	3.11 ± 0.09	3.37 ± 0.04
Fat	2.90 ± 0.06	2.68 ± 0.03	2.71 ± 0.02	2.46 ± 0.05	2.57 ± 0.09
Ash	1.33 ± 0.03	0.92 ± 0.01	0.73 ± 0.01	0.77 ± 0.01	0.87 ± 0.02
Starch	0.15 ± 0.01	0.12 ± 0.03	0.16 ± 0.04	0.11 ± 0.004	0.14 ± 0.01
Other sugars[c]	16.3 ± 0.04	6.37 ± 0.05	3.03 ± 0.05	1.67 ± 0.02	0.81 ± 0.01
Organic acids[d]	8.41 ± 0.04	2.96 ± 0.02	1.24 ± 0.004	0.54 ± 0.003	0.19 ± 0.003
Glucose	8.18 ± 0.09	2.60 ± 0.02	0.96 ± 0.004	0.32 ± 0.001	0.07 ± 0.001
Anthocyanins[e]	0.42 ± 0.02	0.27 ± 0.003	0.13 ± 0.05	0.06 ± 0.03	0.04 ± 0.001
Total[g]	73.8 ± 0.5	65.5 ± 1.1	62.8 ± 0.7	61.1 ± 0.9	61.4 ± 1.3
Soluble solids					
Other sugars[c]	34.0 ± 0.8	39.4 ± 0.3	39.8 ± 0.06	42.4 ± 0.5	34.2 ± 0.2
Organic acids[d]	17.5 ± 0.1	18.3 ± 0.1	16.3 ± 0.1	13.8 ± 0.1	8.15 ± 0.13
Glucose	17.1 ± 0.2	16.1 ± 0.1	12.7 ± 0.1	8.04 ± 0.01	2.91 ± 0.05
Pectin	18.3 ± 0.9	15.8 ± 1.3	14.0 ± 0.8	17.8 ± 1.1	26.4 ± 0.7
Other polysaccharides[f]	4.49 ± 0.27	3.29 ± 0.10	3.89 ± 0.20	6.35 ± 0.09	11.6 ± 0.2
Total[g]	91.4 ± 1.3	92.9 ± 1.4	86.7 ± 1.0	88.4 ± 1.5	83.3 ± 0.8

[a] Values are mean wt-% ± standard error for triplicate determinations
[b] Number of times washed with water
[c] As arabinose
[d] As citric acid
[e] Duplicate determinations
[f] As arabans
[g] Standard error of total = $\sqrt{\Sigma(\text{standard error})^2}$

ϕ_A/ϕ_{cwm} = ratio of aggregate volume fraction to cell wall material volume fraction was used to interpret the settling data. ϕ_{cwm} was determined for each of the cell wall material suspension concentrations by the Michaels and Bolger (1962) relationship,

$$\phi_{cwm} = \frac{\rho_s - \rho_w}{\rho_{cwm} - \rho_w}, \qquad (2)$$

where ρ_s = density of the cell wall material suspension. ρ_{cwm} was determined by the pycnometer method to be 1.4 ± 0.03 g/ml. A plot of $Q_0^{1/4.65}$ versus the corresponding value of ϕ_{cwm} resulted in a straight line (Fig. 5, r = 0.998) from which

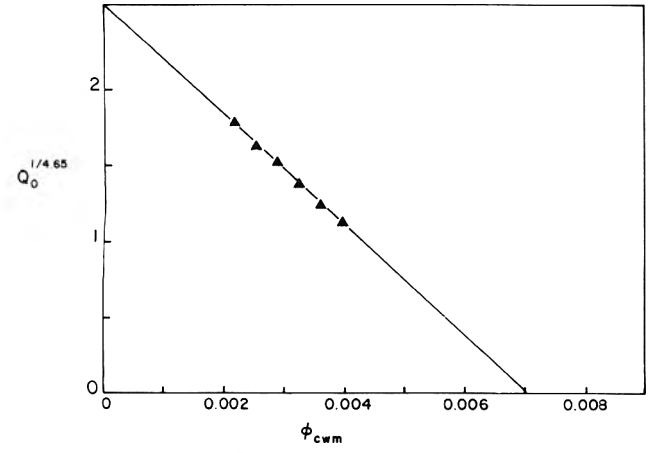

Fig. 5—Plot of $Q_0^{1/4.65}$ versus ϕ_{cwm} for cranberry cell wall material suspensions.

$\bar{d}_A$ and $C_{A/cwm}$ could be derived (Michaels and Bolger, 1962).

The derived mean aggregate diameter of 360 microns was larger than that actually observed for cell wall material particles (Fig. 1) since the tissue concentrations used had to be large enough to prevent the smaller cell wall material particles from forming a slower settling layer. Sedimentation of cell wall material concentrations less than 0.30% produced diffuse interfaces with small particles suspended in the supernatant. The derived value of 143 for $C_{A/cwm}$ indicated that one volume of cell wall material was capable of carrying with it 142 volumes of fluid during sedimentation. Such a large "hydrodynamic" volume for hydrated cell wall material would appear to be caused by the occlusion of fluid in the cellular cytoplasmic cavities in addition to entrapment between loosely packed cellular aggregates. Such a large value would seem valid since a value of 91 for $C_{A/cwm}$ can be calculated for undiluted sieve screen recovered cell wall material from the insoluble solids content (Holmes et al., 1977) and ρ_{cwm}. A diluter cell wall material suspension which is not compressed by the weight of material above it or allowed to drain 30 min on a sieve (Holmes et al., 1977) should have more fluid associated with the settling tissue.

Settled bed volume fractions were larger than the corresponding ϕ_A by a factor of 1.09–1.11 probably due to additional small amounts of water entrapped in the packed bed. The ϕ_A values were larger than the corresponding cell wall material concentrations by a factor of 1.02. A tissue concentration of 0.98% would thus be expected to occupy the total suspension volume. Actual samples close to this concentration were observed to settle slightly probably due to compressive forces. Increases in concentration above 0.98% should significantly increase yield stress as cell wall material particles are increasingly compressed against one another. The observed increases in cell wall material suspension yield stress (Holmes et

Table 2—Retention of puree constituents in centrifugally recovered cell wall material[a]

Constituent	Cell wall material			
	1[b]	2	3	4
Insoluble solids	99.1 ± 0.03	100.2 ± 0.02	101.7 ± 0.08	99.5 ± 0.02
Soluble solids	20.8 ± 0.04	8.97 ± 0.03	4.54 ± 0.09	2.62 ± 0.03
Total solids				
Cellulose	107.9 ± 3.9	109.7 ± 1.8	114.2 ± 2.5	111.0 ± 3.1
Pectin	58.1 ± 1.3	55.2 ± 1.2	51.3 ± 1.5	51.8 ± 2.0
Protein	84.7 ± 0.6	84.3 ± 1.6	79.2 ± 2.4	82.6 ± 1.1
Fat	56.8 ± 0.7	52.7 ± 0.4	46.7 ± 1.0	46.9 ± 1.7
Ash	42.4 ± 0.4	30.9 ± 0.4	32.1 ± 0.4	34.6 ± 0.6
Starch	48.7 ± 10.6	58.7 ± 13.0	39.4 ± 1.3	48.6 ± 2.6
Other sugars[c]	24.0 ± 0.2	10.5 ± 0.2	5.65 ± 0.07	2.63 ± 0.02
Organic acids[d]	21.7 ± 0.1	8.34 ± 0.03	3.57 ± 0.02	1.22 ± 0.02
Glucose	19.6 ± 0.1	6.66 ± 0.03	2.14 ± 0.01	0.45 ± 0.01
Anthocyanins[e]	40.0 ± 0.4	17.1 ± 6.3	8.40 ± 3.58	4.89 ± 0.05
Soluble solids				
Other sugars[c]	24.0 ± 0.2	10.5 ± 0.2	5.65 ± 0.07	2.63 ± 0.02
Organic acids[d]	21.7 ± 0.1	8.34 ± 0.03	3.57 ± 0.02	1.22 ± 0.02
Glucose	19.6 ± 0.1	6.66 ± 0.03	2.14 ± 0.01	0.45 ± 0.01
Pectin	17.9 ± 1.4	6.86 ± 0.40	4.40 ± 0.25	3.78 ± 0.10
Other polysaccharides[f]	15.2 ± 0.4	7.75 ± 0.39	6.42 ± 0.09	6.77 ± 0.12

[a] Values are mean percentage retention ± standard error for triplicate determinations
[b] Number of times washed with water
[c] As arabinose
[d] As citric acid
[e] Duplicate determinations
[f] As arabans

al., 1977), corresponds well with this prediction.

Chemical composition of cell wall material

The chemical composition of the total and soluble solids fractions of pressed cranberry puree and cell wall material are listed in Table 1. Other sugars are expressed as arabinose since only small amounts of sucrose or fructose are present in cranberries in comparison to glucose (Widdowson and McCance, 1935) and arabans known to occur with cranberry pectin (Pintauro, 1967) would be expected to partially hydrolyze during puree preparation (Aspinall, 1970). Assays not reported here have indicated that the majority of the undetermined total solids are hemicellulose. The major constituents of puree and cell wall material are therefore polysaccharide in nature with only small amounts of protein, fat, ash and starch present. Table 2 indicates that washing of cell wall material reduces the content of all constituents except cellulose. Those listed from pectin to starch have significant portions retained while other constituents are removed during the centrifugal washing steps. The constituents retained in significant amounts are similar to those found in other primary plant cell walls (Northcote, 1972; Talmadge et al., 1973; Knee, 1973). Cellulose retentions of greater than 100% for the cell wall material samples are believed to be caused by degradation of some puree cellulose during the acid digestion step (Updegraff, 1969). Some constituent of the large amount of soluble solids present (47.9% of total solids) during the acid digestion may have caused loss of cellulose.

The use of a larger or smaller pulper screen to prepare pressed cranberry puree would be expected to appreciably change the size distribution of cell wall material particles. The reduction of pulper screen openings could change the ratio of puree soluble to insoluble solids by shear-induced solubilization of cell wall components. A screen with 0.033-inch (834 micron) holes was used for this study since larger holes could allow seed fragments to pass through whereas smaller holes could reduce cell wall material yield.

The data in Table 1 indicate that cell wall material contains insufficient quantities of protein and starch to exert any major influence on cell wall material functionality in food systems. It would appear that cell wall material functionality in food systems will be dependent on its water occluding capability and/or the utilization of the properties of the three major polysaccharide constituents, cellulose, pectin and hemicellulose.

CONCLUSIONS

THE FOLLOWING conclusions can be made concerning cell wall material recovered from pressed cranberries:

1. Microscopic examination indicated that particles consisted of single or attached parenchyma cell walls that approached spherical or ellipsoidal shapes capable of occluding significant amounts of water.
2. Analysis of settling data confirmed that large volumes of water are associated with cell wall material and that increases in yield stress would be expected when tissue concentration exceeds 1%.
3. Cell wall material is composed primarily of primary plant cell wall polysaccharides, containing only small amounts of protein, starch and other constituents.
4. Functionality of cell wall material in food systems will be dependent on the ability to occlude fluid and/or the utilization of the properties of the cell wall polysaccharides.

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MARJORIE B. MEDINA
Dept. of Food Science, Cook College
VICTOR A. GREENHUT
Departments of Ceramics and Mechanics & Materials Science
College of Engineering
and PAUL A. LACHANCE
Dept. of Food Science, Cook College
Rutgers—The State University, New Brunswick, NJ 08903

IDENTIFICATION OF STARCH AND PROTEIN COMPONENTS OF GREEN BANANA TISSUE BY SCANNING ELECTRON MICROSCOPY AND USING SELECTIVE STAINS FOR X-RAY FLUORESCENT MICROANALYSIS

ABSTRACT

Starch and protein components of green banana tissue (*Musa cavendish*) were selectively complexed with iodine and copper respectively. The tissue was characterized with a scanning electron microscope by employing X-ray fluorescent emission (EDS) to locate starch and protein components by their relevant stain and secondary emission. Starch granules in the tissue gave strong iodine signals and revealed a structure similar to isolated granules. Electron-opaque substances surrounding the starch granules provided a strong copper signal indicating its proteinaceous nature. Other elements used in tissue preparation revealed no specificity to the tissue components. It is apparent that specific tissue components can be identified by selective labeling with elements for electron-microbeam X-ray fluorescent analysis.

INTRODUCTION

SELECTIVE COLOR STAINING is a well established method for identification of tissue components in light microscopy (Thompson, 1966; Conn et al., 1960). In view of the growth of scanning electron microscopy (SEM) for characterizing the morphology of plant and animal tissues beyond the light optical resolution limit, an analogous selective staining approach could prove to be a valuable tool for characterizing tissue components. Such a tool is available in X-ray fluorescent analysis combined with SEM.

The use of energy and wavelength dispersive system for obtaining chemical composition and localization has been proven valuable in characterizing inorganic microstructures (Lifshin, 1974). The energy dispersive methods (EDS) permit easy and rapid identification of an atomic specie by collecting and processing the characteristic X-ray fluorescent spectrum produced when high voltage electrons in the SEM beam interact with the sample. Depending upon the specimen examined, an X-ray spatial resolution of less than one micrometer can be obtained. A resolution of 100 Å is attainable when combined with the morphological information obtained with secondary electron signals. The application of such X-ray microanalysis methods to elemental composition analysis and localization of atomic species in biological systems has been reviewed briefly by Chandler (1973) and Hantsche (1974).

Tissue components composed of specific molecular groups such as proteins, carbohydrates and lipids could be identified in situ by labeling or complexing them with elements or compounds having a selective affinity to such groups. This study was undertaken to identify the starch and protein components of green banana (*Musa cavendish*) by employing such selective stains. A starch-iodine complex stain was investigated by EDS to provide elemental maps locating the starch structures. The copper chelating property of polypeptides at alkaline condition, commonly known as the biuret reaction, was employed to provide selective localization of proteins. These results could then be compared directly with morphological information obtained from the same area by secondary electron signals.

MATERIALS & METHODS

Sample preparation

Starch isolation. The green banana tissue was homogenized at 0°C in the presence of 0.01M mercuric chloride and 1% sodium metabisulfite. The suspension was filtered and centrifuged at 200–300 × G. The starch was further purified by repeated suspension in water and centrifuged at 1500 × G. The starch granules were then sedimented at 0.1M sodium chloride and suspended overnight in saline with toluene (0.1 volume). The toluene layer was removed and the starch was dried under vacuum at 50°C (Badenhuizen, 1964).

Iodine staining. Sections (1 × 2 × 4 mm) of green banana tissue were immersed in an iodine solution (1% iodine + 0.2% potassium iodine) for 20 min. The excess iodine was rinsed four times (30 min each) with 0.02M sodium phosphate buffer, pH 6.9.

Biuret reaction. Sections (1 × 2 × 4 mm) of green banana tissue were placed in 1 ml of isopropyl alcohol, 0.5g of powdered cupric carbonate and 25 ml of alkaline-alcohol solution (5.61g of potassium hydroxide pellets in 600 ml of isopropyl alcohol made up to 1000 ml with distilled water). The mixture was stoppered and agitated for 15 min and stood undisturbed for an additional 15 min (Johnson and Craney, 1971). The excess reagents were removed by rinsing with 0.01M phosphate buffer (pH 6.9).

Deproteinization. Some samples were deproteinized by suspending the tissue slices in a 10% buffered toluene containing 4% NaCl, 0.01M $HgCl_2$ and 0.001M $Na_2S_2O_5$ (Badenhuizen, 1964). The deproteinized samples were subsequently treated with biuret reagents and also stained with iodine.

Dehydration. The stained and complexed tissues were freeze dried. Prior to freeze drying, tissues were immersed in isopentane cooled by an acetone and dry ice mixture to provide rapid freezing and to facilitate drying. In another study, the use of isopentane alone appeared to cause alteration of the protein structure in the tissues.

X-ray analysis and scanning electron microscopy

Freeze-dried tissues were mounted on graphite sample stubs employing conductive carbon cement and subsequently coated with approximately 300Å of carbon in a vacuum evaporator. This provided for needed electrical conduction in the SEM without producing spurious X-ray spectra on severely limiting resolution in secondary electron micrographs.

The tissues were examined in an ETEC Autoscan SEM (ETEC Corp., Hayward, Calif.) with an attached Canberra-Energy Dispersive X-ray Analyzer. Satisfactory secondary electron images and X-ray signals were obtained when operating with a 20 KV accelerating voltage, 200Å objective aperture and 45° tilt of the overall specimen surface relative to the electron beam.

RESULTS & DISCUSSION

SPECIFIC STUDIES on starch granules in situ by light microscopy using histochemical tests were extended to electron microscopy studies (Hess and Mahl, 1954; Hess et al., 1955;

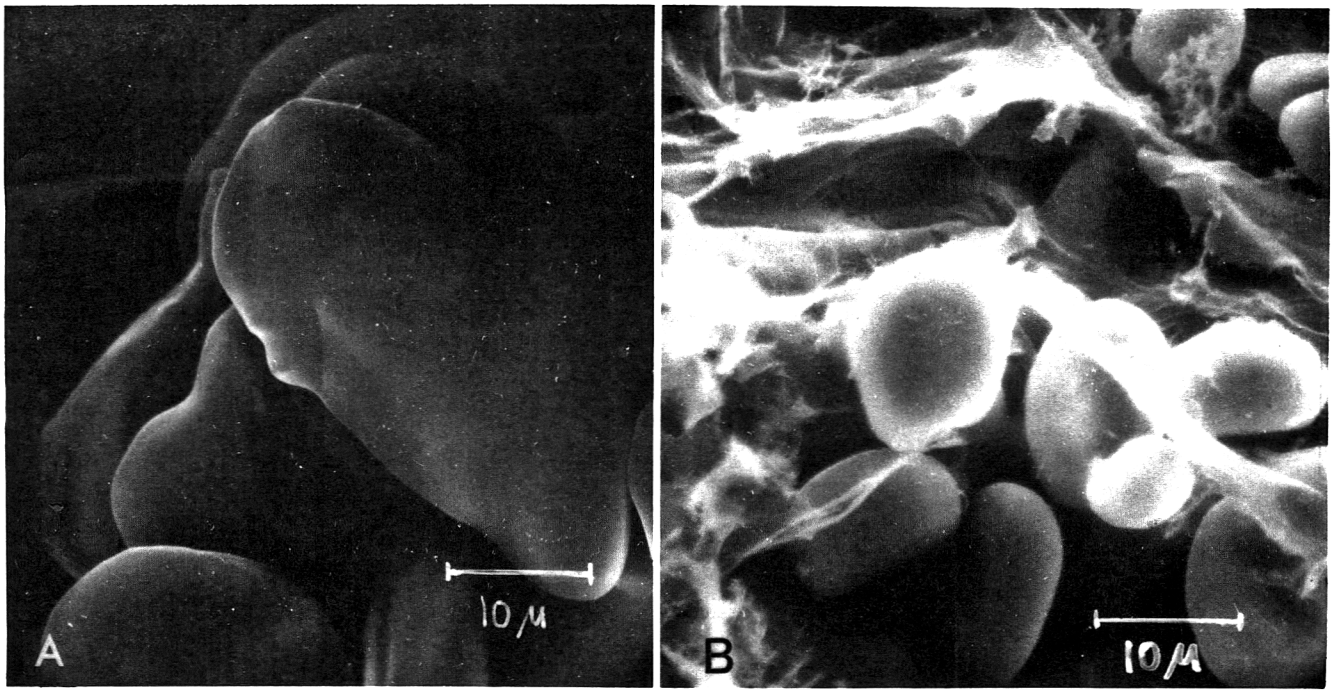

Fig. 1—Scanning electron micrographs of green banana starch granules (A) banana starch isolate (original magnification 2000X); (B) banana starch in situ (original magnification 2000X).

Buttrose, 1963). In current scanning electron microscopy studies, starch granules in situ have been identified with the use of amylolytic enzymes (Dronzek et al., 1972; Gallant et al., 1972). Figures 1A and 1B show the electron micrographs of green banana starch isolate and the starch in situ. No apparent differences in structure can be detected as a result of preparation.

It has been demonstrated (Banks and Greenwood, 1975; Hollo and Szeitli, 1968), that iodine staining is specific for starch and its component. A typical qualitative X-ray spectra (Fig. 2) from the untreated and iodine-stained green banana tissue show that detectable iodine spectral lines were obtained as a result of high voltage electron interactions with the sample. Additional atomic specie present in solutions used for the preparation are also apparent. Aluminum and silicon may not be endogenous to banana tissue but are a result of a background signal from the column. By selecting for the iodine $L_{\alpha 1}$ peak (I $L_{\alpha 1}$) it was possible to contrast an X-ray localization mapping (Fig. 3A and 3C) with morphological secondary electron images (Fig. 3B and 3D). An increase in iodine signal above background is displayed in the X-ray map by an increase in counts or dots on the micrograph. It may be noted that the starch granules, as determined by the comparison with starch isolate, dc indeed demonstrate preferential absorption of iodine. Residual elements from sample preparation such as sodium, while distinct in the X-ray spectrum showed no specificity or localization at the starch granule or other location. A close examination of Figures 3A and 3C reveals a variation in iodine signal for an individual starch granule. This might indicate a variation of the proportion of amylose, amylopectin, and other shorter chained polymers.

In present electron microscopy studies, proteins in situ are localized and detected by histochemical tests (Hess and Mahl, 1964; Hess et al., 1955); fluorescent antibody technique (Barlow et al., 1973); and proteolytic digestion (Inglett, 1976). The proposed SEM-X-ray method for the identification of protein components of the banana tissue is based on the complex formation of copper with the polypeptide chain under alkaline condition. Phenolic compounds are present in plant tissues in relatively high concentration and may bind

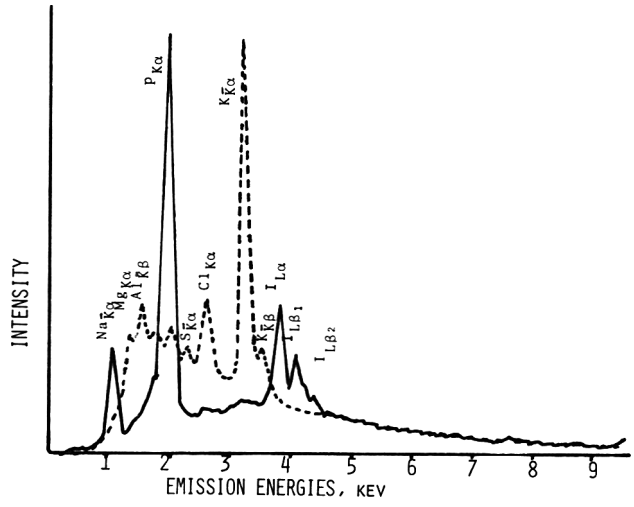

Fig. 2—Qualitative X-ray spectra of iodine-stained (—) and untreated (- - -) green banana tissue. Atomic species present in relatively high concentration are identified. The spectral scan of untreated tissue has background on expanded scale to show minor constituents. $K\overline{K}\alpha$ peak is relatively same as in the iodine-stained specimen.

with copper in a similar manner. However, this possible interference with the biuret reaction is inhibited by the extraction of the phenolics with alcohol (Johnson and Craney, 1971). The alcohol in turn also acts as a nonadditive fixing agent (Hayat, 1970).

Tests conducted on the green banana tissue shows a typical qualitative X-ray spectra (Fig. 4) of the copper-stained and untreated banana tissue which demonstrates a considerable amount of copper signals from the sample. Residual elements from sample preparation and other elements in detectable concentration are also shown. The spectral scan of the un-

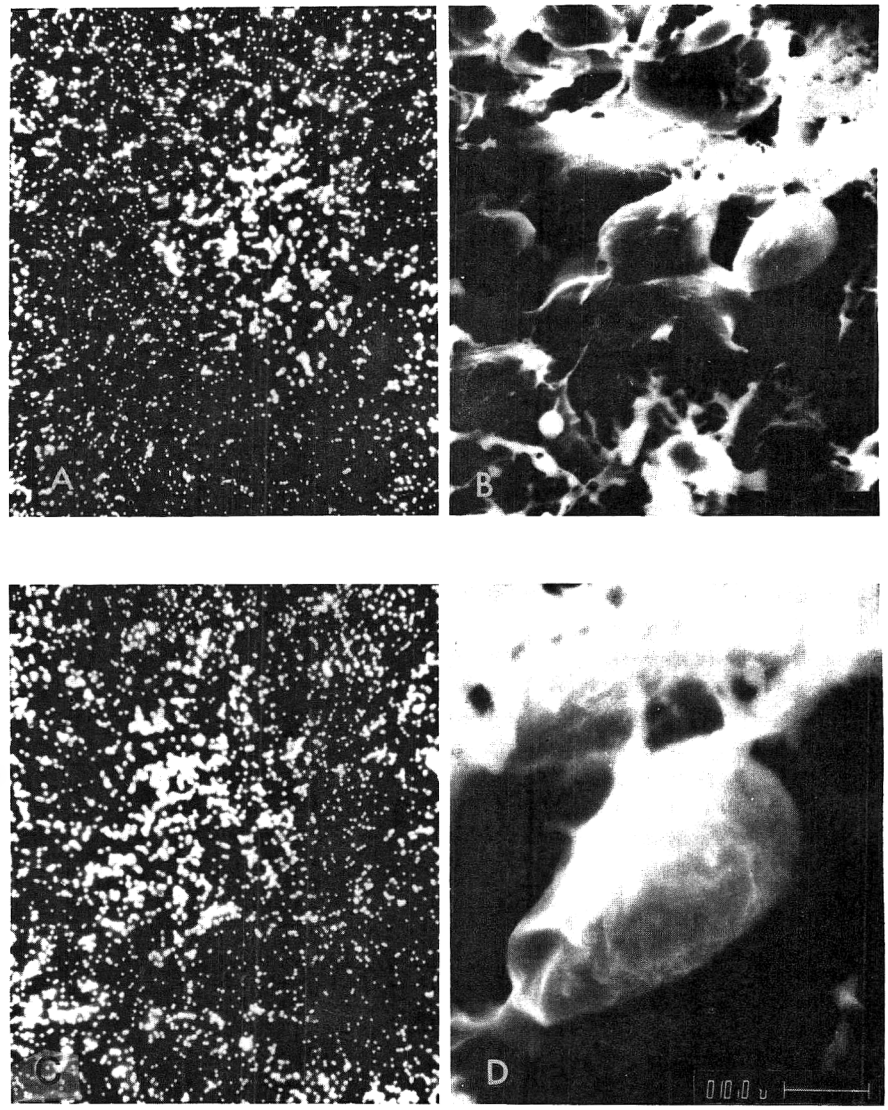

Fig. 3—Iodine-stained green banana tissue: (A) X-ray distribution image for $I_{L\alpha}$, corresponding to (B) secondary electron signal (1500X); (C) X-ray distribution image for $I_{L\alpha}$, at higher resolution corresponding to (D) secondary electron signal (5000X).

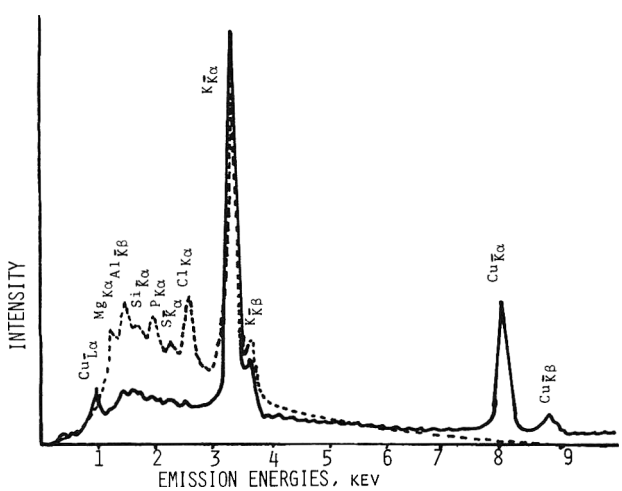

Fig. 4—Qualitative X-ray spectra of copper-stained (——) and untreated (- - -) banana tissue. Atomic species present in relatively high concentration are identified. The spectral scan of the untreated tissue has background on expanded scale to show minor constituents. $P_{\overline{K}\alpha}$ peak is same as in copper-stained tissue.

treated banana tissue is on expanded scale to show minor constituents. The X-ray mapping (Fig. 5A and 5B) of Cu-$K\alpha$ and the corresponding secondary image of the same spot reveal high concentration of this element at the white-appearing electron-opaque substances surrounding the starch granules (Fig. 5C and 5D). These micrographs clearly reveal the specificity of copper for these structures. It may be noted in Figures 5C and 5D that the starch granule at the lower portion of the secondary electron image has not produced a copper signal while the structure at the top of the micrograph is shown to be proteinaceous. The nature of these two structures although similar in size and shape is clearly discriminated by the biuret reaction.

An examination of the tissue (Fig. 6A and 6B) partially deproteinized with buffered toluene gave very low copper signals compared to the intact tissue (Fig. 5). Further examination of the tissue shows that the cell walls did not bind copper when contrasted with the residual protein structure (Fig. 7A and 7B).

As in the case with the iodine-stained tissues, other elements, such as potassium, present as a result of preparation showed no specificity or localization. This is an indication that the reagent has been uniformly distributed over the specimen surface. Electron micrographs and X-ray mapping of the deproteinized tissue revealed that the starch granules did not respond to subsequent iodine treatment. This result may be

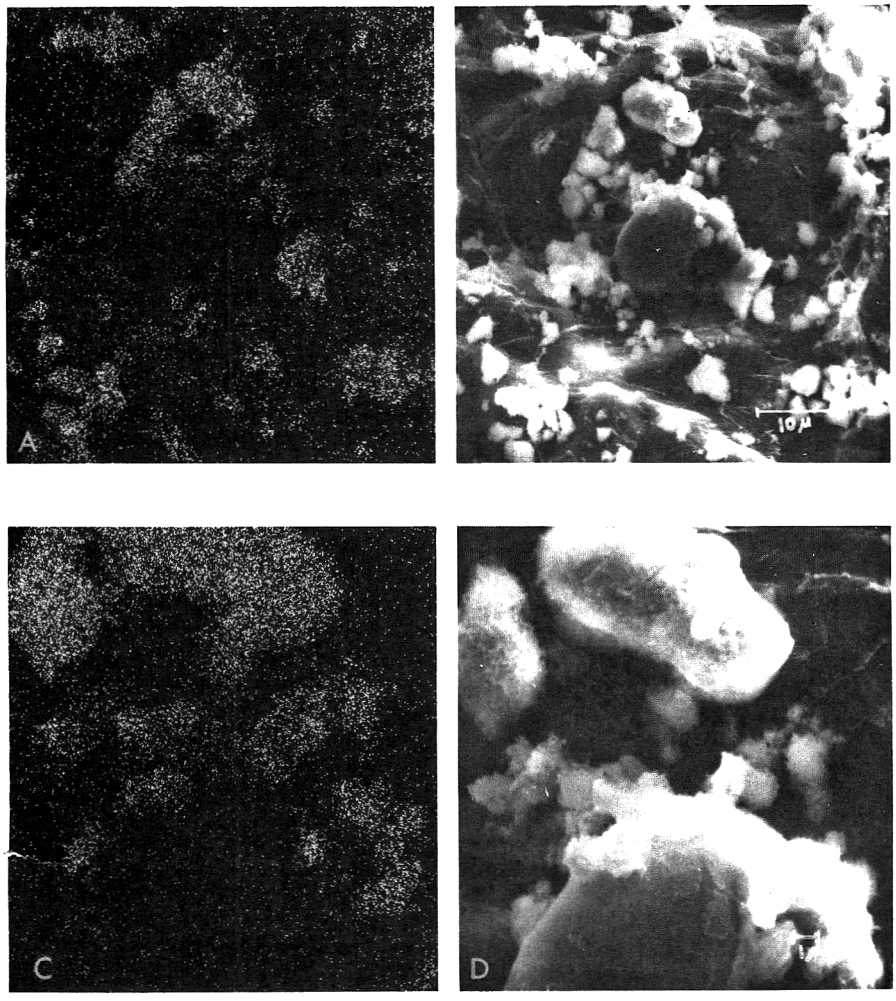

Fig. 5—Copper-stained green banana tissue: (A) X-ray distribution image for $Cu_{\overline{K}\alpha}$ corresponding to (B) secondary electron image (1500X); (C) X-ray distribution image for $Cu_{\overline{K}\alpha}$ at higher resolution and corresponds to (D) secondary electron signal (1500X).

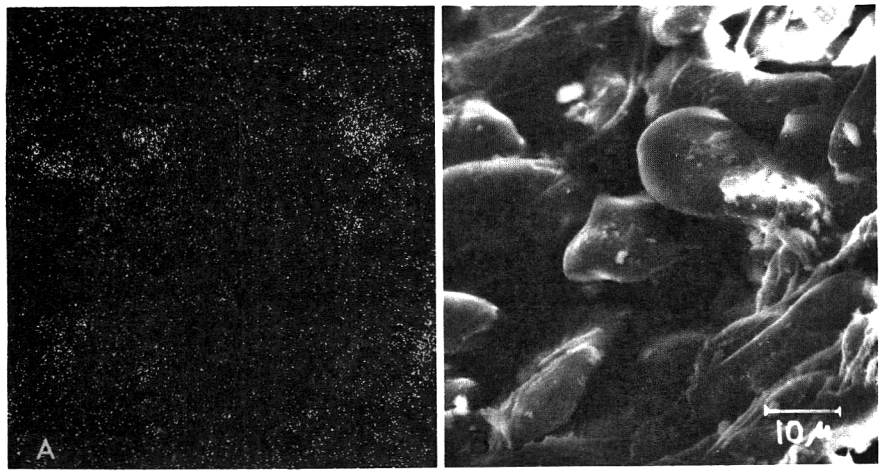

Fig. 6—Deproteinized banana tissue stained with copper: (A) X-ray distribution for $Cu_{\overline{K}\alpha}$; (B) corresponding secondary electron image (1500X).

attributed to the reducing effect of sodium metabisulfite used to prevent darkening of the tissue. These results confirm observations by optical microscopy which show that banana tissue did not produce a blue iodine stain after treatment with metabisulfite.

CONCLUSION

THE RESULTS of this investigation support our hypothesis that macromolecular components of tissues such as starch and protein can be identified by using iodine and copper as selective electron stains for SEM characterization. This tech-

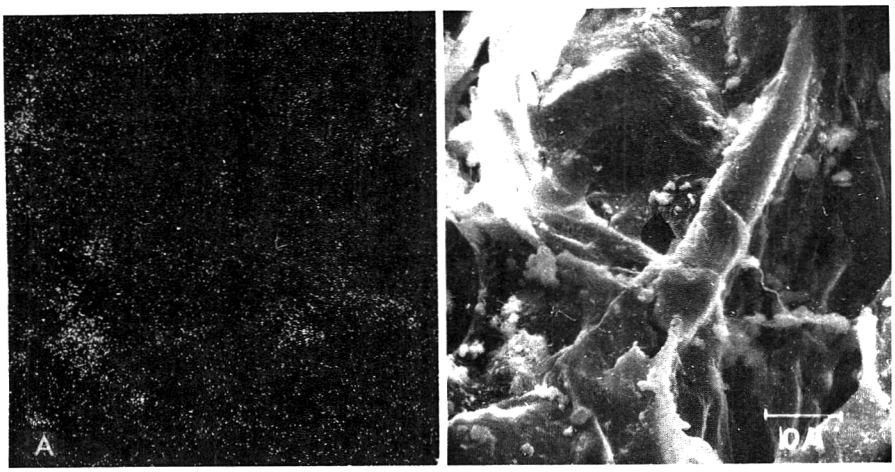

Fig. 7—Deproteinized banana tissue stained with copper: (A) X-ray distribution for $Cu\overline{K}_{\alpha}$ at the cellular interface; (B) corresponding secondary electron image (1500X).

nique can be extended to the identification of specific organelles and molecular groups by proper labeling of such constituents with highly selective stains. The combination of cytochemical tests and X-ray and SEM analyses should not be limited to classical color stains but other elements having specific reaction to a molecule may be employed.

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*COMPOSITION CRANBERRY CELL WALL MATERIAL . . .**From page 115*

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I. SAGUY, S. MIZRAHI and I. J. KOPELMAN
Dept. of Food Engineering & Biotechnology
Technion—Israel Institute of Technology, Haifa, Israel

MATHEMATICAL APPROACH FOR THE DETERMINATION OF DYES CONCENTRATION IN MIXTURES

ABSTRACT

A method is proposed whereby pigments content (in a two or more components admixture) can be determined accurately and directly from the spectra, thus dispensing the time consuming initial step of pigments separation. The procedure is based upon a nonlinear curve fitting of the visible spectrum of the pigments with a predicted function of the individual dyes. The logarithmic normal distribution function showed a remarkable fitting with the pigments tested (Amaranth—Red # 2, Tartrazine—Yellow # 5 and Yellow 2G) thus, used as the mathematical model for the curve fitting process.

INTRODUCTION

COLOR CONTENT and determination is one of the most important features in food technology. The procedures for pigments content determination mostly involved spectrophotometric measurement (AOAC, 1975) of the pure substance. Thus, where a mixture of dyes is involved, prior time-consuming separations in electrophoresis and/or column chromatography are normally required (AOAC, 1975). If however, the components are mutually unreactive, separation may be dispensed with: the visible or UV spectrum of the mixture is used for deriving a set of linear equations with the individual component contents as unknowns (Ewing, 1969).

Proper mathematical description of the spectral data coupled with advances in technology in spectrophotometry, may provide one with the ability of continuously analyzing pigments concentration in a mixture. Thus, the proposed method suggests a procedure whereby mixtures of dyes can be accurately determined for its specific concentration obviating the need for prior time consuming separation. The method is based upon the statistical matching of the predicted mathematical visible spectrum of the pigments with their respective experimental data.

EXPERIMENTAL

AMARANTH RED. NO. 2, Tartrazine Yellow No. 5 (both FD & C) and Yellow 2G (Williams, Hounslow, U.K.) were dissolved in bidistilled water to yield stock solutions of 27, 35 and 14 mg/L respectively. Extinction coefficient of the pure pigments in water solution are given below:

	$E^{1\,cm}_{1\,\%}$	λ_m (nm)
Amaranth	332	521
Tartrazine	286	426
Yellow	428	404

The visible spectra (360–620 nm, read at 10 nm intervals) of ten dilution levels of the pure dyes and seven ternary mixtures (all buffered at pH 5, with McIlvaine's citric phosphate buffer, 0.1M; Anon, 1968) were recorded using a Beckman DB spectrophotometer.

Graphical presentation (Calcomp plotter) and curve fitting (nonlinear least squares (BMDX85—Dixon, 1971) were carried out on the Technion IBM 370/168 computer.

Statistical analyses were performed by linear regression (BMDO2R—Dixon, 1971). Comparison between actual and predicted concentration was carried out by the t test (Volk, 1958) in order to establish the significant level of the differences.

RESULTS & DISCUSSION

THE DESCRIBED METHOD suggests a procedure whereby pigment concentration (in a two or more components admixture) can be accurately determined by the visible spectrum of the mixture. The procedure is based upon curve fitting of the visible spectrum of the mixture with a predicted curve based upon mathematical function of the individual pigment. The selection of a mathematical function, having a similar pattern to that of the pigment's visible spectrum, is obviously the first step of such a procedure. Mathematical functions are often used to interpret and describe spectral data (Porter and Thomas, 1956; Porter and Rosenzweig, 1965; Rosenzweig and Porter, 1960). Of the functions tested (all with skew pattern), the logarithmic normal distribution showed remarkable fitting. Specifically, a variable is said to have a logarithmic normal distribution if the logarithm of the variable is normally distributed (Hald, 1962):

$$p\{x\} = \frac{M}{\sqrt{2\pi}\alpha x} \exp\left[-(\log x - \log\epsilon)^2/2\alpha^2\right] \text{ for } o < x < \infty \qquad (1)$$

where: x = independent variable; $p\{x\}$ = probability; M = log(e) (= 0.4343); α^2 = a measure of the concentration of the distribution around the mean; ϵ = median of distribution.

The value of the abscissa x_m at which the distribution curve reaches its maximum value, is obtained by differentiating Eq (1) and equating the derivate to zero:

$$x_m = \epsilon\, 10^{-(\alpha^2/M)} \qquad (2)$$

Substituting it in Eq (1), ϵ is eliminated and we have:

$$p\{x\} = \frac{M}{\sqrt{2\pi}\alpha x} \exp\left[-(\log\frac{x}{x_m} - \frac{\alpha^2}{M})^2/2\alpha^2\right] \qquad (3)$$

In our case $p\{x\}$ was taken as the absorbance at wavelength referred to an arbitrary level of λ_{ref}, which transforms the abscissa to match the skew pattern occured at lower wavelength. Thus, λ_{ref} was chosen arbitrarily as 650 nm where absorbance is partically zero, so we get:

$$x = \lambda_{ref} - \lambda \quad (620 \geq \lambda \geq 360 \text{ nm}, \lambda_{ref} = 650 \text{ nm}) \qquad (4)$$

$$A_\lambda = \frac{M}{\sqrt{2\pi}\alpha(\lambda_{ref} - \lambda)} \exp\left[-(\log\frac{\lambda_{ref} - \lambda}{\lambda_{ref} - \lambda_m} - \frac{\alpha^2}{M})^2/2\alpha^2\right] \qquad (5)$$

where: λ_m is the wavelength of the peak, at which maximum absorbance is read.

Eq (5) describes the absorbance A_λ as a function of the wavelength for a pigment at a constant concentration. Generalizing this equation and taking into account a different (but constant) concentration can be done by inserting the concentration factor P into Eq (5).

$$A_\lambda^c = PA_\lambda = P\frac{M}{\sqrt{2\pi}\alpha(\lambda_{ref}-\lambda)}\exp\left[-\left(\log\frac{\lambda_{ref}-\lambda}{\lambda_{ref}-\lambda_m}-\frac{\alpha^2}{M}\right)^2/2\alpha^2\right] \quad (6)$$

where: P = concentration factor; A_λ^c = absorbance at concentration c and wavelength λ.

Eq (6) enables predicting the substance absorbance A_λ^c at any given wavelength λ and concentration c. It is seen that three parameters, namely λ_m, α and P, must be known for predicting A_λ^c. The first of those is a characteristic of the pure material (see Table 1). α and P were determined according to the following procedure: The experimental spectra data consist of absorbance vs wavelength for various concentrations for each of the pure substances. The data were curve-fitted (nonlinear least squares technique, see "Methods") with Eq (6), so that the best fitted parameters (P and α) were derived. The results indicated that α is practically constant (having a very low coefficient of variation) thus, independent of concentration (Table 1).

Finally, using $\bar{\alpha}$ (average of α; Table 1) and rearranging Eq (6) resulted with:

$$A_\lambda^c = P\frac{M}{\sqrt{2\pi}\bar{\alpha}(\lambda_{ref}-\lambda)}\exp\left[-\left(\log\frac{\lambda_{ref}-\lambda}{\lambda_{ref}-\lambda_m}-\frac{\bar{\alpha}^2}{M}\right)^2/2\bar{\alpha}^2\right] \quad (7)$$

The best refitted P (using Eq 7 and values of $\bar{\alpha}$, Table 1) is obviously concentration dependent, indicating a linear correlation between concentration c and concentration factor P can be assumed:

$$P = m + nc \quad (8)$$

where: m = intercept; n = slope.

The respective correlation coefficients (r), the values of the intercept (m), and the slope (n) for the three evaluated colors are listed in Table 2.

The very good agreement between the actual visible spectrum data and the predicted curve (based upon Eq 7) is shown in Figures 1, 2 and 3 for Amaranth, Yellow and Tartrazine, respectively.

A further step in the procedure was to show that the concept outlined for the individual pigment can be applied also for the determination of dyes concentration in a ternary mixture.

Table 1—Curve fitted statistical parameters of the pure dyes (10 concentrations)[a]

Dye	Conc range (mg/L)	λ_m (nm)	$\bar{\alpha}$	C.V. (%)	Residual mean squares (X 10^4)
Amaranth	2.7–27.0	521	0.1420002	1.90	0.76–25.3
Yellow	1.8–14.0	404	0.0680192	3.91	0.07–4.2
Tartrazine	4.3–35.0	426	0.0782247	6.20	3.22–8.9

[a] λ_m = wavelength at which maximum absorbance is read; $\bar{\alpha}$ = average of α; C.V. = coefficient of variation ($\frac{\text{standard dev } \alpha}{\bar{\alpha}} \times 100$)

Table 2—Linear correlation between concentration factors and concentrations of pure dyes (10 data points)

Dye	Conc factor	Conc range (mg/L)	Slope n	Intercept m	Corr coeff r
Amaranth	P_1	2.7–27.0	3.75	1.955	>0.999
Yellow	P_2	1.8–14.0	4.34	–0.332	>0.999
Tartrazine	P_3	4.4–35.0	3.02	1.166	>0.999

This can be done simply by determination of the mixture absorbance (A_λ^T) at several wavelengths, and the computation of the total absorbance, A_λ^T in the manner outlined below:

$$A_\lambda^T = \frac{M}{\sqrt{2\pi}(\lambda_{ref}-\lambda)}\left\{\frac{P_1}{\alpha_1}\exp\left[-\left(\log\frac{\lambda_{ref}-\lambda}{\lambda_{ref}-521}-\frac{\bar{\alpha}_1^2}{M}\right)^2/2\bar{\alpha}^2\right]\right.$$
$$+\frac{P_2}{\bar{\alpha}_2}\exp\left[-\log\frac{\lambda_{ref}-\lambda}{\lambda_{ref}-404}-\frac{\bar{\alpha}_2^2}{M}\right)^2/2\bar{\alpha}_2^2\right]$$
$$\left.+\frac{P_3}{\bar{\alpha}_3}\exp\left[-\left(\log\frac{\lambda_{ref}-\lambda}{\lambda_{ref}-426}-\frac{\bar{\alpha}_3^2}{M}\right)^2/2\bar{\alpha}_3^2\right]\right\} \quad (9)$$

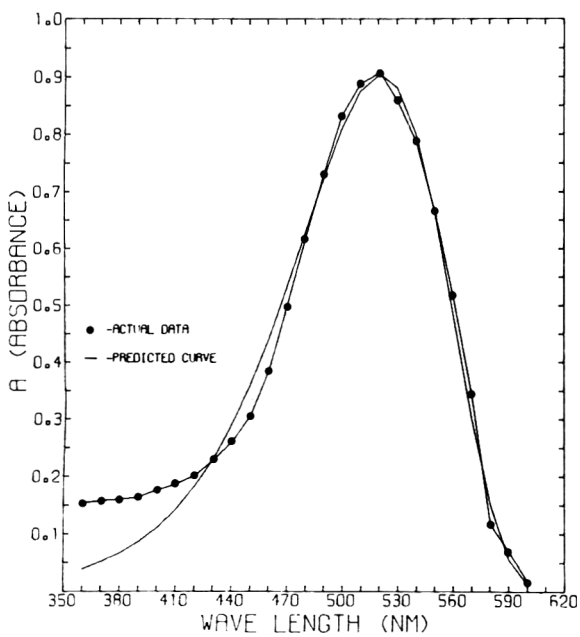

Fig. 1—Predicted and actual visible spectra of Amaranth—a typical sample (c = 27.0 mg/L).

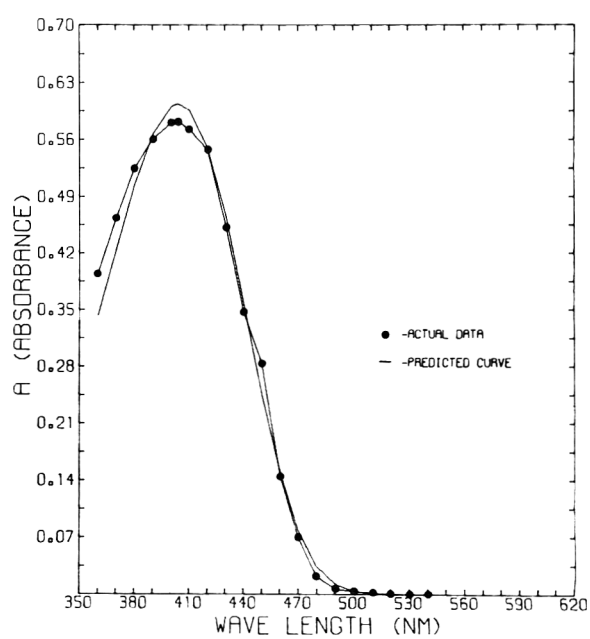

Fig. 2—Predicted and actual visible spectra of Yellow—a typical sample (c = 14.0 mg/L).

Table 3—Predicted vs experimental content of three-component mixtures

Sample No.	Dye content (mg/L): Amaranth Actual	Amaranth Predicted	Amaranth Difference (%)	Yellow Actual	Yellow Predicted	Yellow Difference (%)	Tartrazine Actual	Tartrazine Predicted	Tartrazine Difference (%)
1	10.800	10.763	0.3	3.861	3.939	−2.0	1.701	1.680	1.3
2	6.885	6.701	2.7	4.239	4.307	−1.6	2.241	2.239	0.1
3	6.237	6.350	−1.8	8.721	8.891	−2.0	11.070	11.919	−7.7
4	9.531	9.698	−1.8	8.991	8.693	3.3	8.640	8.160	5.6
5	6.615	6.524	1.4	3.888	3.691	−1.9	1.350	1.359	−0.7
6	19.278	19.101	0.9	5.778	5.434	6.0	1.917	1.919	−0.1
7	16.875	17.210	−2.0	7.020	7.213	−2.7	3.375	3.360	0.4
Absolute mean difference (%)			1.6			2.8			2.3
Standard deviation of difference (%)			0.8			1.5			3.1
Student's t value		0.239			0.136			0.320	
Significance level		0.001			0.001			0.001	

The experimental absorbance data of the three pigments was curve fitted with its respected predicted function (Eq 9). The fitted curve yields the concentration factors for the three components, which can be converted to absolute concentrations by means of the linear relationship referred to earlier.

The procedure was checked by comparing the predicted value with the actual dyes concentration in 7 different mixtures (Table 3).

Statistical comparisons (t test) showed nonsignificant differences ($p < 0.001$) between predicted and actual concentration, thus, indicating the excellent agreement as illustrated in Fig. 4 (Sample No. 3).

Comparing the results with the normal method of three linear equations (Ewing, 1969), showed better accuracy for the proposed method (Table 4). Obviously, the predicted accuracy will be improved with the increase of number of wavelength used for the nonlinear least square curve fitting (Table 4).

The advantages of the nonlinear curve fitting is clearly obvious since it enables to solve multi-equations, yielding a statistically optimal solution, taking into account the entire spectrum further than limited number of wavelengths. Further, nonlinear least squares curve fit techniques are readily avail-

—Continued on page 134

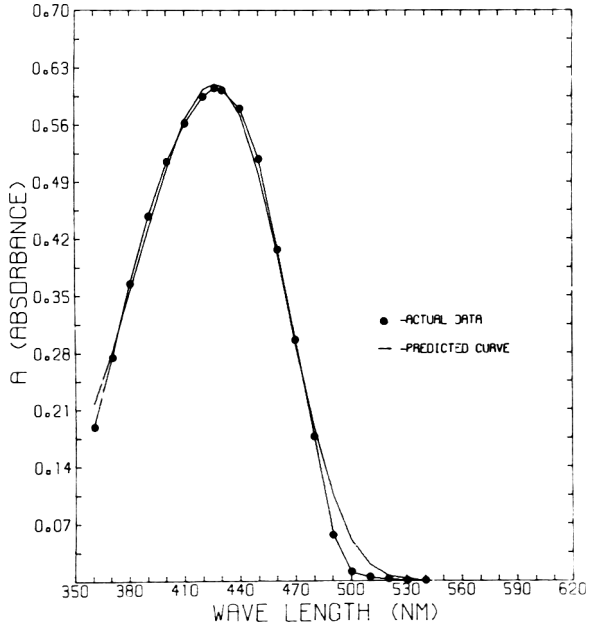

Fig. 3—Predicted and actual visible spectra of Tartrazine—a typical sample (c = 21.0 mg/L).

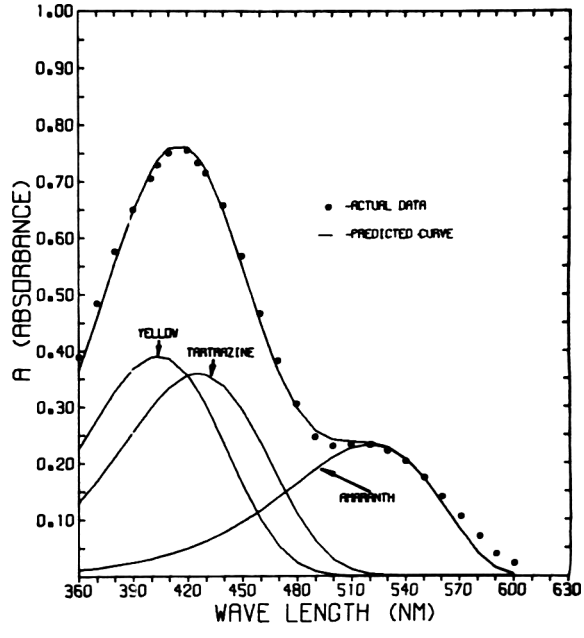

Fig. 4—Predicted and experimental data of a typical three dyes mixture (concentration of 6.24, 8.72, 11.07 mg/L for Amaranth, Yellow and Tartrazine, respectively).

I. SAGUY, I. J. KOPELMAN and S. MIZRAHI
Dept. of Food Engineering & Biotechnology
Technion—Israel Institute of Technology, Haifa, Israel

COMPUTER-AIDED DETERMINATION OF BEET PIGMENTS

ABSTRACT

A quick and accurate method is proposed for determining all major beet pigments (betanin, vulgaxathin -I, betalamic acid) and browning substances, from the visible spectrum of the mixture. The procedure is based on nonlinear curve fitting of the spectrum with a predicted function of the individual pigments, and obviates the need for laboricus and time-consuming separation. The approach is extremely useful in continuous monitoring of time- and temperature-related processes, such as drying and storage.

INTRODUCTION

INTEREST IN NATURAL FOOD colorants has increased markedly in the past few years, particularly because of intensified consumer awareness to the health aspects of some artificial dyes. Dehydrated red beets and beet juice concentrates are permitted in the U.S. as colorants (1960 Color Additive Amendment, Food Drug and Cosmetic Act of 1938) and have been shown to be applicable in some food systems (von Elbe and Maing, 1973; Pasch et al., 1975). Beetroot pigments (so-called betalains), all of them water soluble, comprise two main groups: betacyanins and betaxanthins. In the first group, the principal component is betanin, which contributes 75–95% of the total red color (Nilsson, 1970; von Elbe et al., 1972); in the second, vulgaxanthin-I which contributes approximately 95% of the yellow color (Nilsson, 1970). Another yellow pigment, betalamic acid (Kimler et al., 1971), derives directly from cleavage of betanin and is probably the key intermediate in the biogenesis of all betalains.

Separation of the pigments, so far restricted to laboratory techniques, is based on ion-exchange-resin chromatography and/or paper electrophoresis (Powrie and Fennema, 1963; Piattelli and Minale, 1964; Nilsson, 1970; von Elbe et al., 1972). Their quantitative determination, so far limited to total betacyanins, betanin and vulgaxanthin, involved mainly spectrophotometry whereby the absorbance values obtained at the maximum are translated into concentration by means of the appropriate absorptivities (Nilsson, 1970). Other methods are based on electrophoretic separation of individual pigments with the resulting bands quantified by densitometric methods (von Elbe et al., 1972).

The need for monitoring color changes during processing and storage (further complicated by the variability of the raw material), necessitates a method for rapid and accurate simultaneous determination of the pigments in their mixture, dispensing with the time-consuming separation procedure. The method described below is based on the procedure proposed by the authors in an earlier paper (Saguy et al., 1978).

EXPERIMENTAL

TWO HUNDRED grams of Detroit beetroot were homogenized in a Waring Blendor. The cake was washed with small aliquots of water until all pigments were extracted, and the vacuum-filtered extract purified according to Nilsson (1970) by acidification (pH 4), overnight precipitation, and pigment crude separation over Dowex 50 W X2 (H^+ form) resin. Browning substances and pigments being eluted with 0.1% HCl and double distilled water, respectively. Individual pigments were separated by column chromatography, using Polyclar AT (Polyvinylpyrrolidone, GAF Corp., New York) according to von Elbe et al. (1972).

All procedures involve color purification and separations were carried out in a walk-in refrigerated (4°C) darkroom. Visible spectra (360–620 nm) of the pure pigments and the various known mixtures (all buffered at pH 5, with McIlvaine's citric phosphate buffer, 0.1M; Anon, 1968) were recorded using a DB Beckman spectrophotometer. Quantitative characterization of the pure pigments were based on the following data (Wyler and Dreiding, 1959; Piattelli and Minale, 1964; Piattelli et al., 1965; Kimler et al., 1971):

	$E^{1cm}_{1\%}$	λ_m
Betanin	1120	537 nm
Vulgaxanthin 1	750	478 nm
Betalamic acid	142	430 nm

The spectrum of the browning fraction was typical of that produced in Maillard reaction—namely, monotonic increase in the OD towards the UV region (Meschter, 1954); there is no visible maximum, but the absorbance is commonly read at 420 nm. The fraction was standardized against $FeCl_3$ (B.D.H. product, 60% w/v, s.g. = 1.45) which shows the same visible spectrum thus eliminating variations due to the instrument used. Electrophoresis, visible and UV spectra run on the pure separated pigment were in very good agreement with literature data (Piattelli et al., 1965; Nilsson, 1970; Kimler et al., 1971; Dopp and Musso, 1973).

Graphical presentation (Calcomp plotter) and curve fitting non-linear least squares fit (BMDX85–Dixon, 1971) were carried out on the Technion IBM-370/168 computer.

Statistical analysis were performed by linear regression (BMD02R–Dixon, 1971). Comparison of actual and predicted concentration, in order to establish the significance level of the differences, was carried out by the t-test (Volk, 1958).

RESULTS & DISCUSSION

THE PROCEDURE is based on non-linear curve-fitting of the pigment visible spectrum with a predicted curve based upon mathematical function of the individual pigment. As earlier in the original paper by the authors (Saguy et al., 1978), the log-normal distribution function (Eq. 1) was used for the curve-fitting procedure (Eq. 2).

$$P\{x\} = \frac{M}{\sqrt{2\pi\alpha x}} \exp[-(\log x - \log\epsilon)^2/2\alpha^2] \text{ for } 0 < x < \infty \quad (1)$$

$$A^c_\lambda = PA_\lambda = P\frac{M}{\sqrt{2\pi\alpha}\,(\lambda_{ref} - \lambda)} \exp[-(\log\frac{\lambda_{ref} - \lambda}{\lambda_{ref} - \lambda_m} - \frac{\alpha^2}{M})^2/2\alpha^2] \quad (2)$$

where: $A\lambda$ = absorbance at a given wavelength; A^c_λ = absorbance at concentration c and wavelength λ; M = log(e) (= 0.4343); P = concentration factor; x = independent variable; $P\{x\}$ = probability; α = a measure of the concentration of the distribution around the mean; ϵ = median of distribution; λ = wavelength; λ_m = wavelength at maximum absorbance; λ_{ref} = arbitrary wavelength (650 nm) where absorbance is practically zero.

Eq (2) yields the substance absorbance A^c_λ at any given wavelength λ and concentration c. For predicting it, three parameters must be known, namely, λ_m, α, and P. The wavelength at maximum absorbance λ_m is a characteristic of the pure substance and usually independent of concentration. The values, of λ_m for betanin, vulgaxanthin and betalamic acid are given in Table 1. P and α were determined according to the following method. The experimental results consist of absorb-

Table 1—Curve fitted statistical parameters of the pure pigments[a]

Pigment	N	Conc range (mg/L)	λ_m (nm)	$\bar{\alpha}$	C.V. (%)	Residual mean squares (X 10^4)
Betanin	14	0.41–10.15	537	0.1257375	2.49	0.06–16.7
Vulgaxanthin	9	0.92–4.62	478	0.0671545	1.99	0.21–4.1
Betalamic acid	10	0.25–2.48	426	0.0602323	1.89	0.02–1.1
Browning	13	0.23–4.02	–	0.0173183	9.72	0.21–1.4

[a] N = Number of experiments; λ_m = wavelength at which maximum absorbance is read; $\bar{\alpha}$ = Average of the parameter; C.V. = Coefficient of variation ($\frac{\text{standard deviation of } \alpha}{\bar{\alpha}} \times 100$)

ance vs. wavelengths data for each of the pure substances at different concentrations. The data were curve fitted (nonlinear least squares technique, see "Method") with Eq (2) so that the best fitted parameters (P and α) were derived. The results indicated that α is practically constant (having a very low coefficient of variation) thus, independent of concentration (Table 1). Finally, using α (average of α, Table 1) and rearranging Eq. (2) resulted with:

$$A_\lambda^c = P\frac{M}{\sqrt{2\pi\bar{\alpha}}(\lambda_{ref} - \lambda)}\exp[-(\log\frac{\lambda_{ref} - \lambda}{\lambda_{ref} - \lambda_m} - \frac{\bar{\alpha}^2}{M})^2/2\bar{\alpha}^2] \quad (3)$$

The best refitted P (using Eq 3 and values of $\bar{\alpha}$, Table 1) is obviously concentration dependent, indicating that a linear correlation between concentration c and concentration factor P can be taken:

$$P = m + n\,c \quad (4)$$

where: m = intercept; n = slope.

The respective correlation coefficient (r), the value of the intercept (m) and the slope (n) for betanin, vulgaxanthin and betalamic acid are listed in Table 2.

The curve-fitting model for the browning fraction was:

$$A_\alpha^c = P_4\exp(-\alpha_4\lambda) \quad (5)$$

The experimental data were curve fitted into Eq (5) in the same way described above. The respective α_4 (obtained) is listed in Table 1, while the linear correlation between P_4 –the browning concentration factor–and the concentration is given in Table 2.

Figures 1 through 4 are typical curves, in excellent agreement with the predicted curve according to Eq (3) and (5).

A further step was to show that the above procedure is feasible for the four component mixture. Accordingly, the total absorbance (A_λ^T) of the mixture was represented as:

$$A_\lambda^T = \sum_{j=1}^{4}(PA_\lambda)_j \quad (6)$$

and the curve-fitting procedure was applied to A_λ^T data ob-

Table 2—Linear correlation between concentration factors and concentration of the pure pigments

Pigment	No. of data points	Conc factor	Conc range (mg/L)	Slope n	Intercept m	Correlation coefficient r
Betanin	14	P_1	0.41–10.15	9.74	1.170	>0.999
Vulgaxanthin	9	P_2	0.92–4.62	5.98	0.564	>0.999
Betalamic acid	10	P_3	0.25–2.48	11.05	0.123	>0.999
Browning	13	P_4	0.23–4.02	109.12	0.497	>0.999

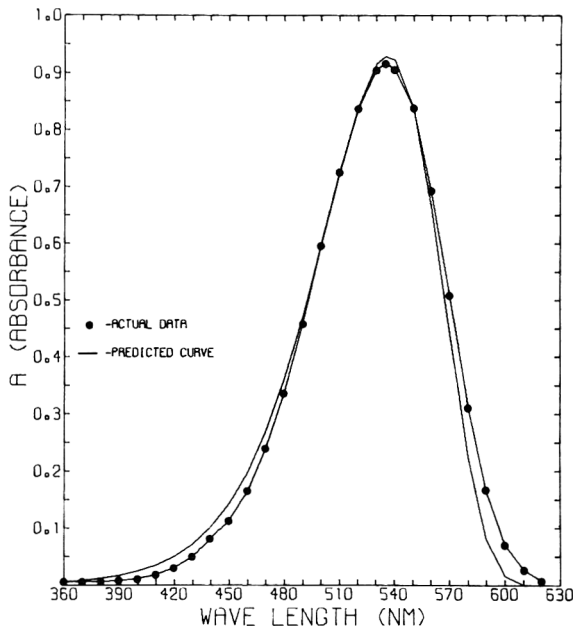

Fig. 1—Predicted and actual visible spectra of betanin—a typical sample (c = 8.13 mg/L).

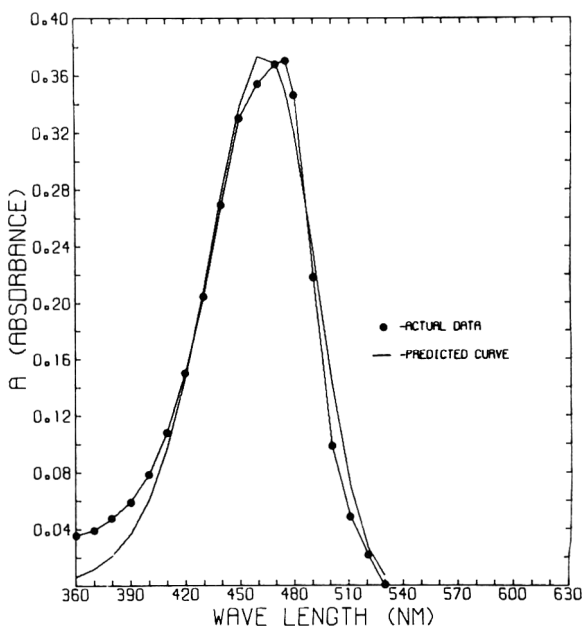

Fig. 2—Predicted and actual visible spectra of vulgaxanthin—a typical sample (c = 4.62 mg/L).

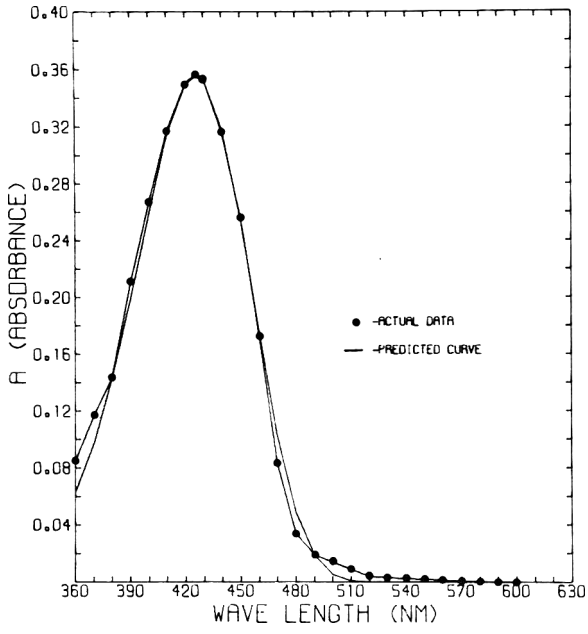

Fig. 3—Predicted and actual visible spectra of Betalamic acid—a typical sample (c = 2.48 mg/L).

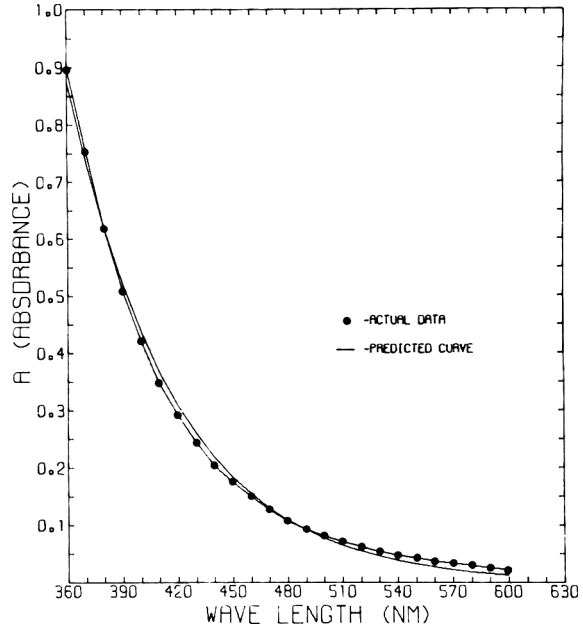

Fig. 4—Predicted and actual visible spectra of browning—a typical sample (c = 4.02 mg/L).

tained for different wavelengths. The best-fitting-curve simultaneously yielded the concentration parameters of the four principal substances, which were in turn, translated into actual concentration by means of the linear relationship referred to earlier.

The procedure was tested statistically (t test) by comparing the predicted values of the known pigment concentrations in 10 different mixtures (Table 3). Results showed nonsignificant differences ($p < 0.001$) thus, indicating the excellent agreement as illustrated in Figure 5 (sample No. 3, Table 3).

Comparing the results obtained using the above outlined technique with those obtained by the Nilsson (1970) method (restricted to betanin, vulgaxanthin and impurities determination) showed improvement in accuracy (Table 4). Obviously, accuracy can be increased with the increase of number of wavelengths used for the curve-fitting.

In conclusion, the proposed procedure is rapid and accurate, and dispenses with the laborious and time-consuming separation steps in the laboratory. Further work on other natural color systems is under way.

Table 3—Predicted vs experimental pigment content in a four-pigment mixtures

	Pigment content (mg/L)											
	Betanin			Vulgaxanthin			Betalamic acid			Browning		
Sample no.	Actual	Predicted	Difference (%)	Actual	Predicted	Difference (%)	Actual	Predicted	Difference (%)	Actual	Predicted	Difference (%)
1	2.132	2.135	−0.1	1.155	1.105	4.3	0.252	0.265	5.2	0.296	0.310	−4.7
2	1.117	1.136	−1.7	1.617	1.641	−1.5	0.436	0.455	−4.4	0.237	0.232	2.1
3	3.198	3.135	2.0	0.600	0.547	8.8	0.194	0.185	4.6	0.261	0.262	−0.4
4	0.853	0.850	0.4	1.732	1.708	1.4	0.204	0.216	−5.9	0.415	0.392	5.5
5	0.640	0.638	0.3	0.531	0.531	0.0	0.436	0.441	−1.1	0.296	0.307	−3.7
6	3.411	3.454	−1.3	1.155	1.193	−3.3	0.242	0.231	4.5	0.190	0.196	−3.2
7	3.838	3.781	1.5	0.693	0.672	3.0	0.136	0.135	0.7	0.267	0.278	−4.1
8	3.876	3.836	1.0	0.577	0.560	2.9	0.039	0.038	2.6	0.356	0.338	5.1
9	1.706	1.707	−0.1	1.270	1.326	−4.4	0.412	0.427	−3.6	0.190	0.188	1.1
10	5.330	5.371	−0.8	1.501	1.558	−3.8	0.116	0.112	3.4	0.166	0.164	1.2
Absolute mean difference (%)			0.9			3.3			3.6			3.1
Standard deviation of difference (%)			0.7			2.4			1.7			1.8
Student's t value		0.517			0.962			1.805			1.887	
Significance level		0.001			0.001			0.001			0.001	

Table 4—Influence of number of wavelengths used in the curve fitting on the percentage of absolute mean difference (± standard deviation) between actual and predicted pigments concentration (10 mixutres)

Method	No. of wavelengths	Betanin	Vulgaxanthin	Betalamic acid	Browning
	28	0.90 ± 0.62	3.34 ± 2.36	3.75 ± 1.78	3.08 ± 1.86
	20	1.32 ± 0.64	4.99 ± 2.93	5.24 ± 3.09	3.38 ± 2.07
Proposed method	10	1.38 ± 0.66	5.36 ± 3.45	5.63 ± 3.64	3.96 ± 2.97
	5	2.27 ± 0.80	6.53 ± 3.68	9.65 ± 6.20	7.12 ± 4.16
Nilsson (1970) method	3	6.59 ± 3.09	10.79 ± 10.33	—	—

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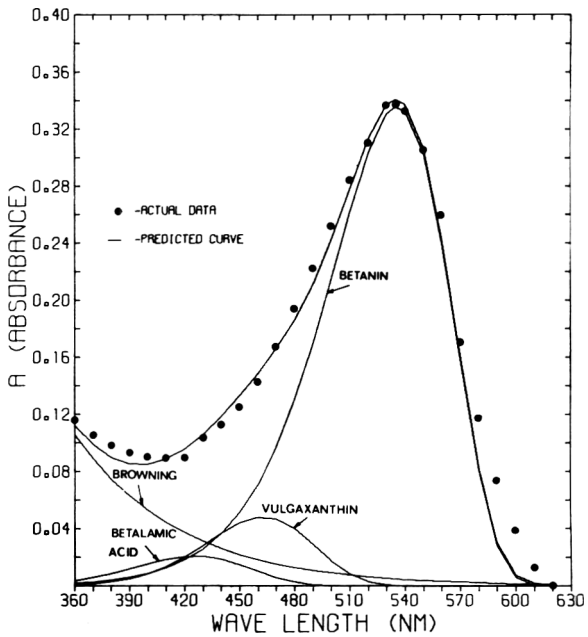

Fig. 5—Predicted and experimental data of a typical four pigments mixture (concentration of 3.20, 0.60, 0.19, 0.26 mg/L for betanin, vulgaxanthin, betalamic acid and browning, respectively).

S. D. SENTER and W. R. FORBUS JR.
USDA, Richard B. Russell Agricultural Research Center, ARS, Athens, GA 30604
and C. J. B. SMIT
Dept. of Food Science, University of Georgia, Athens, GA 30604

LEUCOANTHOCYANIDIN OXIDATION IN PECAN KERNELS: RELATION TO DISCOLORATION AND KERNEL QUALITY

ABSTRACT

The red-brown discoloration in the testa of pecan [*Carya illinoensis* (Wang) K. Koch] kernels appears to be caused by the oxidation of endogenous leucoanthocyanidins to phlobaphenes, cyanidin and delphinidin. Oxidation was progressive in kernels of the Stuart and Schley varieties during storage for 16 wk at 32°C, 50% RH. The levels of leucoanthocyanidins in the two varieties differed significantly, however, rates of transformation were not different. Correlations between weekly increases in these compounds and peroxides formed in the pecan oils were low. Changes in peroxide values and Hunter values correlated well; thus, total color change of the testa may be a useful index of lipid quality. Differences between varieties in quantities of phenols suggest the influence of these compounds on the stability of pecan kernels.

INTRODUCTION

SEED COAT (testa) color is a major criterion in the quality determination of shelled pecans. The U.S. Standards for Grades of Shelled Pecans (USDA, 1969) defines four grades of kernels on the basis of color classification. These classifications are: (1) light, (2) light amber, (3) amber, and (4) dark amber—terms that imply the colors golden, light brown, medium brown, and dark brown, respectively. A light (golden) color is indicative of a fully mature pecan that has been properly harvested, processed, and stored; and pecans so colored command a premium price in the market channels. However, the degree of lightness is a varietal characteristic (Woodroof, 1967) and should not be confused with the amber and dark colors that are caused by exposure of the kernels to adverse conditions.

Several factors are known to affect the coloration of pecan kernels. Woodroof (1967) observed seasonal differences in the darkness of kernels within varieties and attributed the differences to growing conditions. The availability of moisture, location of trees within an orchard, and differences in horticultural practices were implicated. These factors also influenced the overall quality of the pecans. Time of harvest is highly implicated in the color quality of kernels. Quality is highest in nuts harvested as soon as possible after maturation (Heaton et al., 1975). While premature harvesting results in underdeveloped, white kernels, delayed harvesting can result in the exposure of nuts to the damaging effects of weather, which includes heating by the sun, often to internal temperatures exceeding 38°C, and wetting by fog, rain and soil. Such conditions darken, discolor, and promote rancidity of the kernels (Heaton, 1974). Studying the effects of time of harvest on the quality attributes of pecan nuts, Heaton et al. (1975) found that within a harvest period of 4 wk, the patterns of changes in the Hunter a and L values were similar for kernels of three varieties even though their testa characteristics differed. The changes were attributed to a natural weathering process and were found to be more rapid in the first 2–3 wk than in the last 3–4 wk. In this study, the early harvested nuts were characterized by a harsh, pungent flavor that was not present in nuts harvested later in the season. This study and one by Woodroof and Heaton (1961) showed that a minimal amount of discoloration is associated with the development of a desirable pecan flavor. High moisture content has also been shown to accelerate kernel discoloration (Heaton et al., 1975), and prompt removal of moisture to the desired 4–5% level by appropriate means is recommended to maintain color quality.

Pecans are a semi-perishable product, and as such, must be refrigerated to maintain quality. At temperatures below −7°C, pecans can be kept free from insect damage and molding indefinitely, and protected against discoloration and rancidity for at least 30 months. At temperatures above 4.5°C, kernels are subject to severe quality damage including discoloration (Heaton, 1974).

Many factors have been implicated in the development of discoloration in pecan kernels and efforts have been made to prevent this defect. However, the chemical changes causing this transformation have not been explained. Short-time exposure of kernels to faint traces of ammonia has been shown to cause irreversible black discoloration of the testas (Woodroof and Heaton, 1961; Woodroof, 1967; Heaton, 1974).

Comparison of this finding with those of Mathew and Parpia (1970), who reported the discoloration of cashew nuts, suggested to us that phenolics may be implicated in the discoloration of pecans. We therefore undertook to identify the discoloring pigments and their precursors in pecan kernels, and to determine the relationship, if any, between the formation of these pigments and the peroxidation of the oils in the kernels.

EXPERIMENTAL

Preliminary analyses

The characteristic discoloration of pecans was rapidly produced in kernels of the Stuart and Schley varieties. Kernel halves from in-shell samples that had been maintained at 0°C were placed in a forced-air oven for 7 days at 70°C. Portions of the samples were then removed daily, placed in polyethylene bags, and stored at 0°C. The pecans were progressively discolored from a light amber (0 day) to a dark amber (7 day) color and were used to optimize extracting conditions and analytical methods.

To extract the pigments, we tested the solvent systems used by Mathew and Parpia (1970). We then tested pigment extraction by 0.1% HCl in methanol at reflux temperature. Finally, we established that the pigments responsible for the discoloration produced within the 7-day period were effectively removed from all kernels by a 3-hr soak at ambient temperature in the methanolic HCl solution. Also, testas were separated from the kernels, and both these kernel parts were separately extracted. The spectra of these extracts and subsequent isolates were determined with a Beckman Acta III, double-beam scanning spectrophotometer. For isolation of the pigments from these extracts, the methanolic extract was evaporated to dryness at reduced pressure, and the residue suspended in water. The pigments in the suspension were then extracted with amyl alcohol and analyzed by paper and thin-layer chromatography (TLC). The thin-layer plates were of microcrystalline cellulose, type 20 (Sigma Chemical Co.), and were developed two-dimensionally for 15 cm with formic acid-HCl-water (10:1:3) and amyl alcohol-acetic acid-water (2:1:1), respectively. Paper chromatographic analysis was with Whatman No. 1 chromatography paper (W. & R. Balston, Ltd.) developed two-dimensionally for 16 hr each with butanol-acetic acid-water (4:1:2) and acetic acid-HCl-water (30:3:10), respectively. The isolates obtained were identified on the basis of

solubilities, Rf values, absorbance spectra, color, and reactions with specific sprays and reagents.

Stored samples

Inshell pecans of the Stuart and Schley varieties that had been commercially cleaned and sized to ca 2.4 cm in diameter were obtained from a commercial shelling plant in Georgia in November 1974. The nuts were obtained in 45.4-kg lots of each variety and were from different trees and orchards. Both lots were stored at 0°C in woven bags until removed for shelling and use in this study.

In July 1975, the pecans were removed from storage, equilibrated to ambient temperature, and shelled mechanically. Intact halves of each variety were selected for uniformity of color and freedom from defects. The selections within each variety were divided into three replicate samples of ca 4.54 kg each and spread evenly in aluminum trays to a depth not exceeding 5 cm. The samples were stored in darkness at 32°C, 50% RH.

Sixty halves were removed weekly from each replicate sample during 16 wk of storage for Hunter color measurements, pigment extraction, and peroxide value determinations. At 3-wk intervals for 15 wk, halves that were taken from storage and not damaged by analyses were sealed in polyethylene bags and stored at −34.5°C until the end of the storage study for analysis of flavanols and phenols.

Hunter color measurements. The 60 halves taken weekly from each replicate sample were divided into three subsamples for color difference measurements with a Hunterlab D25D Measuring Unit equipped with a 2-inch optical head. Each subsample of 20 halves was placed in a 2-inch diameter plexiglass holder, the kernels being arranged so that the flat side of the half faced up. The L (lightness), a (red), and b (yellow) values for each subsample were determined from four positions; the sample holder was rotated 90° between readings. From these values, total color change (ΔE) during storage for each variety was determined by the equation $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$ (Scofield, 1943), where ΔE represents total color difference between storage periods, and ΔL, Δa, and Δb represent differences between the mean values of the samples before storage and after the cumulative storage period.

Phlobaphene and anthocyanidin quantitations. About 20g (8–10 halves) of the 60 halves was extracted with 200 ml methanol-HCl, as previously described. The extract was filtered through Whatman No. 2 filter paper and diluted so that a 1:10 ratio of kernel weight to solvent volume was maintained. Absorbance of the extract was determined at 450 and 550 nm against (1) solvent, for determination of total absorbance, and (2) similar extracts of fresh kernels for determination of net increase in absorbance. Means of these analyses were determined within each variety and are reported.

The concentrations of anthocyanidins (A_{550}) and phlobaphenes (A_{450}) (Ribéreau-Gayon, 1972; Robinson, 1967) were determined from standard curves established with chromatographically pure cyanidin (courtesy of L. Jurd, WRRL) and phlobaphenes. The phlobaphene standard was prepared from hydrolyzed methanolic-HCl extracts of pecan kernels. After extraction with the acid-alcohol and subsequent hydrolysis with 1N HCl for 1 hr, a portion of the hydrolysate was applied to a 0.5- × 10-cm column of Porpak, type Q (Waters Associates, Inc.). The column was washed successively with copious amounts of 0.1% HCl, ether, and ethyl acetate. The phlobaphenes were then eluted with 0.1% HCl in methanol, taken to dryness with a rotary-evaporator, and dried to constant weight over P_2O_5 in a desiccator evacuated to 100 mm Hg pressure. The purity of methanolic solutions of this standard was established by TLC on microcrystalline cellulose with specific reagents for visualization, and by spectrophotometric analyses (Ribéreau-Gayon, 1972; Robinson, 1967).

Peroxide values. Peroxide values (PV), expressed as meq O_2/kg oil, were determined weekly on the replicate samples and used as indices of quality. About 20g of kernels was extruded from a perforated aluminum block with a plunger and Carver laboratory press by application of ca 70 kg/cm² pressure that forced the kernels through the holes (0.08-cm diam) in the block. The macerate was collected in a beaker, transferred to centrifuge tubes and centrifuged for 10 min at 10,000 × G. The separated oil was decanted into a glass vial, which was then flushed with N_2, capped, and stored in the dark for no longer than 1 hr before analysis. Peroxide values were determined according to Holloway (1966) on 75-mg aliquots of oil (±2 mg).

Flavanols and total phenols. At the end of the 16-wk storage period, the halves that had been taken at 3-wk intervals were removed from storage, warmed to ambient temperature, extracted with methanolic HCl, filtered, and diluted to 200 ml with solvent as previously described. A 1-ml aliquot (representative of 100 mg of kernel) was diluted 1:10 with 0.1% HCl, and 1-ml aliquots of this dilution were analyzed for flavanols and total phenols according to Swain and Hillis (1959). Concentrations, as mg/g kernel, were determined from standard curves established with the cyanidin standard.

Statistical analysis. The statistical analysis system (SAS) developed by Barr and Goodnight at N.C. State University, Raleigh, was used to determine differences between values obtained in the weekly analyses. For the entire data set, all possible correlations between variables were calculated for each variety and for the varieties combined. Also for each variety, the regression of each parameter as a function of storage time was determined.

RESULTS & DISCUSSION

Preliminary analyses

The red-brown discoloration of the pecan halves stored for 7 days at 70°C was confined to the testa, as determined by the separate analysis of testa and kernel residue. Since these storage conditions would tend to inhibit enzymatic activity, we believe that the color development was caused by nonenzymatic oxidation of colorless compounds. Pigment extraction from pecans by the solvent systems used by Mathew and Parpia (1970) to extract cashew nut pigment was not rewarding. Partial success was achieved only with the ethyl acetate-ethyl alcohol system. However, all the extracts showed the presence of phenolic compounds when tested.

Since flavanoids form the largest group of phenolic compounds in the plant kingdom and are sufficiently polar to be extracted with methanol (Robinson, 1967), discolored pecan halves were taken from the 7-day sample and refluxed for 1 hr in 0.1% methanolic HCl. At the end of the extraction, the kernels were devoid of pigment and were a light golden color. Although this procedure successfully removed the pigments from the 7-day sample, the extract was similar in color to that of fresh kernels. Spectrophotometric analyses of these extracts showed maximum absorbances (A) at 550 and 450 nm. The intensities of absorbances were about the same between both extracts; further, A_{550} was about one-fifth A_{450}. The transformation of colorless compounds in the fresh sample to the colored derivatives suggested that leucoanthocyanidins were the precursors of the developed color (Harborne 1973). This was supported by the absorbances at 550 and 450 nm, which are reported to be specific for anthocyanidin and phlobaphene compounds formed from the oxidation of leucoanthocyanidins (Geissman, 1962; Robinson, 1967). Additionally, the solubility of the compounds in methanol and the ratios of A_{450} to A_{550} were indicative of leucoanthocyanidin precursors (Ribéreau-Gayon, 1972; Harbourne, 1973).

Leucoanthocyanidins have high reducing potential and tend to polymerize during analyses; therefore, they are usually studied indirectly through their oxidative products—anthocyanidins and phlobaphenes—on the basis of solubilities, absorbance spectra, and Rf values (Geissman, 1962; Goldstein and Swain, 1963; Harbourne, 1973). For study of the leucoanthocyanidins of pecans, the methanol-HCl extract obtained at ambient temperature was evaporated with vacuum, and the residue was hydrolyzed in 1N HCl for 1 hr at 100°C. An intense red pigmentation was extracted into amyl alcohol from the cooled hydrolysis mixture that had an absorbance maxima at about 550 nm. It was separated by paper chromatography into two compounds, one red and the other magenta color in visible light. Rf values of these compounds on paper developed with Forestal reagent were 0.49 and 0.31, respectively, and corresponded to Rf values for cyanidin and delphinidin (Harbourne, 1973). The absorbances of the red and magenta compounds were 534 and 546 nm, respectively, in methanol. These maxima were shifted to 552 and 569 nm, respectively, with the addition of 2 drops of 5% $AlCl_3$ in methanol.

The data (Table 1) indicated that the compounds were cyanidin and delphinidin, and that they were formed by the oxidation of leucocyanidin and leucodelphinidin.

Table 1—Analysis of the anthocyanidins from pecan kernels

Spot no.	Rf[a]	Color[b]	λ max (nm) (methanolic-HCl)	Δ λ max (nm) (with $AlCl_3$)	Identity
1	0.49	red	534	+18	cyanidin
2	0.31	magenta	546	+23	delphinidin

[a] In Forestal reagent
[b] Visible light

Table 2—Weekly values for peroxide[a], phlobaphene[b], anthocyanidin[c] and Hunter[d] determinations on Stuart pecan kernels stored at 32°C, 50% RH

Week	Peroxide value	Absorbance 450	Absorbance 550	Hunter values L	a	b	a/b	ΔE
0	0.10	0.00	0.00	32.6	8.60	15.0	0.57	0
1	0.27	0.03	0.01	31.6	8.81	14.7	0.60	1.08
2	0.72	0.15	0.02	30.4	9.07	14.1	0.65	2.51
3	0.41	0.09	0.03	30.1	9.50	13.9	0.68	2.92
4	0.21	0.11	0.03	28.2	9.74	12.9	0.76	4.97
5	0.17	0.08	0.02	28.8	9.80	12.9	0.75	4.51
6	0.33	0.08	0.02	28.6	9.76	13.0	0.75	4.64
7	0.37	0.09	0.02	27.8	9.94	12.6	0.79	5.49
8	0.94	0.15	0.04	27.1	10.20	12.5	0.81	6.23
9	2.13	0.16	0.05	27.4	10.10	12.5	0.80	5.94
10	1.16	0.16	0.05	27.9	10.40	12.7	0.82	5.58
11	1.53	0.16	0.05	26.6	10.60	11.8	0.88	7.00
12	1.27	0.16	0.05	25.4	10.70	11.4	0.94	8.31
13	1.38	0.13	0.04	25.6	10.70	11.5	0.93	8.08
14	0.54	0.18	0.06	25.1	10.70	11.0	0.97	8.77
15	2.91	0.18	0.06	25.2	10.70	11.0	0.98	8.71
16	1.16	0.22	0.06	25.2	10.80	11.2	0.97	8.61

[a] Expressed as meq O_2/kg oil. Each value is the mean of triplicate analyses.
[b] Absorbance @ λ450. Each value is mean of triplicate analyses.
[c] Absorbance @ λ550. Each value is mean of triplicate analyses.
[d] Each value is the mean of 12 determinations.

Table 3—Weekly values for peroxide,[a] phlobaphene,[b] anthocyanidin[c] and Hunter[d] determinations on Schley pecan kernels stored

Week	Peroxide value	Absorbance 450	Absorbance 550	Hunter values L	a	b	a/b	ΔE
0	0.19	0.02	0.01	33.7	8.88	15.3	0.58	0
1	0.40	0.03	0.01	31.4	9.25	14.3	0.64	2.57
2	0.18	0.05	0.01	31.4	9.93	14.3	0.69	2.76
3	0.34	0.12	0.03	31.2	9.82	14.1	0.70	2.94
4	0.31	0.05	0.01	30.9	10.30	14.1	0.72	3.36
5	1.35	0.11	0.02	29.1	10.50	13.3	0.79	5.30
6	1.57	0.10	0.03	29.2	10.70	13.3	0.80	5.29
7	2.24	0.12	0.02	28.7	10.90	13.1	0.83	5.85
8	2.09	0.12	0.02	27.8	10.80	12.5	0.87	6.90
9	2.94	0.14	0.04	27.6	11.30	12.4	0.91	7.26
10	2.82	0.16	0.05	27.4	11.40	12.3	0.93	7.45
11	3.39	0.15	0.05	25.4	11.50	11.1	1.02	9.70
12	3.76	0.17	0.05	25.3	11.40	10.7	1.04	9.81
13	2.89	0.20	0.05	24.8	11.30	10.6	1.07	10.41
14	3.53	0.22	0.09	24.4	11.30	10.3	1.10	10.86
15	3.36	0.20	0.06	24.3	11.50	10.1	1.14	11.16
16	3.91	0.24	0.07	24.3	11.60	9.9	1.16	11.22

[a] Expressed as meq O_2/kg oil. Each value is the mean of triplicate analyses.
[b] Absorbance @ λ450. Each value is mean of triplicate analyses.
[c] Absorbance @ λ550. Each value is mean of triplicate analyses.
[d] Each value is the mean of 12 determinatons.

Phlobaphenes are formed by the polymerization of leucoanthocyanidins (Bate-Smith and Swain, 1953; Hillis, 1956; Hathway and Seakins, 1957; Roux, 1958; Swain, 1964; Ribéreau-Gayon, 1972) and are included in the broad classification of compounds commonly referred to as condensed or compound tannins. The tannins are known to be composed of several groups, i.e., oligomers of polyhydroxyflavan-3,4-diols, compounds of hydroxyflavan-3-ols (catechin tannins) (Schanderl, 1970), and dimers of hydroxyflavan-3,4-diols and hydroxyflavan-3-ols (biflavans) (Ribéreau-Gayon, 1972). No standard method has been established for studying the tannins because they are complex and not easily isolated. They are usually studied in respect to their total quantities present, degree of polymerization, and constituent monomers.

We undertook to determine whether tannins other than polymers of hydroxyflavan-3,4-diols contribute to pecan discoloration. Methanolic-HCl extracts of fresh pecan kernels were concentrated with vacuum under N_2, and the residue was resuspended in 0.1% HCl. This suspension was extracted with three 10-ml portions of ether and then with three 10-ml portions of ethyl acetate (Mathew and Govindarajan, 1964). The combined ether extracts and combined acetate extracts were concentrated to ca 1 ml with vacuum and a stream of N_2; and portions of these concentrates and the aqueous residue were spotted on cellulose plates and developed one-dimensionally for 15 cm with formic acid-HCl-water (10:1:3). The plates were sprayed with vanillin-sulfuric acid (1g vanillin in 100 ml concentrated H_2SO_4) and with *p*-toluenesulfonic acid (20% in $CHCl_3$) (Stahl, 1962; Robinson, 1967). Results with both spray reagents indicated that catechins were not present. The chromatogram of the ethyl acetate extract sprayed with the vanillin reagent showed an intense pink streak from the origin to an Rf of ca 0.75, indicating the presence of phlobaphenes polymerized to various extents. Chromatograms sprayed with *p*-toluenesulfonic acid showed similar coloration and distribution of the compounds with no evidence of brown color which would have indicated the presence of catechins (Robinson, 1967). Chromatograms of the aqueous residue sprayed with the above reagents showed an intense pink spot at the origin with slight streaking for ca 1 cm, indicating highly polymerized phlobaphenes. No brown color was apparent with the *p*-tolunenesulfonic acid reagent.

The results of the preliminary study indicated that discoloration of pecan testa is caused by the oxidation of leucocyanidin and leucodelphinidin to their respective derivatives—phlobaphenes of varying degrees of polymerization, and, to a lesser extent, cyanidin and delphinidin.

Stored samples

Peroxide values (PV). Regression analyses of data (Table 2,3) show that peroxidation of the oils in both the Stuart and Schley varieties increased linearly with storage time. Also, the varieties differed significantly in the rate of peroxidation ($P < 0.01$). In these analyses, the best fit equations were determined for the dependent variables in relation to time. Plots of these equations and the means of weekly PV are presented in Figure 1.

Forbus and Senter (1976) reported that hedonic ratings of Stuart and Schley kernels decreased significantly at 30 and 33 wk of storage at 21°C respectively, when their PV were ca 1.0. This finding suggests that in the present study, rancidity would have been detected in the Schley variety after 4–5 wk in storage and in the Stuart variety after 8–9 wk. Initially, the PV for both varieties were similar; however, after 16 wk, that of the Schley kernels was 3.91 in contrast to 1.16 for kernels of the Stuart variety.

Phlobaphenes. The absorbance of the methanolic-HCl extracts at 450 nm did not vary significantly between varieties in either the weekly rates of increase or the intensity at the end of 16 wk in storage. Differences among the weekly values in both varieties were significant ($P < 0.01$). Regression

analyses of these data showed that absorbance increases linearly with time. Since variation in the rates of increase between the two varieties was not significant at the 5% level, we pooled the weekly values for both varieties in a linear regression analysis to establish the best fit line for these data (Fig. 2).

Anthocyanidins. The absorbance of the methanolic-HCl extracts at 550 nm increased significantly ($P < 0.01$) with time in both varieties; however, neither the rates of increase nor interaction between varieties and time were significant at the 5% level. The linear regression of the dependent variable (A_{550}) vs time suggests a relationship between the formation of phlobaphenes and anthocyanidins from the oxidation of endogenous leucoanthocyanidins. Linear regression analysis of pooled data from both varieties was used to establish the best fit line for these data (Fig. 3).

Hunter color values. The values given in Tables 2 and 3 for L, a, b, a/b and ΔE determinations indicate the color changes that occurred in the stored pecan kernels. Decreases in the L and b values of both varieties were significant ($P < 0.01$) in relation to time and indicated progressive loss of the golden kernel color. With decrease in lightness and yellow color, a and a/b values increased progressively in both varieties, signifying increasing redness of the kernels. Differences were significant ($P < 0.01$) between varieties, weeks in storage, and in the interaction between varieties and weeks with respect to the a, b, a/b, and ΔE values. Differences in the L values were not significant at the 5% level between varieties but were significant ($P < 0.01$) for both varieties in relation to time. Although the initial a and a/b values were essentially the same for both varieties, the rates of increase were significantly higher in the Schleys than in the Stuarts. Weekly rates of increase in the ΔE values (Fig. 4) reflected the darkening of the kernels. The values varied significantly ($P < 0.01$) between varieties and among weeks, with interaction between varieties and weeks significant at the 0.1% level.

Correlations coefficients. From the entire data set, correlation coefficients were calculated between all variables within each variety and for the varieties combined (Table 4).

In the Hunter analyses, correlations were high (ca 0.95) between all variables in both varieties. The results showed the interrelationship of the L, a, b, a/b, and ΔE values in pecans in that increases in red color were accompanied by decreases in yellow color and lightness.

This study and the report of Forbus and Senter (1976) showed that Stuarts were more resistant to discoloration than Schleys. If resistance to peroxidation were related to the oxidation of leucoanthocyanidins, correlations among these variables would be expected to be higher in the Stuart variety than in the Schley. However, this trend was not observed in the analyses. For the Stuart variety, correlations were rather low among all Hunter values and anthocyanidin analyses (ca 0.62), phlobaphene analyses (ca 0.70), and peroxide values (ca 0.44). Correlation coefficients between the formation of phlobaphenes and anthocyanidins and the peroxide values were low, with values of 0.40 and 0.42, respectively.

For the Schley variety, correlations were acceptable among all variables. The high correlation coefficients, ca 0.95, between all Hunter values, again emphasized the interrelationship of these variables. Correlation coefficients between all Hunter values and phlobaphene formation were ca 0.86, and between anthocyanidin formation, ca 0.79. In this variety, the correlation of phlobaphene and anthocyanidin formation was high (0.92). Additionally, correlation coefficients between Hunter values and peroxide values were ca 0.90; between anthocyanidin formation and peroxide values, 0.77; and between phlobaphene formation and peroxide values, 0.77.

In the combined analyses, these values were, as expected, midrange between values obtained for the two varieties. Correlations between peroxide values and the variables in the Hunter analyses were ca 0.72.

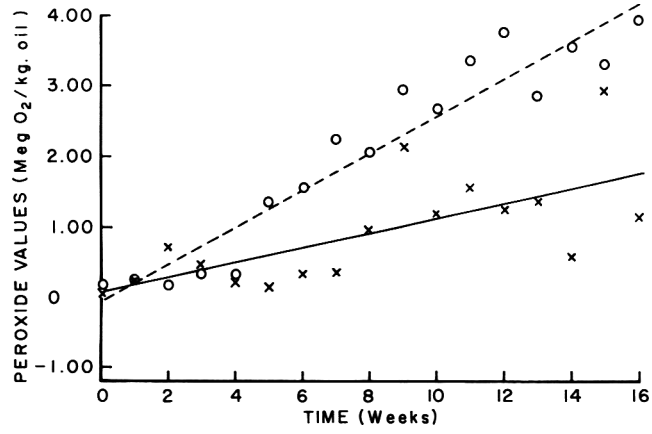

Fig. 1—Relationship between time and peroxide content of oil in Stuart (—,x) and Schley (- - -, o) pecan kernels stored for 16 wk at 32° C, 50% RH. [Stuarts: PV = 0.085 + 0.104 (no. wk); r = 0.68; P = <0.01; S.E. estimate = ≠ 0.55. Schley: PV = −0.052 + 0.25 (no. wk); r = 0.96; P = <0.01; S.E. estimate = ≠ 0.40.]

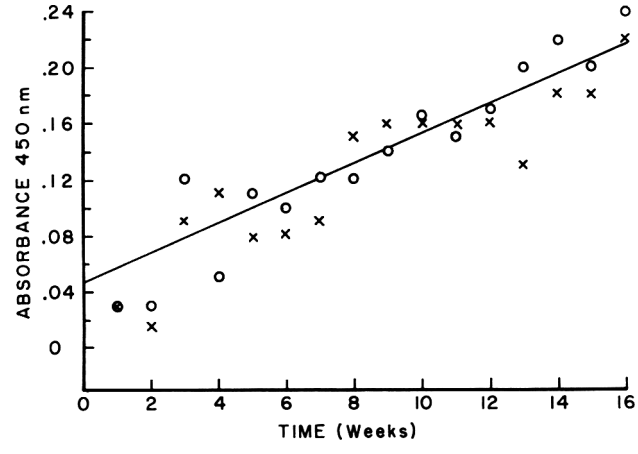

Fig. 2—Relationship between time and phlobaphene formation (A_{450}) in Stuart (x) and Schley (o) pecan kernels stored for 16 wk at 32° C, 50% RH. [A_{450} = −0.048 + 0.01 (no. wk); r = 0.85; P = <0.01; S.E. estimate = ≠ 0.032.]

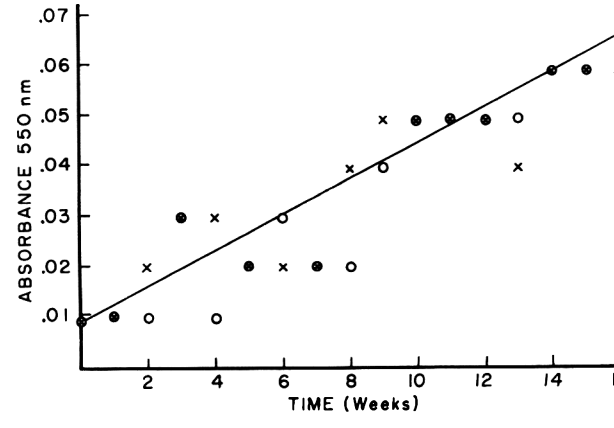

Fig. 3—Relationship between time and anthocyanidin formation (A_{550}) in Stuart (x) and Schley (o) pecan kernels stored for 16 wk at 32° C, 50% RH. [A_{550} = 0.0067 + 0.0036 (no. wk); r = 0.92; P = <0.01; S.E. estimate = ≠ 0.0073.]

These data indicate that the transformation of leucoanthocyanidins to phlobaphenes and anthocyanidins could influence the stability of pecan kernels. However, other factors were present, which, alone or in conjunction with the leucoanthocyanidins, accounted for the difference between the two varieties in storage stability.

Flavanols and total phenols. Tables 5 and 6 show the weekly changes in the leucoanthocyanidin, phlobaphene, and anthocyanidin contents of the stored pecans. Phenolic compounds, which are good reducing agents, were more abundant in the Stuart variety than in the Schley variety. The difference in abundance may explain the greater resistance of the Stuart kernels to peroxidation. Although the potential to discolor was greater for the Stuart pecans, their greater quantities of other phenolic compounds might have protected the leucoanthocyanidins through preferential oxidation. Although the quantities of total phenolic and flavanol compounds varied in the six analyses made on each variety, these differences could be explained by: (1) variation in the quantity of phenolic compounds among nuts within a variety, and (2) increased polymerization of the flavanols to compounds not readily soluble with increasing storage time. Decreasing solubility with increasing polymerization was reported by Geissman (1962) and Ribéreau-Gayon (1972), and was indicated in our study by decreases in the quantities of flavanols with concomitant increases in the formation of phlobaphenes.

The relationship of the leucoanthocyanidins, phlobaphenes, anthocyanidins, and total flavanols are presented in Tables 5 and 6. Increases in phlobaphenes and anthocyanidins reflect decreases in leucoanthocyanidins; and these three classes of compounds collectively comprise the flavanols. Quantitative differences in these compounds between the two varieties reflect differences previously shown in Tables 2 and 3. In Tables 5 and 6, the net increases in the quantities of phlobaphenes and anthocyanidins as compared to the amounts present in fresh kernels are indicative of the more rapid rate of formation of these compounds in the Schley variety.

CONCLUSIONS & RECOMMENDATIONS

OUR STUDY INDICATES that the discoloration in the testa of stored pecan kernels was caused by the oxidation of endogenous leucocyanidin and leucodelphinidin to their respective phlobaphenes and anthocyanidins. The oxidation was progressive in kernels of the Stuart and Schley varieties during 16 weeks' storage at 32°C, 50% RH. Although kernels of the Stuart variety contained larger quantities of leucoanthocyanidins, the two varieties did not differ significantly in either the rate or the extent of phlobaphene formation or of anthocyanidin formation.

Correlation coefficients between the formation of phlobaphenes, or anthocyanidins, and extent of peroxidation were low for both varieties. Although the correlates were acceptable for the Schley variety, they were especially low for the Stuarts. Therefore neither phlobaphene or anthocyanidin value would be a useful index of lipid quality in pecans. The Hunter values, indicative of the total color changes that occur in the kernels, correlated reasonably well with peroxide values. Color measurements can be made nondestructively; hence, they would be useful as an objective means for determining kernel quality.

The study also showed that significant quantities of phenolic compounds other than leucoanthocyanidins, phlobaphenes,

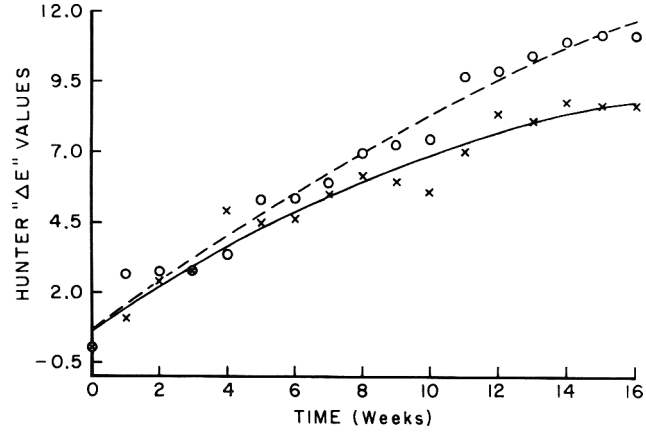

Fig. 4—Relationship between time and Hunter ΔE values of Stuart (—, x) and Schley (- - -, o) pecan kernels stored for 16 wk at 32°C, 50% RH. [Stuart: ΔE = 0.57 + 0.84 (no. wk) − 0.021 (no. wk)²; r = 0.97; P = <0.01; S.E. estimate = ≠ 0.62. Schley: Δ = 0.71 + 0.86 (no. wk) − 0.011 (no. wk)²; r = 0.99; P = <0.01; S.E. estimate = ≠ 0.57.]

Table 4—Correlation coefficients[a] of combined variables for Stuart and Schley pecan kernels

	L[b]	a[b]	b[b]	a/b[b]	ΔE[b]	Anthocyanidins[c]	Phlobaphene[c]	Peroxide
L	1.00	−0.82	0.99	−0.95	−0.97	−0.68	−0.77	−0.65
a			−0.82	0.91	0.90	0.54	0.66	0.76
b				−0.97	−0.98	−0.70	−0.76	−0.70
a/b					0.99	0.65	0.75	0.77
ΔE						0.67	0.77	0.75
Anthocyanidins							0.67	0.51
Phlobophene								1.00
Peroxide								

[a] Each of these probable values are significant at the 0.01 level.
[b] Hunter values
[c] From net absorbance of sample extracts vs. extract of fresh sample

and anthocyanidins were present in the testa of pecan kernels. The compounds appeared to markedly influence the stability of the kernels. Further studies are needed to identify them and to determine their relation to pecan stability.

Table 5—Changes in total phenols and flavanols of Stuart pecan kernels stored for 16 wk at 32° C, 50% RH

	mg/g of Kernel						
						Net increase[d]	
Weeks	Total phenols[a]	Total flavanols[a]	L.A.[b]	Phlobaphene	Anthocyanidins[c]	Phlobaphene[c]	Anthocyanidins
0	10.65	4.43	3.71	0.13	0.19	0.00	0.00
1			3.12	0.85	0.06	0.16	0.03
2			2.05	1.86	0.12	0.81	0.05
3	11.70	4.08	2.37	1.53	0.13	0.49	0.06
4			2.31	1.59	0.13	0.60	0.06
5			2.54	1.36	0.13	0.44	0.05
6	14.20	4.15	2.37	1.53	0.13	0.44	0.05
7			2.26	1.64	0.13	0.49	0.05
8			2.02	1.86	0.15	0.81	0.08
9	12.90	40.3	2.06	1.80	0.17	0.88	0.10
10			2.15	1.75	0.13	0.88	0.10
11			1.97	1.91	0.15	0.88	0.10
12	11.15	3.88	2.11	1.75	0.17	0.88	0.10
13			2.02	1.86	0.15	0.71	0.08
14			1.93	1.91	0.19	0.99	0.12
15	11.55	3.58	1.93	1.91	0.19	0.99	0.12
16			1.58	2.24	0.21	1.20	0.12

[a] Quantities determined by Folin-Ciocalteau and vanillin analyses of samples removed from storage at 3-wk intervals and maintained at −34°C.
[b] Leucoanthocyanidin values determined by difference of mean flavanol value and sum of phlobaphenes and anthocyanidins.
[c] Values determined from means of weekly spectrophotometric analysis of extracts vs extracting solvent.
[d] Values determined by difference in absorbance of extract from stored kernels minus absorbance of extract from fresh kernels.

Table 6—Changes in total phenols and flavanols of Schley pecan kernels stored for 16 wk at 32° C, 50% RH

	mg/g of Kernel						
						Net increase[d]	
Weeks	Total phenols[a]	Total Flavanols[a]	L.A.[b]	Phlobaphene[c]	Anthocyanidins[c]	Phlobaphene	Anthocyanidins
0	8.30	3.63	2.37	1.20	0.10	0.00	0.00
1			2.42	1.15	0.10	0.16	0.03
2			2.29	1.26	0.12	0.16	0.03
3	9.45	3.65	1.94	1.59	0.14	0.65	0.06
4			2.37	1.20	0.10	0.28	0.03
5			2.00	1.53	0.14	0.60	0.05
6	9.30	3.65	1.89	1.64	0.14	0.55	0.07
7			1.52	2.03	0.12	0.65	0.05
8			1.91	1.64	0.12	0.65	0.05
9	9.25	3.73	1.93	1.59	0.15	0.76	0.08
10			1.75	1.75	0.17	0.87	0.10
11			1.70	1.80	0.17	0.82	0.10
12	9.10	3.70	1.59	1.91	0.19	0.92	0.10
13			1.45	2.03	0.19	1.10	0.12
14			1.18	2.28	0.21	1.20	0.12
15	7.80	2.78	1.57	1.91	0.19	1.10	0.12
16			1.23	2.23	0.21	1.31	0.14

[a] Quantities determined by Folin-Ciocalteau and vanillin analyses of samples removed from storage at 3-wk intervals and maintained at −34°C.
[b] Leucoanthocyanidin values determined by difference of mean flavanol value and sum of phlobaphenes and anthocyanidins.
[c] Values determined from means of weekly spectrophotometric analyses of extracts vs extracting solvent.
[d] Values determined by difference in absorbance of extracts from stored kernels minus absorbance of *extract* from fresh kernels.

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DETM OF DYES CONC IN MIXTURES . . .From page 123

able in most computer libariers, thus making the usage of the proposed color determination technique a fairly simple matter to proceed.

In conclusion the proposed technique enables one to use mathematical models for the determining and describing pigment visible spectrum. The usage of such procedure showed a remarkably good accuracy in determined pigments concentration in mixtures. Further studies on the usage of different and modified mathematical functions, and the application of the technique for other natural and artificial colors mixtures is being undertaken.

Table 4 — Influence of number of wavelengths used in curve fitting on mean difference (± standard deviation) between predicted and experimental concentration (7 mixtures)

Method	Number of wavelengths	Amaranth	Yellow	Tartrazine
Proposed method	28	1.54 ± 0.76	2.82 ± 1.56	2.25 ± 3.04
	20	1.59 ± 0.79	3.05 ± 1.82	2.64 ± 3.43
	10	1.63 ± 0.82	3.23 ± 1.89	3.07 ± 4.05
	4	1.76 ± 0.85	4.09 ± 2.98	4.31 ± 2.91
Linear equations (Ewing, 1969)	3	1.09 ± 0.71	8.27 ± 6.50	8.24 ± 4.73

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K.A.V. RETZLAFF, C. KIES and H.M. FOX
Dept. of Food & Nutrition, University of Nebraska, Lincoln, NE 68583

BLOOD LIPID LEVELS OF ADULT MEN ON VITAMIN D-SUPPLEMENTED AND UNSUPPLEMENTED DIETS

ABSTRACT

A single blind study 57 days in length was conducted to determine the effect of dietary supplementation of vitamin D upon serum lipid levels of older men. The experimental group each received a capsule dose daily of 2000 IU of activated 7-dehydrocholesterol. The control group subjects each received a placebo. Fasting blood samples were taken on days 0, 28 and 57. The blood serum was analyzed for cholesterol, triglyceride and phospholipid content. Results supported the theory that low levels of vitamin D supplementation do have a depressing effect on blood serum cholesterol levels but little or no influence on serum triglyceride or phospholipid levels.

INTRODUCTION

ATHEROSCLEROSIS/CORONARY HEART DISEASE is a major cause of premature death among American men. The possible influence of vitamin D on blood lipid levels of humans has not been extensively studied. Vitamin D consumption of Americans has increased over the last 50 yr through the intake of vitamin supplements and fortification and enrichment of products, such as milk and margarine. Vitamin D is a form of cholesterol and therefore a possible precursor of circulatory and deposit forms of cholesterol in the human body (Myasnikov, 1958; Bajiwa et al, 1971; Kummerow et al., 1976).

Conflicting results have been reported on effects of vitamin D intake on blood lipids, particularly cholesterol. Most of the research involves giving vitamin D at very high levels such as 10,000 IU/day to rabbits (Myasnikov, 1958), 1.25 million USP units in rat rations (Bajiwa et al., 1971), or 100,000 IU per pound of ration for swine (Kummerow et al., 1976) which suggests a positive correlation between dietary vitamin D and circulating and tissue levels of cholesterol.

Studies done by Jurgens and Peo (1970) resulted in decreased serum cholesterol levels when high levels of vitamin D (1,100 IU vitamin D_2/kg diet) were fed in combination with cholesterol. Similar results were obtained by Jurgens et al. (1971) when swine were fed vitamin D in combination with cholesterol. The objective of the current project was to study the effect of vitamin D_3 supplementation of diets fed to middle-aged men on their blood serum cholesterol, triglyceride and phospholipid levels.

EXPERIMENTAL

INMATES of the Nebraska Penal and Correctional Complex for Men served as subjects for a single blind study, 57 days in length. All volunteers were adult males 40 yr of age or older. Descriptions of subjects are given in Table 1. Questionnaires were used to determine past health records and dietary habits. Subjects were allowed to select their own meals at random from the usual institutional menu–a process of acceptance or rejection of included items from a single menu composing each meal. Subjects were randomly divided into two groups of approximately equal size. The experimental group (24 individuals) received a capsule dose daily of 2000 IU of activated 7-dehydrocholesterol at the noon meal, while the control group (22 individuals) were given a placebo.

Fasting blood samples were taken on day 0, day 28 and day 57 of the study. Blood samples were analyzed for total serum cholesterol (McDougal and Farmer, 1957; Albers and Lowry, 1955; Long, 1961; Turner, 1970), triglycerides (Turner, 1970) and phospholipids (Bloor, 1914; Boumann, 1924; Fiske and Subbarow, 1925). Data were subjected to analysis of variance and Student Neumar. Keul Test.

Table 1–Description of subjects

Subject No.	Age (yr)	Height (cm)	Weight[a] (kg)
Experimental group			
231	48	182.9	93.2
233	52	172.7	70.0
235	55	162.6	68.2
237	43	179.1	86.4
239	45	182.9	81.9
241	52	182.2	111.4
243	51	177.8	75.0
245	47	180.3	65.9
247	58	168.9	81.8
249	51	180.3	81.8
251	52	185.4	79.5
253	40	198.1	90.0
255	40	172.7	84.1
257	54	175.3	59.1
259	63	170.2	61.4
261	40	177.8	84.1
263	60	170.2	82.7
265	52	177.8	86.4
267	55	172.7	68.2
269	67	175.3	65.9
271	43	172.7	72.7
273	60	175.3	68.2
275	56	185.4	63.6
277	55	180.3	88.6
Control group			
232	50	179.1	83.2
234	46	180.3	77.3
236	49	177.8	84.1
238	43	190.5	83.2
240	50	154.9	84.1
242	45	175.3	63.6
244	50	167.6	68.2
246	45	186.7	117.3
248	48	167.6	77.3
250	43	182.9	72.7
252	48	179.1	84.1
254	64	172.7	82.7
258	46	167.6	59.1
260	43	172.7	69.1
262	40	176.5	70.9
264	43	172.7	65.9
266	44	172.7	54.5
268	45	177.8	79.5
270	43	172.7	72.7
272	44	180.3	77.3
274	44	170.2	68.2

[a] Mean of beginning and final weights. Weights of subjects did not vary significantly with time.

RESULTS & DISCUSSION

CHARACTERISTICS of the two groups were found to be similar in the parameters measured. Only one individual indicated that he had previously had a heart attack. Incidence of high blood pressure was also found to be low.

Table 2—Comparison between groups on dietary patterns, smoking practices and health characteristics

Parameter	Experimental group[a]	Control group[a]
Breakfast, about every day	66.7	77.3
2–3 times weekly	8.3	0
hardly ever	8.3	9.1
Butter, pats/day		
0–1	41.7	18.2
2–3	54.2	63.6
4–6	4.2	13.6
Bread, slices/day		
0–1	20.8	13.6
2–3	20.8	22.7
4–5	25.0	31.8
6	33.3	0
Milk, glasses/day[b]		
0–1	37.5	50.0
2–3	45.5	27.3
4–5	16.7	9.1
6	4.2	13.6
Eggs/day[b]		
0–1	33.3	27.3
2–3	37.5	27.3
4–5	20.8	31.8
6	8.3	13.6
Sugar, tsp/day		
0–1	37.5	36.4
2–3	33.3	13.6
4–5	8.3	9.1
6–7	16.7	31.8
8	4.2	0
Desserts, servings/day		
0	16.7	18.1
1–2	83.3	72.7
3–4	0	9.1
Potatoes, servings/day		
0–1	41.7	27.3
2–3	54.2	72.7
4	4.2	4.5
Coffee, cups/day		
0–1	29.2	27.3
2–3	50.0	59.1
4–5	16.7	9.1
6	4.2	4.5
Tea, cups/day		
0–1	64.2	54.5
2–3	37.5	40.9
4	0	4.5
Smoking		
Cigarettes	75.0	68.0
Cigars	4.6	13.6
Pipe	0	9.1
Heart Attack		
Individual	4.2	0
Close relative	16.7	31.8
High Blood Pressure	16.7	0
Low Blood Pressure	0	4.5
Diabetes	4.2	0
Medicine for High Blood Pressure	4.2	0

[a] Percent of group giving positive answer. Totals do not equal 100% because of failure of some individuals to respond to specific questions.

[b] Milk was served 4–6 times a week (1 glass per serving) and eggs were served 3–4 times per week (1–2 egg(s) per serving limit); hence, these figures represent desires for consumption rather than true consumptions.

If given completely free choice, subjects indicated wide variations in dietary preferences (Table 2). However, quantitative and qualitative limitations were imposed through institutional living. Therefore, while an individual might indicate that he would prefer to eat six eggs per day the total number consumed would be limited by whether or not eggs appeared on the menu and the number of servings allowed (one or two eggs per individual). In calculating individual and group intakes of vitamin D from the institutional diet, it was assumed that each subject would consume the full helping if it were within his indicated normal choice. Thus, if two eggs were offered, individuals indicating a willingness to eat two eggs or any figure greater than two eggs were credited with eating two eggs. Conversely, individuals indicating a desire to eat less were credited with eating less. The institution used a 4-wk cycle menu. The time period covered by the current study covered the second and third repeats of the Spring cycle. Hence, the same series of menus was served during the 4 wk before the first blood sample was drawn and before the second and third blood drawings.

If subjects chose to eat all foods containing vitamin D in the maximum amounts allowed, a mean intake of 48 IU of vitamin D would have been provided. Not all individuals indicated this as being their choice. The mean intake for vitamin D was calculated to be 39 IU per day for the experimental group and 37 IU per day for the control group from the ordinary foods in the diet. The Food & Nutrition Board of the National Research Council, National Academy of Sciences (1974) has made no recommendations for vitamin D intake by adult men since it is assumed that adults with exposure to sunlight do not require a dietary supplement. Although institutionalized, the men in the present study were allowed outdoor exercise and sun exposure.

Mean energy intake of subjects for both the experimental and control groups was calculated. Mean intake for both the control and experimental groups was estimated to be 2450 kilocalories per day over the entire period. This is less than recommended intake figures for this age/sex group given by the Food & Nutrition Board, NRC-NAS (1974); however, weights of subjects did not vary significantly from the beginning to the end of the study indicating an adequacy of energy intake. Mean weights of subjects are shown in Table 1. The generally sedentary lives led by individuals in institutionalized circumstances probably indicate a reduced energy need in comparison to similar individuals in "free-living" society.

Dietary fat was calculated to provide approximately 47% of the energy in the institutional diets. This is similar to estimates made of usual American diets.

The mean cholesterol level of subjects receiving vitamin D supplements at day 0 was 229.71 mg/100 ml, day 28 was 241.25 mg/100 ml and on day 57 mean levels fell to 203.13 mg/100 ml (Table 3). Mean values for the control group receiving no supplementary vitamin D were as follows: day 0, 215.14; day 28, 241.45; day 57, 214.73. Changes in blood cholesterol for the experimental group were found to be statistically significant at the $P < 0.005$ level whereas in the

Table 3—Effect of dietary vitamin D supplementation on serum cholesterol values of adult men

Subject no.	Cholesterol level (mg/100 ml blood serum)		
	Day 0	Day 28	Day 57
Experimental group			
231	213	222	180
233	223	197	175
235	209	175	168
237	222	210	181
239	189	241	206
241	192	214	146
243	216	266	230
245	210	194	228
247	245	279	227
249	327	197	121
251	189	239	198
253	222	215	221
255	250	252	210
257	239	190	189
259	313	347	285
261	255	314	235
263	231	257	257
265	285	258	225
267	203	220	197
269	226	206	183
271	176	221	185
273	254	336	204
275	202	259	210
277	222	281	214
Mean[a]	229.71y	241.3y	203.1z
Control group (no vitamin D)			
232	204	238	204
234	215	234	225
236	180	193	241
238	207	241	206
240	186	260	197
242	309	285	286
244	229	198	159
246	262	233	129
248	175	285	269
250	170	171	202
252	197	221	227
254	228	303	222
258	177	222	240
260	258	323	208
262	208	261	262
264	219	270	225
266	121	215	192
268	266	210	250
270	314	281	245
272	228	278	200
274	160	219	160
276	242	171	175
Mean[a]	216.1y	241.5v	214.73z

[a] Different letters indicate values significantly different from one another ($t > 0.005$), Analysis of Variance and Student Neuman Keuls Test.

Table 4—Effect of dietary vitamin D supplementation on serum triglyceride values of adult men

Subject no.	Triglyceride level (mg/100 ml blood serum)		
	Day 0	Day 28	Day 57
Experimental group			
231	146.25	110.00	83.50
233	66.00	61.00	112.00
235	42.50	70.00	45.50
237	109.00	223.50	123.50
239	99.00	73.00	87.50
241	120.00	373.00	186.00
243	47.00	87.50	55.50
245	65.50	86.00	48.00
247	95.50	176.00	176.50
249	105.50	82.00	70.25
251	90.00	127.50	88.25
253	94.50	105.50	112.00
255	102.50	81.50	150.75
257	103.50	66.50	64.50
259	148.00	178.50	128.00
261	115.50	153.25	101.00
263	157.50	195.50	102.50
265	99.00	143.00	130.50
267	95.25	98.50	86.50
269	162.50	161.00	69.75
271	59.00	95.00	63.00
273	56.25	159.00	60.00
275	60.25	97.00	117.50
277	105.50	138.00	91.50
Mean[a]	97.73y	130.91z	98.08y,z
Control group			
232	136.25	191.25	170.25
234	95.00	167.50	102.75
236	101.00	109.00	145.00
238	144.50	133.00	166.25
240	70.50	170.00	188.50
242	117.25	64.00	88.50
244	98.50	108.50	87.50
246	70.50	115.50	39.00
248	60.50	100.00	74.00
250	74.50	123.00	65.00
252	99.50	178.50	135.75
254	84.00	126.50	130.50
258	71.50	153.50	99.00
260	95.25	82.50	41.00
262	83.00	105.75	101.50
264	77.00	130.00	102.00
266	63.75	63.00	85.25
268	119.00	133.50	88.50
270	121.50	106.00	158.75
272	113.00	124.00	137.50
274	106.50	174.00	76.00
276	79.50	84.00	92.50
Mean[a]	94.64y	124.68	107.98y,z

[a] Different letters indicate values significantly different from another ($t > 0.025$, control group; $t > 0.05$, experimental group), Analyses of Variance and Student Neuman Keuls Test.

control group there were no significant changes. The increase in cholesterol levels on day 28 was probably apparent rather than real since the control group showed an even greater increase.

Mean blood serum triglyceride levels of the experimental subjects on day 0, 28 and 57 were 97.73, 130.91 and 107.98 mg/100 ml, respectively (Table 4). Mean values for the control group were 94.64, 124.68 and 107.98 mg/100 ml respectively. Mean blood phospholipid levels of the experimental group were day 0, 279.0; day 28, 281.1; and day 57, 256.0 mg/100 ml. Mean blood phospholipid values for the control group were day 0, 282.0; day 28, 287.0 and day 57, 256.0 mg/100 ml.

Observations from cholesterol and triglyceride data suggest day 28 figures may not represent true values. This may also be true of the phospholipid values; however, the dramatic fall on day 57 is difficult to explain. Other authors (Truswell and

Mann, 1972; Fredrickson et al., 1967) have reported that phospholipid level changes tend to be directionally similar to those of cholesterol. This might explain the change in the experimental group, but may not that of the control group.

If results of day 28 are ignored, this study would then suggest that vitamin D supplementation of diets may result in the lowering of blood cholesterol levels. Little if any effect attributable to vitamin D supplementation on blood serum triglycerides and phospholipid levels was found (Table 5).

Table 5—Effect of dietary vitamin D supplementation on serum phospholipid values of adult men

Subject no.	Phospholipid level (mg/100 ml blood serum)		
	Day 0	Day 28	Day 57
Experimental group			
231	288.46	262.82	258.16
233	283.12	267.10	226.02
235	280.45	232.91	262.25
237	289.92	298.61	262.76
239	263.66	312.50	276.64
241	291.50	333.34	252.57
243	276.26	273.38	241.29
245	252.63	255.21	257.17
247	275.74	337.50	309.96
249	284.67	210.42	205.95
251	255.43	277.55	281.25
253	264.74	304.56	253.98
255	253.65	308.26	261.75
257	273.44	249.94	212.50
259	348.58	331.04	320.12
261	248.99	341.49	240.00
263	306.91	279.26	262.50
265	296.81	280.32	242.50
267	278.20	256.39	206.67
269	270.22	243.09	221.11
271	254.26	265.96	242.49
273	341.30	300.53	292.92
275	259.57	252.66	272.53
277	258.01	271.28	245.18
Mean[a]	279.02y	281.09y	254.51z
Control group (no vitamin D)			
232	267.10	308.51	248.94
234	288.46	303.96	264.80
236	250.00	269.23	254.28
238	262.59	265.96	226.02
240	240.02	239.84	258.20
242	353.47	312.50	306.87
244	254.15	264.59	242.83
246	281.52	320.31	238.73
248	289.13	305.90	303.36
250	204.79	268.01	166.86
252	293.23	268.01	251.14
254	317.71	302.34	277.78
258	243.48	325.21	233.91
260	353.15	342.99	290.00
262	286.98	311.17	311.70
264	281.92	294.72	252.50
266	260.64	307.45	207.78
268	360.11	286.17	315.04
270	286.77	276.60	273.07
272	270.21	271.28	265.56
274	246.81	243.62	205.48
276	311.17	226.06	236.05
Mean[a]	281.97y	287.02y	255.95z

[a] Different letters indicate values significantly different from one another ($t > 0.025$ for control group; $t > 0.005$ for experimental group); Analysis of Variance and Student Neuman Keul Test.

In rabbits, rats, swine and humans, vitamin D supplementation of rations or diets has been shown to have variable effects on blood serum cholesterol levels (Myasnikov, 1958; Vijayakumar and Kurup, 1974; Bajiwa et al., 1971; Jurgens and Peo, 1970; Jurgens et al., 1971; Kummerow et al., 1976; Ross and Campbell, 1961; Eskelson, 1967; Linden, 1974). In general, those studies showing an increase in serum cholesterol levels in response to vitamin D additions involved extremely high supplementation levels. The supplementary level used in the present study was fairly low in comparison to these animal studies. Toxic symptoms of vitamin D have been shown in humans at an intake level of 4,000 IU/day (Kutsky, 1973); thus, the 2000 IU daily doseage used in the present study for experimental purposes could not have been raised with impunity even though higher levels might have maximized differences in response. In swine studies showing detrimental effects of vitamin D, levels of 100,000 IU of vitamin D per pound of rations were used (Kummerow et al., 1976). Only 1,100 IU per kg (500 IU per/pound) of ration were used in Jurgens and Peo study (1970) in which swine showed lowered blood cholesterol levels when vitamin D was fed in cholesterol-containing rations.

In conclusion, the results of this and earlier studies suggest that the directional influence of vitamin D on blood serum cholesterol levels may be dose and/or species related. Vitamin D supplements for humans at nontoxic levels seemingly present no hazard in relationship to circulating blood lipids and may have a slight beneficial effect. These results should not be interpreted to indicate that no damage from excessive vitamin D intakes exist at higher dose levels or in parameters not measured.

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M. I. FARBOOD and J. H. MacNEIL
Dept. of Food Science, The Pennsylvania State University, University Park, PA 16802

LIMITATIONS OF LEAD ACETATE FOR SEPARATION OF METHANETHIOL AND HYDROGEN SULFIDE FROM FOOD SYSTEMS

ABSTRACT

The principle involved in the quantification of methanethiol in the presence of hydrogen sulfide is based on the efficient separation of hydrogen sulfide from methanethiol. The removal of hydrogen sulfide from a mixture containing methanethiol is dependent upon the selective reaction of hydrogen sulfide with crystalline lead acetate under dry conditions. It was found that crystalline lead acetate is not as selective as it was previously thought. It was also concluded that methanethiol either reacted or was absorbed by the crystalline lead acetate under dry conditions.

INTRODUCTION

THE IMPORTANCE of methanethiol (CH_3SH) in flavor and off-flavor production in various food stuffs has been well documented in the literature. For example, Ziemba and Malkki (1971) reported that extremely high levels of CH_3SH and dimethyl disulfide were formed in beef under extreme sterilization conditions which consequently resulted in off-flavor development. Because of the importance of CH_3SH in flavor and off-flavor formation, an evaluation of the accuracy of the widely used method for quantification of CH_3SH seemed appropriate.

Reith (1934) reported that methanthiol is absorbed by lead sulfide which is produced in a lead acetate solution. He also pointed out that methanethiol will not react with lead acetate crystals in a dry condition. Later Dateo et al (1957), Ueno and Nobuhara (1960) and Takai and Asami (1962) confirmed Reith's finding. They reported that crystals of lead acetate in a dry condition would separate hydrogen sulfide completely from methanthiol. Reith's finding was used by Dateo et al (1957), Grill et al (1966, 1967), O'Palka (1973) and Takai and Asami (1962) for the determination of methanthiol in cabbage, cheese, chicken meat and paddy soil, respectively. It should be mentioned that Ryland and Tamele (1970) discussed the fact that thiols form water-insoluble mercaptides with many inorganic and organic compounds of heavy metals, of which silver, mercury and copper are the most important.

The principle involved in the quantification of CH_3SH in the presence of hydrogen sulfide (H_2S) is based on the efficient separation of H_2S from CH_3SH. This separation is dependent upon the selective reaction of H_2S with crystalline lead acetata under dry conditions. This investigation was undertaken in order to evaluate the effectiveness of crystalline lead acetate in separating H_2S from CH_3SH.

EXPERIMENTAL

Preparation of chicken meat and radioactive material

Seven to eight-week old broilers were obtained from the poultry farm of The Pennsylvania State University. The birds were sacrificed and bled. The meat was removed and ground with a Hobart Model 4812 Chopper (Troy, OH) using a plate with holes 1/8-inch in diameter. The meat was stored at 4°C overnight. L-methionine-methyl-carbon-14 (Met-Cl4; spec. act. 55 mci/mM) and L-methionine-sulfur-35 (Met-S35; spec. act. 536.2 ci/mM) were obtained from Schwarz/Mann (Orangeburg, NY) and New England Nuclear (Boston, MA) respectively. Prior to each experiment, the radioactive materials were purified using descending paper chromatography on Whatman chromatography paper No. 1 (46 × 57 cm) for 15 hr against standard cold methionine. Among several solvent systems tested, it was found that butanol:propionic acid:water (3:2:2) containing 10 mM of mercaptoethanol/ml to be the most effective system for the purification of radioactive methionine.

Distillation

The distillation apparatus was the same as that used by Dateo et al. (1957). Two classes of sulfur compounds, H_2S and thiols, were separated and collected with the distillation apparatus. The compounds and their chemical reactions involved are listed in Table 1.

A 1200-g chicken meat slurry (1 part meat, 2 part water) was transferred into a 3-L, 3-neck distillation flask. The distillation flash was heated using heating mantles. The amount of current reaching the mantles was regulated by Variac (Superior Electric Co., Bristol, CT). Prior to the addition of the radioactive methionine, the slurry was preheated to about 80°C and then the portion of the chromatographic paper containing 10 uci of Met-Cl4 or 45 mci of Met-S35 was added directly to the chicken meat slurry. The slurry was subsequently heated to 101°C. The radioactive volatile sulfur compounds producted during 6 hr of cooking at 101°C were carried through the train of traps using purified N_2 at a flow rate of 20–25 ml/min. At the end of 6 hr of distillation the content of the lead acetate trap was dissolved in distilled water and filtered through a Whatman filter paper No. 1. The remaining black precipitate (lead sulfide, PbS) was weighed and its radioactivity determined by liquid scintillation counting.

Interaction of methanethiol with crystalline lead acetate

Qualitative evaluation. The possible reaction or absorption of methanethiol by lead acetate was evaluated as follows: An amount of lead methylmercaptide equivalent to 202μg of methanethiol was weighed into a 1-L 3 neck distillation flask. The apparatus was the same as Dateo et al (1957) except for the insertion of an additional gas wash bottle between the gas wash bottle containing pyrogallol and the distillation flask. The additional gas wash bottle contained 200 ml of 0.1N HNO_3. In this manner the HNO_3 would flow into the distillation flask by the nitrogen pressure and thus the regenerated methanethiol would not escape while adding HNO_3. The flask temperature was maintained at 80°C during the 4 hr distillation. During distillation the nitgrogen flow rate was 20–25 ml/min. At the end of the experiment the lead acetate trap content was transferred to another distillation flask. Methanethiol which possibly reacted with the lead acetate was regenerated by HNO_3 using the same procedure as mentioned above and trapped in 2,4–dinitrofluorobenzene (DNFB) reagent.

The details for preparation of the DNFB-reagent and purification of the obtained derivatives are given by Libbey and Day (1963). The isolation and identification of DNFB-derivatives was performed using descending paper chromatography on Whatman chromatographic paper No. 1 (20 × 57 cm) against a known DNFB-derivative of CH_3SH. The known DNFB-derivative of CH_3SH was prepared according to the method of Day and Patton (1959). The spots were detected under UV light. Afterward the spots were cut and the DNFB-derivative was extracted with absolute ethanol. Maximum absorption of each extracted spot was also determined in the UV region using a Beckman DBG Grating Spectrophotometer (Beckman Instruments Inc., Fullerton, CA).

Quantitative evaluation. The extent of the interaction of CH_3SH with crystalline lead acetate was evaluated using a quantity of lead methyl mercaptide equivalent to 204 μg of CH_3SH. The same pro-

Table 1—Sequence of traps for identification and quantification of selected sulfur compounds

Trap	Type	Substance absorbed	Reaction product
$CaCl_2$ (12 mesh)	Solid	H_2O	Absorbtion
Lead acetate (25g)	Solid	H_2S	PbS
Identification: DNFB[a]	Liquid	CH_3SH	Dinitrophenyl sulfide
Quantification: 4.0% $Hg(CN)_2$	Liquid	CH_3SH	$Hg(CH_3S)_2$

[a] 2,4-dinitro-fluorobenzene

cedure as that used in the qualitative evaluation was used except the DNFB-reagent was replaced by a 4.0% $Hg(CN)_2$ solution. The CH_3SH content of this trap was determined using the colorimetric method of Takai and Asami (1962).

RESULTS & DISCUSSION

THE BLACK PRECIPITATE recovered from the crystalline lead acetate trap in experiments with Met-S35 and Met-Cl4 were found to contain radioactive compounds (Table 2). Averages of 82 and 63 mM/100M of the original radioactive methionine were found in the crystalline lead acetate using Met-Cl4 and Met-S35 respectively. Since methionine is not a direct precursor of H_2S (Hamm and Hofmann, 1965) the radioactivity observed in the lead acetate traps of experiments with Met-S35 could not result from H_2S. The black precipitate recovered from experiments using Met-C14 was also found to be radioactive. Under the conditions of this experiment it was then logical to assume that the precipitates obtained in these experiments contained radioactive sulfur and carbon. Considering the nature of the methionine structure, its breakdown pattern (Strecker degradation), and the position of the labeled element in the methionine, it would appear that CH_3SH could have interacted with the crystalline lead acetate. Therefore, the possible interaction of CH_3SH with crystalline lead acetate was further investigated using pure CH_3SH.

The nitrophenyl sulfide derivative obtained from regenerated lead acetate was purified and then identified (Fig. 1). The paper chromatography of the derivative revealed the existence of CH_3SH in the lead acetate and maximum absorption confirmed the existence of CH_3SH in this trap. Therefore, methanethiol was either absorbed by crystalline lead acetate or reacted with lead acetate.

Table 2-Moles of radioactive volatile sulfur compounds originated from 100 moles of radioactive methionine which were trapped in crystalline lead acetate during 6 hr of cooking at 101° C[a]

	Radionuclide	
Experiments	Methionione-Cl4	Methionine-S35
I	0.092	0.065
II	0.072	0.061

[a] Average of duplicate samples

Fig. 1—Paper chromatography of the DNFB-derivatives obtained from regeneration of crystalline lead acetate: (a) Known DNFB-derivatives of methanethiol; (b,c) DNFB-derivatives obtained from regeneration of lead acetate.

Reith (1934) reported that methanethiol would not react with crystalline lead acetate in a dry condition. Later Dateo et al (1957), Ueno and Nobuhara (1960), and Takai and Asami (1962) confirmed Reith's finding. Our experimental results do not agree with Reith's finding.

The quantification aspect of such an interaction was also investigated. It was found that 10 μg of the total 202 μg methanethiol was trapped in the lead acetate. However, one must remember that these results present the inaccuracy of the quantification method of methanethiol. The reason for this is the rate of methanethiol generation in an actual experiment is much slower than that of a model system. In the model system, the methanethiol was generated during the first few minutes of the experiment, while the generation of methanethiol from chicken meat was much slower and continued during 6 hr of distillation. Thus the chance of absorption of reaction between methanethiol and lead acetate is much higher. In addition to the slower rate of methanethiol generation in an actual experiment, one must consider the possibility of methanethiol absorption by PbS which is continuously formed during the distillation. Reith (1934) found that the PbS produced in a lead acetate solution would absorb methanethiol. Such a finding could also support the possibility of methanethiol absorption by PbS in a dry condition. During our experiments with radioactive compounds the treatment of the black precipitate with diluted HNO_3 did not result in the loss of radioactivity. This indicates that possibly CH_3SH was absorbed by PbS and treatment with acid would not result in its loss.

It is suggested that reliable results could be obtained using this method if the following precautions are considered:

1. Use an exact amount of crystalline lead acetate with the lead acetate having specified mesh size.
2. The standard curve should be established using identical conditions as that used in experiments and a constant flow of nitrogen should be maintained at all times.
3. The preparation of a standard curve in the presence of H_2S would reduce errors.

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H. E. SCHIFF and J. ADLER-NISSEN
Novo Industri A/S, Enzymes Division
Novo Allé, DK-2880 Bagsvaerd, Denmark

QUANTITATIVE ASSAY OF SPECIFIC PROTEOLYTIC ACTIVITY IN FOOD PROTEIN HYDROLYZATES BY SINGLE RADIAL IMMUNODIFFUSION

ABSTRACT

The increasing use of proteolytic enzymes in food production has established the need for a quantitative and sensitive assay for proteolytic activity in protein foods. The classical methods are not suitable because of the presence of large amounts of proteins and peptides. A quantitative procedure based on single radial immunodiffusion was developed and found to be applicable to the determination of subtilisin Carlsberg activity in protein hydrolyzates. Less than 0.5 ppm subtilisin Carlsberg could be detected in a 7% soy protein hydrolyzate. With minor modifications the method should be applicable to many of the other enzymes used in food processing.

INTRODUCTION

THE CLASSICAL METHODS for the determination of proteolytic activity are all based on either a hydrolysis under specific conditions followed by a measurement of the amount of degraded protein, or the estimation of a particular parameter associated with the reaction between the enzyme and a synthetic substrate or inhibitor.

The presence of large amounts of foreign proteins and peptides generally render the classical methods unfeasible due to the extremely high blank values obtained. However, the increasing use of proteolytic enzymes in food production has established the need for a quantitative and sensitive assay for proteolytic activity in protein foods. Thus, Adler-Nissen (1976) observed that residual proteolytic activity was detrimental to the storage stability of soya protein hydrolyzates. These considerations led us to examine an immunochemical approach to the problem.

Recently, Rothe et al. (1976) demonstrated that quantitative immunoelectrophoresis of commercial preparations of milk-clotting enzymes is a reliable, direct, and highly specific method for the determination of the individual proteolytic enzymes in bovine rennets. As observed by these authors and by Verbruggen (1975), the quantitative immunoelectrophoresis often results in blurred and double precipitates when unfractionated rabbit antisera are used. Formation of complexes between the enzyme and other serum proteins, probably inhibitors of the same type as found in normal human serum (Verbruggen, 1975), seems to be the cause of these problems. In our own attempts to use immunoelectrophoretic techniques for the quantitative determination of microbial alkaline proteases, e.g., subtilisin Carlsberg, we encountered further problems, because the isoelectric point of the antigen was too close to that of the precipitating antibodies. In order to get correct results without tailing of the antigen towards the "wrong" electrode, we found it sufficient in certain cases to change the pH of the buffer system (Verbruggen, 1975; Verbruggen and Baeck, 1976). In other cases, however, carbamylation (Axelsen et al., 1973) of the antiserum seemed necessary.

The above mentioned problems led us to use the much more simple, albeit slower, technique of single radial immunodiffusion, as described by Mancini et al. (1965). This choice is further substantiated by the fact that the microbial alkaline proteases of commercial interest have a fairly small molecular size (~30,000 daltons), so that equilibrium is attained in a reasonable time (Laurell, 1972). Using single radial immunodiffusion we developed a reliable, simple, and highly specific method for quantitative determination of residual subtilisin Carlsberg activity in food protein hydrolyzates.

MATERIALS & METHODS

Immunochemical procedure

Unfractionated rabbit antiserum against Alcalase® (a commercial enzyme preparation containing subtilisin Carlsberg) was used as antibody source. The animals were Danish Land strain rabbits bred at Novo's experimental farm, Nyvangsgaard, Ganloese. Several buffer systems were tried and proved successful; generally we recommend a phosphate buffer of ionic strength, μ = 0.2 and pH 7.6 (0.0325M K_2HPO_4, 0.0026M KH_2PO_4, 0.100M NaCl). A gel layer of 1.5 mm thickness (0.05–0.15 ml antiserum in 15 ml buffer with 1% agarose and 0.1% NaN_3) was cast on 10 × 10 cm glass plates and 16 equally spaced, circular wells of 2.5 mm diameter were punched in the gel. Five μl of antigen solution were applied to each well and the plates were stored for 2 days in a humid chamber. The precipitates were then stained with Coomassie Brilliant Blue R and the areas were measured by use of a magnifier. The product of two perpendicular diameters, measured to the nearest 0.1 mm, was conveniently used as a quantitation of the area. The immunological activity was then calculated as $d_1 \times d_2 - 6.25$ mm^2.

Proteolytic activity

The proteolytic activity was determined by the procedure originally developed by Anson (1939) and slightly modified as described elsewhere (Novo Industri A/S, 1976). The procedure is based on a measurement of the amount of denatured hemoglobin which is hydrolyzed under specific conditions (pH 7.5, 25°C, 10 min reaction time).

Heat-inactivation experiments

From a stock solution of Alcalase®, containing approximately 650 AU/L, 1 ml samples were transferred to test tubes, containing 10 ml of the phosphate buffer described above and held in a water bath at 70°C. Termination of the heat-treatment was achieved by transferring 1 ml of these dilutions to 10 ml of phosphate buffer held at 0°C. The immunological and proteolytic activities were then determined as a function of the duration of the heat-treatment.

Preparation of protein hydrolyzates

Hydrolyzates of soy protein isolate (Ralston Purina 500 E) and casein (according to Hammarsten) were prepared by use of a microbial alkaline protease, Esperase® (Novo Industri A/S), which does not cross-react with Alcalase® (Schiff, 1975). The following hydrolysis parameters were used: Substrate concentration = 8% protein, enzyme concentration = 1.0 AU/L, temperature = 50°C, and pH = 8.0. The pH was maintained with 4N NaOH during hydrolysis by means of Radiometer pH-stat equipment. The hydrolysis was terminated after 150 min by addition of 6N HCl until pH reached 4.0–this procedure effectively inactivates the Esperase®. The suspension was filtered and pH in the supernatant was adjusted to 7.6. Part of the soy protein hydrolyzate was concentrated in a rotary evaporator. The protein and peptide concentration in all hydrolyzates was determined as Kjeldahl-N × 6.25.

Determination of standard curves

From a stock solution of Alcalase® (40 AU/L) in phosphate buffer, dilution series were prepared with the following eight different concentrations: 3.2-2.4-1.6-1.2-0.8-0.4-0.2-0.1 AU/L. The stock solution was diluted with either phosphate buffer or one of the hydrolyzates, and the dilutions were analyzed by the immunochemical procedure described above. To each plate two series, one with buffer and one with hydrolyzates, were applied. In each of the series a linear regression analysis of the area of the precipitation rings towards the proteolytic activity was performed.

Table 1—Residual enzymic and immunological activity after heat-treatment at 70°C, pH 7.6

Heat-treatment, (min)	Residual enzymic activity (AU/L)	Residual immunol. activity (mm^2)	Heat-treatment (min)	Residual enzymic activity (AU/L)	Residual immunol. activity (mm^2)
0	5.40[a]	115[a]	15	0.16[a]	4[a]
1	4.38[a]	93[a]	20	0.07[a]	3[a]
2	3.28[a]	69[a]	30	0.06	0
3	2.15[a]	46[a]	40	0.04	0
4	1.72[a]	33[a]	50	0.06	0
6	0.79[a]	16[a]	60	0.06	0
8	0.31[a]	9[a]	90	0.03	0
10	0.22[a]	7[a]	120	0.01	0

[a] Linear regression analysis was performed on the 10 first points, for which the enzymic and immunological activity were significantly greater than zero. The regression line has a very high correlation coefficient (r = 0.9992). The point of origin lies well inside the confidence area of the regression line.

RESULTS & DISCUSSION

THE TYPE OF INACTIVATION to which a proteolytic enzyme is exposed during and after its application in food processing is generally a heat-denaturation. It is therefore of practical importance that a high correlation exists between enzymic and immunological activity during heat-inactivation of the enzyme. Table 1 shows the results from the heat-inactivation experiment performed on Alcalase®. Figure 1 as well as a linear regression analysis (r = 0.9992) shows that the enzymic and immunological activities are strictly proportional to one another.

The next step was to show that the presence of large amounts of protein and peptides does not interfere with the immunological assay. Table 2 shows that the presence of hydrolyzate does not have a statistically significant effect on the slope, intercept or uncertainty of the standard curves drawn from the dilution series.

Finally, the sensitivity of the method was estimated by running a series of plates with varying concentrations of Alcalase® and antiserum. The highest sensitivity was obtained with antiserum concentrations of 50–150 μl per plate, where the limit of detection was 0.02 Anson units/liter (AU/L), regardless of the presence of soy protein hydrolyzate. By using a (hypothetical) activity of 44 AU/g for 100% pure subtilisin Carlsberg, this limit corresponds to approximately 0.5 ppm of immunologically active enzyme protein.

The results reported here have demonstrated an alternative and feasible way of assaying proteolytic activity in protein foods. With minor modifications the single radial immunodiffusion technique should be applicable to other types of enzymes used in food materials. The relative simplicity of the method makes it suitable as a routine check method for residual activity.

Table 2—Standard curves for Alcalase® in immunodiffusion assay[a]

Hydrolyzate	Protein content N × 6.25%	Plate No. B = buffer[b] H = hydr[c]	Slope mm^2 AU × L^{-1}	Intercept mm^2	Correlation coeff.r	Std dev mm^2	Degrees of freedom
		B 1	43.2	9.8	0.982	10.0	6
		H 1	48.9	7.7	0.994	6.3	6
Casein	6.29						
		B 2	28.0	6.7	0.947	7.8	6
		H 2	24.3	8.5	0.984	5.2	6
		B 3	28.2	7.7	0.987	5.5	6
		H 3	26.0	9.5	0.978	6.6	6
Soya	5.19						
		B 4	24.8	11.1	0.981	5.8	6
		H 4	22.4	12.2	0.995	2.7	6
		B 5	28.9[d]	8.5	0.993	4.2	6
		H 5	24.1[d]	14.5	0.996	2.5	6
Soya	6.94						
		B 6	19.5	13.6	0.942	8.3	6
		H 6	20.8	13.8	0.994	2.8	6

[a] Based on a dilution series: 3.2-2.4-1.6-1.2-0.8-0.4-0.2-0.1 AU/L
[b] Phosphate buffer, pH 7.6, ionic strength = 0.2
[c] Protein hydrolyzate, adjusted to pH 7.6 with HCl and NaOH
[d] Significant difference on 95% level

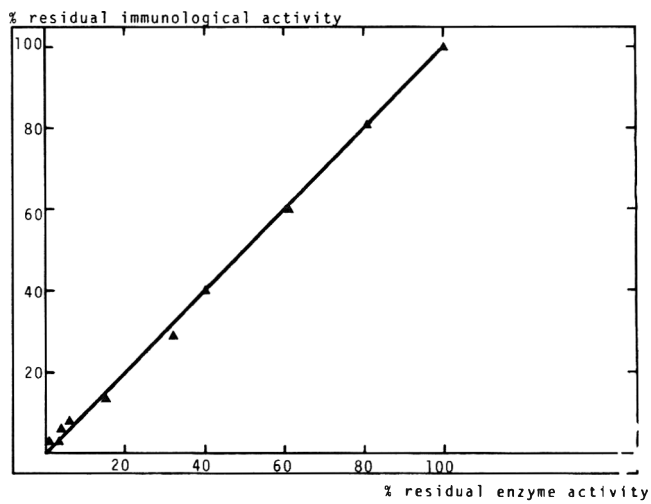

Fig. 1—Correlation between immunological and enzymic activity.

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LEONARD F. BJELDANES and IN-SUK KIM
Dept. of Nutritional Sciences
University of California, Berkeley, CA 94720

SEDATIVE ACTIVITY OF CELERY OIL CONSTITUENTS

ABSTRACT

The central depressant activities of 3,n-butylphthalide and a new compound, sedanenolide, were studied in mice. While neither compound affects ethanol sedation, they exhibit similar activities in both prolonging pentobarbital narcosis by prior administration of the test compounds and in inducing sleep immediately following recovery from a prior treatment with barbiturate. Weak sedative activity is also shown to reside in both compounds without potentiation.

INTRODUCTION

SEEDS OF COMMON CELERY, *Apium graveolens* L., contain up to 2.5% by weight of a steam distillable oil which, in addition to finding extensive use as a flavoring agent, has been employed in folk remedies as a carminative, a diuretic, an abortifacient, an antiseptic, and as a nerve tonic and sedative (Guenther, 1950). While the efficacies of most of these uses remain unsubstantiated by controlled studies, experiments by Kohli et al. (1967) have shown that crude celery oil fractions do indeed exhibit depressant effects on the central nervous system of mice and rats. Maximum activity was observed in the fraction (bp 180–265°C) said to contain sesquiterpene alcohols, sedanolide (I), and sedanonic anhydride (II) (Fig. 1). Although the names of the latter two compounds, coined by Ciamician and Silber (1987) in their classic studies of celery constituents, seem to suggest some involvement in sedative activity, to our knowledge the studies by Kohli et al. (1967) are the first experimental indications of possible sedative activity of these compounds.

Our investigation of the biologically active components of celery oil has resulted in the isolation of two central depressants: the known constituent of celery oil, 3,n-butylphthalide (III) and a previously unreported compound, 3,n-butyl-4,5,-dihydrophthalide (IV). The chemical characterization of the latter compound, for which we propose the trivial name sedanenolide, is reported elsewhere (Bjeldanes and Kim, 1977). We report here an evaluation of the central depressant activity of 3,n-butylphthalide and sedanenolide in mice.

MATERIALS & METHODS

Isolation of sedative components

Steam distillation of ground celery seed (1 kg) produced 27l of aqueous distillate which was extracted consecutively with petroleum ether (bp 30–60°) (18L), ethyl ether (18L) and chloroform (18L). Evaporation of the extracts in vacuo gave a combined yield of 16.8g of essential oil. This material was placed on a silica gel 60 (partical size less than 0.63 mm, E.M. Reagents) column and nine fractions were eluted with 4.5L of hexane-diethyl ether (1:1, v/v). A tenth fraction was eluted with 500 ml of methanol. The solvents were removed in vacuo and the oily residues were assayed for sedative activity by potentiation of pentobarbital narcosis in mice as described by Brodie (1956). Subsequent gas chromatographic resolution of the active fractions (5 and 6) resulted in the isolation of two active components, 3,n-butylphthalide and sedanenolide. The gas chromatographic conditions used were as follows: aluminum column (20 ft × 3/8 in.), packed with 15% S.E. 30 on dimethylchlorosilane treated 60/80 mesh gas chromosorb W, a helium flow rate of 100 ml/min, and column, injector and detector temperatures, 200°, 218° and 235°C, respectively. 3,n-butylphthalide and sedanenolide had retention times of 57 and 75 min under these conditions. The compounds gave single peaks on GLC and were characterized by their chemical and spectral properties. These data are reported in detail elsewhere (Bjeldanes and Kim, 1977).

CNS depressant activity.

Pentobarbital sleeping time. (Test compound administered prior to pentobarbital).

Compounds were dissolved in an aqueous 5% ethanol–3% Tween 80 solution to give concentrations by weight of 0.5, 1.0 and 2.0%. Doses of 25, 50 and 100 mg compound/kg mouse wt, respectively, of these solutions were administered by intraparitoneal (ip) injection to groups (6–13 animals per group) of albino, male mice (22–40g). Thirty minutes after each injection, sodium pentobarbital (50 mg/kg mouse wt) was administered and the sleeping time from loss of righting reflex to recovery was noted. A control group received equivalent doses of the ethanol-Tween 80 solution used as a vehicle for the celery components. Each animal was kept in a separate, constant-temperature (32°C) compartment for the duration of the experiment. The results are recorded in Table 1.

Ethanol sleeping time. A similar procedure was followed as with the determination of pentobarbital sleeping time. However, an aqueous 10% ethanol-3% Tween 80 solution was used as a vehicle and two doses of each compound (50 and 100 mg/kg) were used. In addition, an aqueous 35% ethanol solution was employed at a dose of 3.5 g/kg mouse weight to produce sleep 30 min after injection of the test solutions or the 10% ethanol-3% Tween 80 control. The results are recorded in Table 2.

Pentobarbital potentiation. (Pentobarbital administered prior to test compound). Albino, male mice weighing between 25 and 40g were employed in groups of 6–19 as described by Brodie (1965). Administration (ip) of an aqueous 1% solution of sodium pentobarbital as a dose of 50 mg/kg body weight produced loss of righting reflex in all animals. Immediately upon recovery of the righting reflex, test compounds, as 2% solutions in 10% aqueous Tween 80, and the control vehicle, were administered (ip) to mice in doses indicated in Table 3. The percentage of animals which again lost the righting reflex was noted. Mice were kept in separate compartments maintained at 32°C during the experiments. The ED_{50} values were computed according to Litchfield and Wilcoxon (1949).

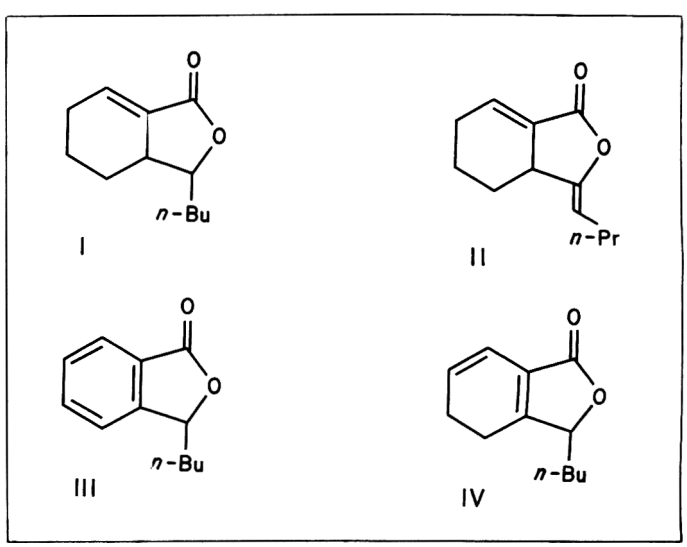

Fig. 1—Selected phthalide compounds reported to be constituents of celery oil.

Table 1—Effect of essential oils on sleeping time after pentobarbital administration

Compound	No. of animals	Dose mg/kg	Sleeping time (min)[a]	% Control
Control	8	–	33.13 ± 6.33a	
	13	100	52.46 ± 7.23b	158
III	6	50	41.83 ± 3.60ac	126
	7	25	30.43 ± 6.70ad	92
	11	100	62.64 ± 5.57	189
IV	7	50	48.00 ± 6.51bc	145
	7	25	38.00 ± 6.22acd	115

[a] The mean ± standard deviation. Means followed by the same letter are not significantly different from one another at the 5% probability level.

Table 2—Effect of essential oils in 10% ethanol solution on sleeping time after ethanol (3.5 g/kg) administration

Compounds	No. of animals	Dose mg/kg	Sleeping time (min)[a]
Control	9	–	40.11 ± 4.43
III	15	100	51.60 ± 21.38
	16	50	41.31 ± 4.92
IV	13	100	38.00 ± 10.61
	10	50	37.60 ± 12.69

[a] The mean ± standard deviation. Values are not significantly different from control at 5% probability level.

Effects of oils alone. Compounds III and IV as 4 and 6% solutions in 10% Tween 80 were administered (ip) to groups of four mice in doses of 200 and 300 mg/kg body weight. Mice were kept in separate compartments at 32°C for 1 hr after injections and in groups according to dose at room temperature for 1 wk thereafter. Behavior and appearance of the animals were normal for this 1-wk period. The sedative effects observed during the first hour following injection are recorded in Table 4.

RESULTS & DISCUSSION

THESE EXPERIMENTS reveal similar mild central depressant activities of 3,n-butylphthalide (III) and sedanenolide (IV). Both compounds prolong sleeping times for pentobarbital (Table 1) and exhibit weak sedative activities by themselves (Table 4). Administration of these compounds immediately following recovery from barbiturate narcosis leads once again to loss of consciousness (Table 3). This suggests that they may increase the sensitivity of the central nervous system to barbiturates. Thus, these compounds appear to be true potentiators as defined by Brodie (1956). True potentiators are distinguished from prolonging agents which act only by decreasing the rate of metabolism of various sedatives.

An analysis of data presented in Tables 1, 3 and 4 reveals that the effects of the celery components of pentobarbital sleeping time cannot be ascribed to simple addition of activities. Thus, in the absence of pentobarbital, doses of 300 mg/kg and 200 mg/kg of compounds III and IV, respectively, resulted in sleeping periods of approximately 6 min. However, when smaller doses of the celery components (e.g. 100 mg/kg) were administered before (Table 1) or after (Table 3) dosing with pentobarbital, the resulting sleep periods were increased relative to controls by 20–30 min. A potentiation effect is clearly indicated.

Ethanol, like the barbiturates, is a central depressant which is considered to affect most strongly the reticular activating system of the brain (Goodman and Gilman, 1970). However,

Table 3—Effect of essential oils on sleeping time following recovery from pentobarbital narcosis

Compound	Animals affected/ Animals treated (% affected)	Dose (mg/kg)	Sleeping time (min)[a]	ED_{50} (mg/kg)
Control	6		0 ± 0a	
	10/10 (100)	150	40.50 ± 17.93	
	5/6 (83)	100	23.17 ± 6.65b	
III	7/9 (78)	85	11.11 ± 11.19cd	72
	10/19 (53	70	6.53 ± 7.27ad	
	3/14 (21	50	2.43 ± 5.65a	
	9/10 (90	100	22.30 ± 5.46b	
IV	8/11 (73	85	6.00 ± 7.16acd	
	4/10 (40)	70	3.60 ± 4.86ad	69
	1/8 (13)	50	0.38 ± 1.06a	

[a] The mean ± standard devaition. Means followed by the same letter are not significantly different from one another at the 5% probability level.

Table 4—Unpotentiated tranquilizing activity of celery components

Compounds	No. of animals	Dose mg/kg	Sleeping time (min)[a]
	4	200	0 ± 0
III	4	300	7.25[b] ± 0.96
IV	4	200	5.75[b] ± 1.32

[a] The mean ± standard deviation.
[b] Significantly different from zero at 1% probability level.

while the celery components affect barbiturate sleeping time, they do not affect ethanol sedation (Table 2). Since the effect of celery components on barbiturate sleeping time seems to involve a modification of effector sites, it appears that the celery components may differentiate between effector sites in the mouse for ethanol and pentobarbitol.

Additional quantitative pharmacological studies are required to further evaluate the efficacy of celery components as central depressants. However, since celery seed oil is readily available and appears to be relatively nontoxic, as indicated by centuries of human use without reported ill effect, the findings presented here suggests that this ancient spice or its constituents may be medicinally useful.

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GLESNI MAC LEOD and BELINDA M. COPPOCK
Dept. of Food Science & Nutrition
Queen Elizabeth College
Campden Hill Road, London W8 7AH, England

SENSORY PROPERTIES OF THE AROMA OF BEEF COOKED CONVENTIONALLY AND BY MICROWAVE RADIATION

ABSTRACT

Sensory properties of the aroma of cooked beef were analyzed using an odor quality assessment technique based on, though not identical with, that of Persson et al. (1973a) (J. Food Sci. 38: 386). Detailed odor descriptions were obtained for beef samples cooked conventionally and by microwave radiation for different periods of time. Several differences in aroma characteristics were observed. In general, odor qualities rated highly for the aroma of microwave cooked beef were shown to be either associated with "meaty boiled" aroma or else to have negative correlation with the quality "meaty roast."

INTRODUCTION

FLAVOR ANALYSIS has evolved in an interesting manner over recent years. During the last decade, attention initially focussed on the chemical composition of the aroma complex and characterization of the volatile constituents advanced rapidly (van Straten and de Vrijer, 1973). As a result, such analyses have attained extreme sophistication and can now be performed with considerable precision (Ayres and Clark, 1975). The data produced are reliable as long as due care is taken.

Until just recently, however, this could not be said for the sensory evaluation of food aroma, since techniques in this field were limited mainly to relatively simple difference and preference tests. It became necessary to develop this aspect of flavor analysis to obtain precision and reliability of data comparable with those of chemical analyses. The aim was to obtain meaningful detailed sensory descriptions of aroma samples so that significant qualitative and quantitative differences between samples could be defined unambiguously. A vocabulary of odor quality terms was required and Harper et al. (1968a, b) were responsible for much of the successful pioneering work in this field. Such precise data can only be achieved by the use of highly trained sensory panels and the procedures are necessarily rigorous, intensive and time-consuming. Nevertheless the benefits therefrom are currently ensuing as shown by the recent work of von Sydow et al. (1970) and Persson et al. (1973a), culminating in a major breakthrough in the ultimate goal of all flavor researchers, i.e., the correlation of chemical composition with sensory properties of food aroma (Persson et al., 1973b; Persson and von Sydow, 1974).

This represents the current status of flavor research. The intention of the present project was to obtain detailed chemical and sensory data for the aroma of beef samples cooked conventionally and by microwave radiation, with a view to future correlations of the two sets of data. No such information has hitherto been published whereby the effects of the two heating methods are compared. This paper reports the sensory analyses only; correlation of these data with results of chemical analyses will be published later.

The theory and use of microwave ovens for cooking and heating have been described in many publications (Procter and Goldblith, 1951; Copson, 1962; Goldblith, 1972; MacLeod, 1972). In summary, and considering the specific case of a frequency of 2,450 MHz (the frequency of the oven used in this work), microwaves are absorbed by 'lossy' substances such as water, which has molecules possessing a permanent dipole moment. The heating effect is a result of dipole rotation as the dipoles attempt to align themselves with the alternating electric field. In lossy materials with uniform distribution of polar molecules, a dramatic heating effect is almost instantaneous throughout the volume of material. Most foods which are not pure fats contain water and therefore are good absorbers of microwaves at 2,450 MHz. Because microwaves heat very quickly, overcooking can readily occur and this should be remembered in any comparisons. Visible browning of cooked surfaces occurs to a limited extent only, if at all, during the short cooking times and it has been suggested that this leads to a lack of many of the aroma compounds attributed to non-enzymic browning (Reynolds, 1970), accounting for a difference in the flavor of foods cooked conventionally and by microwaves.

Very little previously published work is of direct relevance to the current project, since only one reference can be traced to a comprehensive sensory analysis of beef aroma. This relates to the work of Persson et al. (1973a) who developed a technique for detailed qualitative and fully quantitative odor quality assessment of the aroma of canned beef. A similar, modified procedure was used in this instance. Of fringe relevance, however, is the following resumé of work published on the routine sensory comparison of the flavor of various meats cooked conventionally and by microwave radiation. Taste panel scores for juiciness and flavor of beef, pork, lamb and turkey cooked by microwave and conventional means have been obtained by several workers, with some general agreement and also with some conflict of results, e.g., Bollman et al. (1948); Headley and Jacobsen (1960); Marshall (1960); Kylen et al. (1964); Cipra et al. (1971). The majority opinion however indicates lower scores for the flavor of the microwave cooked product. Both Marshall (1960) and Cipra et al. (1971) reported less off-flavors and stale flavor as a result of microwave cooking. Philips et al. (1960) relate differences detected between the flavor and aroma of chicken and pork cooked conventionally and by microwave radiation alone, to browning of the surface of conventional roasts, whilst Zayas et al. (1973) found that small beef pieces heated by microwaves for 4–5 min from the frozen state were not inferior organoleptically to the cenventional control. When heated for more than 10 min however, oxidative changes in the fats occurred and quality deteriorated.

The following describes techniques used to establish whether a significant difference in aroma exists between samples cooked by the two heating methods, together with a detailed description of the sensory differences.

EXPERIMENTAL

Materials and methods

The meat used for all experimental comparisons was standardized as far as possible on breed, age, sex, feeding regime and slaughtering conditions. Longissimus dorsi muscles from Friesian steers of similar age and history were used. The beef was frozen immediately after dissection and stored at −15°C until required, when it was allowed to thaw at

room temperature. All visible fat and connective tissue were removed and the lean meat minced (aperture 4 mm). Removal of visible fat avoided differences in the composition of meat samples used for each experiment. Lean meat contains 4–6% intramuscular fat which is sufficient to give rise to the characteristic species flavor (Patterson, 1974). To eliminate errors further, for any one comparison, the mince was halved, one portion used for conventional heating analysis and the other half for microwave heating.

Preparation and presentation of samples

The following cooking procedure was adopted in order to simulate the Likens and Nickerson extraction technique used for aroma isolation in the chemical analysis performed in parallel with these sensory evaluations (MacLeod and Coppock, 1976, 1977). Lean minced beef (1 kg) was cooked conventionally and by microwaves under a water reflux condenser to prevent loss of aroma volatiles. For the microwave samples, only the meat flask was contained within the microwave oven cavity, because of the heating effect which would otherwise occur on the cooling water in the reflux condenser. The latter was situated on top of the oven and was connected to the meat flask by glass tubing via a hole bored in the roof of the oven (Hirst Microwave Industries Oven, 2,450 MHz).

Presentation of samples to the panelists was standardized as follows. As soon as the heating time was completed, 30g cooked meat, with a little broth, was spooned into coded 30 cm^3 wide-necked glass bottles with Bakelite screw-on lids. Three-digit codes were used. The bottles were allowed to reach room temperature spontaneously (approx 1 hr).

Table 1—Significance levels for difference between microwave and conventionally heated samples of boiled beef using trained judges and triangle tests

Microwave boiled beef	Conventional boiled beef			
	15 min	1 hr	1 hr 30 min	2 hr
5 min	$p < 0.001$[a]	$p < 0.001$	$p < 0.001$	$p < 0.001$
15 min	$p < 0.01$	$p = 0.05$[a]	$p < 0.05$	$p < 0.001$
20 min	$p < 0.001$	$p < 0.05$	$p = 0.05$[a]	$p < 0.001$
1 hr	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$[a]

[a] Indicates samples paired on the basis of equivalent degrees of doneness for the two heating methods.

Table 2—Results of paired comparison tests for difference between microwave and conventionally heated samples of boiled beef on the basis of equivalent degree of doneness, using untrained judges

Test no.	Microwave	Conventional	Difference
1	5 min	15 min	n.s.d.
2	15 min	1 hr	n.s.d.
3	20 min	1 hr 30 min	n.s.d.
4	1 hr	2 hr	n.s.d.

Table 3—Results of paired comparison tests for difference between microwave and conventionally heated samples of boiled beef on the basis of equal heating times, using untrained judges

Test no.	Microwave	Conventional	Difference
1	5 min	5 min	$p < 0.001$
2	15 min	15 min	$p < 0.001$
3	30 min	30 min	$p < 0.001$
4	1 hr	1 hr	n.s.d.

Preliminary experiments indicated that the aroma of the meat samples thus prepared truly represented the aroma of the meat when hot. The relatively large number of samples made it impossible to serve all samples hot simultaneously. The other possibility of keeping the meat warm was discarded because of the problem of the development of warmed-over flavor (Sato and Hegarty, 1971). Chilling the samples reduced the volatility of the aroma compounds. Judges were presented with samples for individual odor assessment in sensory evaluation booths, at a room temperature of 20–21°C. They were instructed to unscrew the lid of each sample bottle and assess the meat aroma only, by sniffing. To eliminate any possible effect on aroma assessment of the different colored appearances of the meat samples, the judges wore green-lensed goggles and the booths were illuminated with white light. This gave a better color masking effect than the use of colored lights.

Sample assessments

Samples cooked for different periods of time by each heating method were assessed. Before analyzing for qualitative and quantitative differences in aroma, simple difference tests were performed to establish whether significant detectable difference existed between samples. These are described initially below. No rewards were offered. Comparisons of microwave and conventional heating were made of samples cooked to equivalent degrees of doneness, for equal heating time and by procedures simulating the aroma extraction technique used in the parallel chemical analysis. For convenience, these latter samples will hereinafter be called equal "extraction" time samples.

Difference tests

Equivalent degrees of doneness comparisons. From preliminary experiments the following cooking times were selected to represent different degrees of doneness for boiled beef (1 kg) for each heating method:

	Underdone	Just done	Acceptable doneness	Overdone
Conventional	15 min	1 hr	1 hr 30 min	2 hr
Microwave	5 min	15 min	20 min	1 hr

Trained judges (see below) compared sample combinations as shown in Table 1 using triangle tests for difference. Thirty-eight judgements were made for each combination.

Age and sex composition of the trained assessors were as follows: 19 assessors (4 male, 15 female); age range 19–35 yr (75% between 20 and 22 yr). Positional effects were controlled for each test by randomization achieved by taking numbers out of a container. Order of presentation was similarly randomized.

Untrained judges compared sample combinations as shown in Table 2, using paired comparison tests for difference. One assessment of each pair was made by each judge. For each of the 40 judgements made, where difference was detected, the panelists were asked to describe the difference. The position of the microwave sample within each pair was randomized as also was the order of presentation of each test, in a manner similar to that already described. Age and sex composition of the untrained assessors were as follows: 40 assessors (18 male, 22 female); age range 18–55 yr. The untrained judges were lay members of the public visiting the laboratories on 'open-day' and they had no detailed experience of sensory evaluation.

Equal heating time comparisons. Untrained judges, using paired comparison tests, compared boiled beef samples cooked for equal heating times of 5, 15, 30 min and 1 hr by the two heating methods. Paired combinations assessed are shown in Table 3. For each of the 40 judgements made, where a difference was detected, the panelists were asked to describe the difference. Randomization of position within a pair and order of presentation of each pair was achieved as described.

Equal "extraction" time comparisons. During the aroma isolation stage of the chemical analysis (MacLeod and Coppock, 1976) using standardized rates of heating for boiled beef, extraction commenced after an initial warm-up time of 30 min (conventional) and 5 min (microwave). For samples used for sensory assessments, replacement of the Likens and Nickerson extractor by a water reflux condenser enabled these pre-extraction warm-up times to be replicated and the "extraction" period timed such that total heating time = warm-up time + extraction time.

To achieve roasting conditions, the meat was 'roasted' by a preliminary standardized open heating period (1 hr conventional; 20 min microwave) to volatilize much of the endogenous water, after which "extraction" commenced immediately and heating continued—in the

case of the conventionally heated samples by means of an oil bath at 160°C (MacLeod and Coppock, 1977).

Fifteen trained judges compared boiled and roast beef samples heated for equal "extraction" times of 5, 15, 30 min and 1 hr by the two heating methods. Paired comparisons were made for differences (see Table 4). Duplicate judgements were obtained for each pair and four pairs were assessed per session. The position of the microwave sample within each pair and the order of presentation of each test were randomized as described. Where difference was detected, the judges were asked to describe the difference.

Odor quality assessment

The odor quality assessment technique used was based on the original work of Harper et al. (1968a, b) and modified later by Persson et al. (1973a).

Selection and training of judges. Meaningful evaluations, particularly of a descriptive nature cannot be expected of untrained panels. Training was therefore essential. The judges used were students who were following a course in sensory evaluation of foods in the same laboratories. This provided a strong degree of interest and motivation–two essential criteria. Since only aroma was being assessed, judges were initially screened for their sensitivity and ability to describe the aromas of 20 odorants recommended by A.D. Little Inc. (1958), which are commonly used for recognition tests. Ability to discriminate between aromas was also established using paired comparison and triangle tests on meat samples. Further specific training involved the use of samples to be encountered in the final experiment. Judges were presented with three samples of cooked beef. These were selected, according to the experience of the experimenter, as being fairly representative of all samples for:

(1) a very weak overall odor strength sample–microwave boiled beef, "extraction" time 5 min;
(2) a very strong overall odor strength sample–conventional roast beef, "extraction" time 4 hr;
(3) a sample deemed to be intermediate between (1) and (2) with respect to both odor strength and odor qualities perceived and which would be a suitable reference sample for future assessments–conventional boiled beef, "extraction" time 1 hr.

The samples were accompanied by a preliminary list of odor description terms selected from the lists compiled by Harper et al. (1968a, b) and Persson et al. (1973a). Judges were requested to assess the aroma of each sample and to indicate which terms were applicable to describe the quality of the odors of each beef sample. Any additional odor qualities considered necessary to describe the total aroma were also requested. This procedure was repeated three times, culminating in a detailed discussion on the exact meaning of several terms which were clarified by comparison with reference products or chemicals. From the frequency of the terms used, a revised list was compiled of 41 odor qualities deemed by the panel to be relevant to the overall project.

Quantitative assessment of the intensity of each odor quality was then introduced. A linear 0–9 scoring scale of intensity was implemented where 0 represented absence of a particular odor quality and 9 represented extreme intensity. Several more sessions followed, during which the judges assessed the aromas of the three samples already described for the intensity of each of the 41 odor qualities. An overall odor strength score was also attributed to each sample. When score sheets for each panelist showed acceptable reproducibility, each judge compiled his/her reference profile for the reference sample (C_4–see Table 4) in terms of its individual odor qualities, their intensities and overall sample odor strength. Judges were given the opportunity to reassess the aroma of this sample at one subsequent occasion, but thereafter these reference profile sheets were retained and presented to each judge at all subsequent panel sessions, together with the reference sample. In this way, each assessed sample could be compared with the reference for intensity of odor qualities.

Odor description of samples. Trained judges assessed 16 samples as shown in Table 4. At any one session, two samples were presented plus the reference sample and profile sheet. Each sample was assessed in duplicate and scored by direct comparison with the reference for each of the 41 odor qualities and for overall odor strength.

RESULTS & DISCUSSION

Difference tests

Equivalent degrees of doneness comparisons. Table 1 presents the results of the trained judges and shows that meat samples boiled to equivalent degrees of doneness conventionally and by microwave radiation are significantly different in aroma.

Table 4–Results of paired comparison tests for difference between microwave and conventionally heated samples of boiled beef on the basis of equal extraction times, using trained judges

	Test no.	Extraction times: Conventional	Extraction times: Microwave	Difference
BOILED BEEF	1	5 min (C_1)	5 min (M_1)	$p < 0.001$
	2	15 min (C_2)	15 min (M_2)	$p < 0.001$
	3	30 min (C_3)	30 min (M_3)	$p < 0.001$
	4	1 hr (C_4)	1 hr (M_4)	$p < 0.001$
ROAST BEEF	5	5 min (C_5)	5 min (M_5)	$p < 0.001$
	6	15 min (C_6)	15 min (M_6)	$p < 0.001$
	7	30 min (C_7)	30 min (M_7)	$p < 0.001$
	8	1 hr (M_8)	1 hr (M_8)	$p = 0.01$

Results of paired comparison tests by untrained judges are given in Table 2. These differ from the above in that no significant difference between pairs is shown. Thus, differences deemed to be significant (and in some cases highly significant) by the trained panel remained undetected by the untrained judges.

Equal heating time comparisons. As shown in Table 1, trained judges indicated highly significant differences in aroma between conventional and microwave samples boiled for equal heating times of 15 min and 1 hr.

Results of the paired comparisons by untrained judges for difference are shown in Table 3. Comparisons 1–3 inclusive showed a highly significant difference. Comparison 4 however showed no significant difference, disagreeing once more with the result for this pair of samples in Table 1, using trained judges.

Therefore, from both these experiments involving untrained judges, it appears that, although they were instructed to search for qualitative differences in aroma, they in fact only indicated gross differences in doneness so that where there was little or no difference in doneness, they recorded no significant aroma difference. The microwave samples were generally described as stronger, more cooked, more meaty, spicy and of 'fuller' aroma than the conventional samples, which in comparison were described as of little odor, fatty, weak, uncooked, sickly, raw, blood-like.

Equal "extraction" time comparisons. Table 4 shows that trained judges indicated very significant aroma differences between all pairs of samples. Determination of the detailed nature of these differences was the object of the odor quality assessment work. However, at this stage, the judges were requested to describe any difference detected between pairs. For paired comparisons 1–6 inclusive the microwave sample–in comparison with the conventional sample–was described as

Table 5—Correlation coefficients between all odor qualities and odor strength

	Almond-like	Ammonia-like	Animal, goaty	Aromatic	Blood-like	Bovril-like	Broth-like	Burnt	Buttery	Cool, cooling	Cooked cabbage	Cured meat, bacon	Earthy soil	Flat, dull	Fragrant	Garlic, onion	Herbal, dried herbs hay	Irritating on nose	Marmite-like	Meaty raw
Almond like	0.30	0.11	−0.29	0.63	−0.42	−0.31	−0.55	0.67	0.67	0.27	0.20	0.29	0.59	−0.08	−0.30	0.27	0.43	−0.02	0.65	
Ammonia like		0.70	−0.52	0.26	−0.08	−0.37	0.02	0.06	0.28	−0.18	0.35	0.44	0.06	−0.36	−0.04	−0.03	0.48	0.25	0.28	
Animal, goaty			−0.17	0.13	0.27	−0.17	0.45	−0.22	0.18	−0.07	0.10	0.54	−0.22	−0.00	−0.13	−0.00	0.60	0.43	0.12	
Aromatic				−0.26	0.67	0.71	0.09	0.03	−0.22	0.10	−0.33	−0.12	−0.27	0.77	0.10	0.44	−0.13	0.36	−0.29	
Blood-like					−0.33	−0.20	−0.44	0.34	0.50	0.58	0.37	0.27	0.37	−0.36	−0.12	0.03	0.46	−0.03	0.66	
Bovril-like						0.73	0.29	−0.08	−0.40	−0.23	−0.23	−0.04	−0.39	0.67	0.07	0.23	0.26	0.69	−0.55	
Broth-like							0.01	0.06	−0.32	0.25	−0.08	−0.11	−0.37	0.57	0.07	0.18	0.04	0.58	0.59	
Burnt								−0.83	−0.52	−0.15	−0.09	0.02	−0.79	0.04	0.09	−0.37	−0.10	0.13	−0.42	
Buttery									0.49	0.09	−0.04	0.14	0.75	0.24	−0.19	0.43	0.32	0.24	0.23	
Cool, cooling										0.30	0.28	0.40	0.57	−0.20	−0.46	0.52	0.11	−0.05	0.70	
Cooked cabbage											0.07	0.16	−0.09	−0.26	−0.12	−0.16	0.14	0.07	0.17	
Cured meat, bacon												0.11	0.24	−0.17	0.15	0.24	0.05	−0.14	0.29	
Earthy, soil													0.12	0.01	0.13	0.34	0.48	0.25	0.36	
Flat, dull														0.04	0.05	0.48	0.19	−0.26	0.54	
Fragrant															0.10	0.56	0.10	0.39	−0.29	
Garlic, onion																0.04	0.14	−0.16	−0.08	
Herbal, dried herbs, hay																	−0.02	0.13	0.37	
Irritating on nose																		0.43	0.15	
Marmite like																			−0.49	
Meaty raw																				
Meaty boiled																				
Meaty roast																				
Metallic																				
Musty, mouldy																				
Nasty smelling																				
Oily, fatty																				
Paint like																				
Rancid																				
Rubber, burnt																				
Sausage like																				
Savory																				
Sharp, pungent																				
Sickly																				
Spicy																				
Sulphurous																				
Sweaty																				
Sweet																				
Throaty																				
Toasted																				
Vegetables, overcooked																				
Yeasty																				
Odor strength																				

being: more flat, dull, weak, buttery, metallic, cooling, sulphurous, sweet, sickly, sweaty, earthy, meaty boiled (as opposed to meaty roast), unpleasant, bland, musty, mouldy, animal-like, blood-like, paint-like, oily and unappetizing.

The conventional sample was described as being: more savory, Bovril-like, burnt, toasted, pungent, strong, pleasant, meaty roast and appetizing.

The first appearance in the microwave series, of aroma descriptions which might readily be associated with roast beef (as opposed to boiled beef) i.e., roasted, toasted, burnt, savory was in sample M_3. For paired comparisons 7 and 8, the microwave sample of each pair was described as decidedly more burnt, smoky, roasted, toasted, pungent, acrid, irritating on the nose, strong and unpleasant. Decomposition of flavor volatiles and precursors had probably occurred to such an extent as a result of considerable overcooking at this stage that these unpleasant, acrid and pungent qualities predominated.

Odor description of samples

In order to determine which sample(s) differed significantly from the others with respect to each odor quality taken in turn, all scores for odor descriptions and for odor strength were converted into interval scores by subtraction of the reference sample score (sample C_4) from the equivalent score for the unknown or assessed sample. This was done in order to correct for differences in sensory acuity of a judge to various odor qualities and for differences in sensitivity of various judges to any one odor quality. Using these data, an Analysis of Variance was performed, followed by Duncan's Multiple Range Test.

Of the 41 odor qualitites listed in Table 5, only 20 showed significant differences ($\leqslant 5\%$ level) between the samples. The results are given in Table 6 together with the results for odor strength. Samples are grouped together in such a way as to indicate that no significant difference in odor quality intensity exists between the aroma of samples within each group, whereas significant differences exist between each group of samples. Figures in brackets below each sample code are the total panel scores for each odor quality for each sample. It is interesting to note that these odor qualities bear a close resemblance to terms used to differentiate between samples in the paired comparison tests.

Odor qualities generally rated higher for the *conventional* than for the microwave boiled beef samples were:

ammonia-like	Marmite-like
animal, goaty	meaty roast
aromatic	savory
broth-like	spicy
Bovril-like	sweaty
burnt	and odor strength

Odor qualities generally rated higher for the *microwave* than for the conventional boiled beef samples were:

almond-like	nasty smelling
blood-like	oily, fatty
flat, dull	paint-like
fragrant	sickly
meaty raw	

Odor qualities generally rated higher for the *boiled* samples than for the roasted samples were:

almond-like	meaty boiled
blood-like	oily, fatty
buttery	paint-like

Table 5 continued...

Meaty boiled	Meaty roast	Metallic	Musty mouldy	Nasty smelling	Oily, fatty	Paint like	Rancid	Rubber, burnt	Sausage-like	Savory	Sharp, pungent	Sickly	Spicy	Sulphur-ous	Sweaty	Sweet	Throaty	Toasted	Veg. o'cooked	Yeasty	Odor strength
0.48	-0.55	0.36	0.41	0.56	0.69	0.72	0.53	-0.24	0.21	-0.55	-0.03	0.73	0.07	0.11	0.61	0.32	0.20	-0.57	0.32	0.02	-0.43
-0.12	-0.14	0.07	0.18	0.52	0.33	0.26	0.45	0.46	0.46	-0.35	0.55	0.14	-0.03	0.76	0.40	-0.26	0.10	0.05	0.16	-0.19	0.19
-0.07	0.30	0.26	0.48	0.50	0.20	-0.03	0.17	0.69	0.41	0.02	0.63	0.02	0.25	0.56	0.34	-0.15	0.37	0.48	0.39	0.13	0.63
0.27	0.59	0.20	-0.07	-0.57	-0.25	-0.39	-0.58	-0.13	0.08	0.66	0.10	-0.28	0.29	-0.45	-0.09	0.36	0.22	0.16	0.13	0.30	0.37
0.32	-0.50	0.37	0.14	0.52	0.43	0.40	0.37	-0.03	0.22	-0.47	0.27	0.52	-0.20	0.33	0.56	0.22	0.36	-0.37	0.42	-0.17	-0.26
0.15	0.90	0.40	-0.09	-0.54	-0.22	-0.59	-0.58	0.20	0.35	0.86	0.42	-0.44	0.52	-0.07	0.13	0.21	0.25	0.39	0.10	0.32	0.68
0.40	0.53	0.28	-0.31	-0.76	-0.33	-0.67	-0.79	-0.10	0.25	0.76	0.28	-0.54	0.43	-0.47	0.21	0.40	0.29	0.14	0.35	0.16	0.35
0.65	0.31	-0.24	0.38	-0.01	-0.52	-0.44	-0.38	0.73	0.10	0.41	0.28	-0.59	-0.13	0.22	-0.35	-0.21	0.29	0.94	0.18	0.29	0.77
0.72	-0.36	0.34	-0.03	-0.02	0.52	0.45	0.26	-0.62	0.01	-0.27	-0.11	0.65	0.38	-0.25	0.53	0.54	-0.01	-0.77	0.02	-0.16	-0.54
0.49	-0.57	0.24	0.12	0.54	0.70	0.49	0.67	-0.41	-0.15	-0.60	-0.22	0.72	-0.01	-0.07	0.41	-0.10	-0.15	-0.63	0.28	-0.34	-0.47
0.32	-0.21	0.21	0.19	0.13	-0.12	-0.10	-0.27	-0.04	-0.03	-0.18	0.13	0.06	-0.14	-0.24	0.46	0.38	0.43	-0.06	0.63	-0.01	-0.09
-0.08	-0.29	-0.17	-0.20	0.09	0.14	-0.08	0.29	0.11	0.50	-0.39	0.28	-0.05	-0.12	0.35	0.25	-0.05	0.23	-0.20	0.44	-0.41	-0.27
0.44	-0.14	0.22	0.14	0.50	0.53	0.05	0.32	0.30	0.22	-0.23	0.47	0.09	0.01	0.20	0.40	-0.07	0.38	0.00	0.47	-0.50	0.20
0.46	-0.65	0.13	-0.11	0.21	0.58	0.48	0.63	-0.53	-0.05	-0.63	-0.28	0.73	0.21	-0.03	0.31	0.22	-0.17	-0.84	-0.18	-0.34	-0.79
0.37	0.55	0.18	0.00	-0.51	-0.00	-0.26	-0.38	-0.11	0.27	0.57	0.14	-0.14	0.64	-0.38	0.00	0.46	0.26	0.08	0.12	0.29	0.28
-0.11	0.08	-0.00	-0.17	-0.18	-0.26	-0.44	-0.16	0.42	0.38	-0.02	0.34	-0.49	-0.02	0.30	0.03	0.05	0.35	0.13	-0.09	-0.22	0.02
0.53	-0.05	0.20	-0.20	-0.07	0.50	0.04	0.31	-0.36	0.15	-0.02	-0.05	0.27	0.23	-0.22	0.19	0.06	-0.02	-0.45	0.17	-0.28	-0.20
0.37	0.14	0.67	0.41	0.37	0.36	0.03	0.04	0.40	0.61	-0.06	0.67	0.19	0.45	0.49	0.76	0.40	0.64	0.01	0.34	0.10	0.26
0.38	0.63	0.47	0.18	-0.31	0.00	-0.30	-0.37	0.18	0.24	0.58	0.51	-0.21	0.57	-0.07	0.47	0.32	0.38	0.24	0.44	0.05	0.56
0.17	-0.72	0.10	0.11	0.78	0.66	0.65	0.77	-0.11	0.08	-0.72	-0.07	0.68	-0.38	0.34	0.19	-0.19	-0.03	-0.47	0.06	-0.20	-0.42
	-0.13	0.59	-0.13	-0.05	0.59	0.08	0.07	-0.46	0.01	0.03	0.03	0.31	0.50	-0.44	0.55	0.33	0.14	-0.58	0.31	-0.25	-0.25
		0.27	0.15	-0.51	-0.44	-0.66	-0.70	0.40	0.26	0.89	0.37	-0.61	0.43	-0.06	-0.04	0.14	0.31	0.66	0.12	0.42	0.81
			0.32	0.17	0.45	-0.02	0.00	0.02	0.25	0.21	0.22	0.18	0.44	0.08	0.66	0.21	0.37	-0.20	0.21	0.11	0.16
				0.49	0.07	0.28	0.04	0.40	0.12	-0.13	0.12	0.26	0.13	0.23	0.30	0.29	0.50	0.32	0.32	0.43	0.30
					0.58	0.61	0.70	0.27	0.04	-0.66	0.07	0.55	-0.35	0.55	0.22	-0.30	0.09	-0.08	0.10	-0.09	-0.07
						0.62	0.76	-0.29	0.04	-0.39	-0.07	0.63	0.10	0.13	0.32	-0.18	-0.10	-0.62	0.03	-0.37	-0.32
							0.71	-0.32	-0.22	-0.57	-0.33	0.80	-0.25	0.16	-0.01	-0.07	-0.29	-0.47	-0.23	0.02	-0.43
								-0.17	-0.15	-0.72	-0.25	0.67	-0.24	0.35	0.02	-0.47	-0.36	-0.52	-0.20	-0.43	-0.49
									0.52	0.14	0.71	-0.50	-0.05	0.67	0.11	-0.14	0.55	0.79	0.30	0.20	0.71
										0.07	0.79	-0.24	0.20	0.59	0.52	0.32	0.66	0.17	0.42	0.17	0.32
											0.18	-0.60	0.35	-0.31	-0.17	0.09	0.11	0.51	0.04	0.38	0.68
												-0.35	0.18	0.59	0.46	0.24	0.72	0.42	0.51	0.01	0.61
													-0.01	0.01	0.24	0.16	-0.22	-0.63	-0.12	-0.01	-0.54
														-0.25	0.36	0.40	0.19	-0.06	0.12	0.23	0.14
															0.20	-0.23	0.27	0.23	-0.03	-0.00	0.26
																0.49	0.57	-0.26	0.59	-0.02	-0.04
																	0.61	-0.11	0.38	0.29	-0.06
																		0.34	0.72	0.12	0.41
																			0.20	0.42	0.86
																				-0.07	0.27
																					0.37

cool, cooling
flat, dull
rancid
sickly

Odor qualitites generally rated higher for the *roasted* samples than for the boiled samples were:

Bovril-like
burnt
meaty roast
rubber, burnt
savory
sharp, pungent
sweet
throaty
roasted
toasted
and odor strength

In addition to the above generalizations, comparisons were made of selected conventional and microwave boiled beef samples on the basis of equivalent and acceptable doneness, equal heating time and equal "extraction" time.

Equivalent and acceptable doneness comparison—boiled beef. (samples C_4 conventional and M_2 microwave) Table 6 shows that the conventional sample was rated significantly higher than the microwave sample for the odor qualities:

animal, goaty
Bovril-like
fragrant
meaty roast
sharp, pungent
spicy
sweet
and odor strength

The microwave sample was not rated significantly higher than the conventional sample for any odor qualities.

Equal heating time (1 hr) comparison—boiled beef. (samples C_3 conventional and M_4 microwave) Table 6 shows that those odor qualities rated significantly higher for the conventional sample than for the microwave sample were:

animal, goaty
cool, cooling
meaty boiled

Qualities rated significantly higher for the microwave sample were:

Bovril-like
fragrant
meaty roast
savory
sweet
and odor strength

Equal "extraction" time comparison—boiled beef. [samples C_2 conventional vs M_2 microwave, (15 min); C_3 conventional vs M_3 microwave, (30 min); C_4 conventional vs M_4 microwave (1 hr)]. Odor qualities present to a significantly greater extent in each sample compared with its counterpart of equal "extraction" time are given in Table 7.

From all these results it can be concluded that odor descriptions dominating in the microwave boiled beef samples are similar to those rated higher for boiling—whether conventionally or by microwaves. However, conventional boiling gave rise to higher scores for those qualities generally rated higher for roasting. This agrees with common observations that microwave cooking does not achieve 'browned' and savory flavors.

It is also clear that nonmeaty odor descriptions such as almond-like, blood-like, flat dull, nasty smelling, oily fatty, paint-like and sickly occur in the microwave cooked samples

Table 6—Odor qualities showing significant difference between samples (Figures in brackets below each sample code are total panel scores)

Animal, goaty	more intense	C_5 C_6 C_4 M_1 C_2 C_3 M_7 M_5 (52,48,46,39,34,33,32,31)
	less intense	C_8 M_3 M_5 C_1 M_6 C_7 M_2 M_4 (29,27,26,26,22,21,21,15)
Blood-like	more intense	M_3 M_5 M_1 C_5 C_3 M_6 M_2 C_4 C_6 M_4 C_2 C_7 (37,35,33,31,31,31,25,24,21,20,19,18)
	less intense	M_8 C_1 C_8 M_7 (17,17,13,12)
Bovril-like	more intense	C_4 (80)
		C_7 M_8 C_2 C_5 M_7 M_4 M_6 C_6 C_8 M_3 M_2 (52,48,45,40,38,36,36,34,34,32,24)
	less intense	C_3 C_1 M_5 M_1 (21,18,16,12)
Broth-like	more intense	C_4 M_4 C_7 M_6 M_8 C_3 M_2 C_2 C_8 M_5 C_6 M_3 (70,58,56,52,52,51,50,48,47,45,44,44)
		C_5 M_7 C_1 (42,40,34)
	less intense	M_1 (15)
Burnt	more intense	M_7 C_6 (108,104)
		C_5 M_8 C_2 (65,61,48)
		C_7 C_8 M_4 M_6 C_4 M_5 C_3 M_2 M_3 C_1 (31,22,20,19,18,12,10,8, 8, 3)
	less intense	M_1 (0)
Buttery	more intense	C_4 M_1 M_5 C_1 M_6 M_4 C_3 C_8 M_2 (69,60,59,58,51,48,43,41,41)
	less intense	M_3 C_2 C_7 M_8 C_5 M_7 C_6 (35,32,31,28,25,15,13)
Cool, cooling	more intense	C_3 M_1 C_1 M_5 C_4 C_2 C_6 M_3 C_7 M_2 (30,28,21,20,18,17,17,16,13,13)
	less intense	M_6 C_8 C_5 M_4 M_8 M_7 (10, 8, 7, 6, 3, 2)
Flat, dull	more intense	M_1 C_1 M_3 C_3 M_4 (56,45,37,33,33)
		C_8 C_4 M_6 M_5 C_7 C_2 M_2 C_5 M_8 M_7 (29,28,28,23,13,10,10, 8, 6, 4)
	less intense	C_6 (0)
Fragrant	more intense	C_4 M_4 C_7 (38,28,21)
	less intense	C_6 C_8 M_7 M_6 M_1 M_2 M_8 C_1 C_3 M_3 C_2 M_5 C_5 (18,18,18,15,12,11,11,9, 9, 9, 7, 6, 5)
Meaty boiled	more intense	C_4 M_2 M_3 C_3 (120,89,88,87)
		C_1 M_5 M_4 M_6 C_8 C_6 M_1 C_7 C_2 M_8 (86,79,75,69,67,65,64,58,57,57)
	less intense	C_5 M_7 (48,37)
Meaty roast	more intense	C_4 M_7 (144,125)
		M_8 C_2 C_7 C_6 C_5 C_8 M_4 M_6 (109,100,98,91,87,82,72,72)
		M_3 M_2 M_5 C_3 C_1 (62,56,52,49,37)
	less intense	M_1 (20)
Oily, fatty		M_1 C_4 M_2 M_3 C_1 — more intense (60,46,43,40,38)
		C_3 C_2 M_4 C_6 M_5 C_5 C_8 M_8 C_7 M_7 (32,31,24,23,23,18,11,11,9, 9)
		M_6 — less intense (7)
Paint-like		M_1 — more intense (19)
		M_2 M_5 C_1 M_6 C_2 C_3 C_4 C_5 C_6 C_7 C_8 M_3 M_4 M_7 M_8 — less intense (9, 9, 4, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)
Rubber burnt		C_5 M_7 C_6 — more intense (12, 7, 6)
		M_8 C_4 M_3 C_3 C_8 C_1 C_2 C_7 M_1 M_2 M_4 M_5 M_6 — less intense (3, 2, 2, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0)
Savory		C_4 M_8 C_7 M_7 M_2 C_2 C_6 M_4 M_6 — more intense (106,102,87,83,78,75,73,72,71)
		C_8 M_3 C_5 M_5 C_3 C_1 (66,61,60,51,50,40)
		M_1 — less intense (31)
Sharp, pungent		C_5 C_4 — more intense (26,16)
		C_6 M_6 M_4 C_2 C_3 M_3 M_2 M_7 C_8 M_5 M_8 C_7 M_1 C_1 — less intense (10,10, 8, 6, 6, 6, 5, 5, 4, 4, 3, 2, 2, 1)
Sickly		M_1 M_5 — more intense (33,21)
		C_1 M_3 C_4 M_6 C_3 C_2 C_7 C_8 C_6 M_2 M_4 M_8 C_5 M_7 — less intense (14,13,12, 11, 9, 8, 8, 8, 7, 6, 4, 4, 1, 0)
Spicy		C_4 C_8 — more intense (26,20)
		C_3 M_8 M_4 M_7 M_3 M_1 M_6 C_1 C_6 M_5 C_2 C_5 M_2 C_7 — less intense (11, 9, 8, 8, 7, 6, 6, 5, 5, 5, 4, 4, 3, 2)
Sweet		M_5 C_4 M_4 M_6 — more intense (45,40,38,37)
		C_6 C_8 M_7 C_5 M_3 C_7 C_1 C_3 M_1 M_8 C_2 M_2 — less intense (27,25,21,20,19,18,17,17,16,15,11,9)
Toasted		C_6 M_7 C_5 M_8 — more intense (49,45,42,38)
		C_7 C_8 M_6 C_4 C_2 M_5 M_2 M_4 M_3 C_3 C_1 M_1 — less intense (22,19,19,18,17,13,12,11,8, 6, 3, 1)
Odor strength:		C_6 C_5 C_4 M_7 M_8 C_2 — more intense (181,180,178,170,163,156)
		C_7 M_2 M_6 C_8 M_3 M_5 M_4 (154,150,145,140,136,136,131)
		C_3 M_1 C_1 — less intense (127,126,124)

and these qualities decreased with heating time. Although this project did not seek evidence regarding consumer preference for each odor quality, it is not unreasonable to classify these terms as relatively undesirable qualities. This qualifies the use of the term 'undesirable' in this context. Thus, the microwave samples—even the overcooked ones—have more of the relatively undesirable qualities normally associated with underdone flavor. These dominate the qualities characteristic of conventional cooking since, even when high intensity ratings for qualities such as roasted, toasted, savory and Bovril-like did appear in the longer cooked microwave samples, these samples

were also described as pungent, acrid, irritating on the nose and unpleasant. Thus, relatively undesirable qualities are present in microwave samples cooked for the normal required length of time for the mass of meat—for either boiling or roasting. The principal cause of the difference is almost certainly a time effect.

Analysis of variance followed by Duncan's Multiple Range Test was also performed in order to give a gross comparison of the four subseries of samples, i.e., conventional boiled beef, conventional roast beef, microwave boiled beef and microwave roast beef. The procedure was also repeated to obtain a gross "two sample" comparison of the two main series of samples, i.e., conventional vs microwave.

For the "four sample" analysis, eight odor descriptions showed significant difference ($\leqslant 5\%$ level) between groups of samples. These are shown, together with the results for overall odor strength in Table 8. The notes burnt, toasted and overall odor strength are all significantly greater in the roasted samples. The aroma qualities flat dull, meaty boiled, oily fatty are all significantly greater in the boiled samples. The microwave samples are overall significantly greater in the quality paint-like. The microwave boiled group of samples is significantly less meaty roast than all others, a fact which will be discussed later regarding the correlation matrix of odor qualities.

The "two sample" analysis of variance results (see Table 9) also shows that the microwave samples are rated significantly higher for the nonmeaty terms blood-like and paint-like. The terms Bovril-like, burnt, spicy and also overall odor strength are confined to the conventional samples. These findings agree with deductions previously made.

It appeared from the data that several interesting relationships existed between odor qualities. These were therefore tested as follows. Correlation coefficients were computed to show how each odor quality correlated with each of the other odor qualities and with overall odor strength. In each case, the data used were the total panel scores for each of the 16 samples for each of the 41 odor qualities (and for overall odor strength). Thus a correlation matrix (shown in Table 5) was prepared. Positive and negative correlations were interpreted as significant if $r \geqslant \pm 0.5$.

Overall odor strength correlates positively with animal goaty, Bovril-like, burnt, Marmite-like, meaty roast, rubber burnt, savory, sharp pungent and toasted. Thus it would appear that the judges were chiefly influenced in their assessment of odor strength by these few dominating odor qualities, which tend to be those which increased with heating time and were associated with doneness. Negative correlations exist between odor strength and buttery, flat dull and sickly. Therefore although these undoubtedly contribute to the overall aroma, they have had a neutralizing effect on the judges' assessment of 'overall' odor strength. These were the qualities rated higher for the microwave than for the conventionally cooked samples.

Meaty boiled aroma correlates positively with buttery, herbal, metallic, oily fatty, spicy, sweaty and had negative correlations with burnt and toasted.

Meaty roast aroma correlates positively with aromatic, Bovril-like, broth-like, burnt, fragrant, Marmite-like, savory, toasted and odor strength. It correlates negatively with almond-like, cool cooling, flat dull, meaty raw, nasty smelling, paint-like, rancid and sickly. Thus it emerges that odor qualities rated higher for the microwave samples are those either associated with boiled beef aroma or else had a negative correlation with meaty roast.

Comparing overall odor strength scores with the summed scores for the intensity of all the individual odor qualities gave a correlation coefficient of $r = +0.8$. This agreement is reassuring, since it means that the judgements of these sensory parameters were relatively consistent and accurate.

Table 7—Odor qualities showing significant difference between samples of boiled beef on the basis of equal "extraction" time

Extraction time	Odor qualities rated significantly higher		
	Conventional		Microwave
15 min	Sample C_2 animal, goaty burnt meaty roast odor strength	vs	Sample M_2 buttery meaty boiled oily, fatty
30 min	Sample C_3 animal, goaty buttery	vs	Sample M_3 Bovril-like oily, fatty odor strength
1 hr	Sample C_4 animal, goaty Bovril-like cool, cooling meaty boiled meaty roast oily, fatty sharp, pungent spicy odor strength	vs	Sample M_4 flat, dull

Table 8—Odor qualities showing significant difference between four series of samples—conventional boiled beef (CB), conventional roast beef (CR), microwave boiled beef (MB) and microwave roast beef (MR)

Burnt	CR MR	>	CB MB
Buttery	CB MB MR	>	CR
Flat, dull	CB MB	>	CR MR
Meaty boiled	CB MB	>	CR MR
Meaty roast	CB CR MR	>	MB
Oily, fatty	CB MB	>	CR MR
Paint-like	MB MR	>	CB CR
Toasted	CR MR	>	CB MB
Odor strength	CR MR	>	CB MB

Table 9—Odor qualities showing significant difference between two series of samples—conventional (C) and microwave (M)

Blood-like	M > C
Bovril-like	C > M
Burnt	C > M
Paint-like	M > C
Spicy	C > M
Odor strength	C > M

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J. D. CROUSE and GERALD M. SMITH
USDA U.S. Meat Animal Research Center, ARS, Clay Center, NE 68933
and R. W. MANDIGO
Dept. of Animal Science, University of Nebraska, Lincoln, NE 68583

RELATIONSHIP OF SELECTED BEEF CARCASS TRAITS WITH MEAT PALATABILITY

ABSTRACT

Relationships among selected carcass traits and cooked meat palatability were studied on 240 carcasses obtained from steers of different biological types produced under a wide range of feeding regimes. Breed type of steer or feeding regime had little or no effect on correlations among taste panel (TP) scores for tenderness, juiciness, flavor and general acceptability. Treatments also had little effect on nonsignificant ($P > 0.05$) correlations of conformation, lean color, lean texture and final maturity with TP traits. Late maturing breeds of steers and steers fed on low energy regimes were rated more youthful than early maturing breeds of steers, and steers fed on high energy regimes. Marbling, percentage of longissimus muscle (LM) fat, quality grade and adjusted fat thickness independently accounted for 2–3% of the variation in TP tenderness and 6–8% of the variation in TP acceptability. Regression analyses indicate that an increase of 30 units in marbling (scored: low Choice = 10; average Choice = 11; high Choice = 12; etc.) would have been required to make a one-unit increase in TP tenderness (scored 1 through 7). No evidence of quadratic effects was observed for TP traits regressed on marbling or percentage LM fat.

INTRODUCTION

THE USDA has recently implemented three major changes in standards for quality grading carcass beef (USDA 1976a). First, conformation was eliminated in determining final quality grades. Second, marbling requirements for the Good grade were narrowed to include only carcasses with a slight amount of marbling. Third, minimum marbling requirements for A maturity carcasses in the Prime, Choice, Good and Standard grades no longer increase with increasing maturity. These changes were the result of research that has shown that maturity, within youthful carcasses, and conformation have little relationship to palatability.

Campion et al. (1976b) have compared USDA (1965) and USDA (1976a) quality grade standards relative to palatability of steaks from youthful carcasses obtained from steers differing greatly in growth rate and fattening characteristics. No obvious differences were found between the old and the revised quality grades for palatability characteristics.

Low positive correlations between marbling score or longissimus muscle (LM) lipid content with taste panel (TP) acceptability scores have been reported by Campion et al. (1976a), Dikeman and Crouse (1975), Berry et al. 1974, Huffman (1974), Parrish et al. (1973), Dryden and Marchello (1970), Breidenstein et al. (1968), McBee and Wiles (1967), Romans et al. (1965), Walter et al. (1965), Blumer (1963), Tuma et al. (1962b), Naumann et al. (1961), Alsmeyer et al. (1959), Wellington and Stouffer (1959), and Palmer et al. (1958). Breidenstein et al. (1968) and Hendrickson and Moore (1965) have also suggested that fat content within the muscle is unimportant for steaks from youthful carcasses.

Results from a number of studies (Breidenstein et al., 1968; Romans et al., 1965; Walter et al., 1965) have indicated significant relationships between maturity and TP palatability characteristics when evaluated over a range of youthful to mature carcasses. Berry et al. (1974) observed significant correlations ($r > 0.2$) between TP palatability scores and maturity when evaluated over the full range of maturity groups. However, these correlations were observed to be low and nonsignificant ($r < 0.1$) when evaluated within the A maturity group. This very low association between maturity and palatability within a narrow maturity range has also been reported by other workers (Campion et al., 1976a; Norris et al., 1971; Covington et al., 1970; Breidenstein et al., 1968; McBee and Wiles, 1967).

Because more than 97% of the "fed" beef is within A maturity (USDA, 1976b), the effect of maturity within A maturity on palatability of steak and roast meat is of most interest. In most previous studies, carcasses from animals with relatively similar growth rates and fattening characteristics typical of domestic beef breeds finished on medium to high energy density diets, have been sampled to determine the efficiency of carcass criteria in estimating palatability. In the present study, relationships among carcass quality indicating criteria, relationships of carcass quality indicating criteria with palatability and how these relationships were affected by breed groups and nutritional environment were examined. Observations were made on carcasses obtained from steers that varied greatly in growth and fattening characteristics and produced under a wide range of feeding regimes.

EXPERIMENTAL

Design

The experimental design ensured variation in carcass traits such as maturity (within the A maturity classification) and carcass composition with relatively low covariance among these traits. This variation allowed an evaluation of independent as well as multivariate effects of maturity, marbling and other traits on palatability. Carcasses from 120 large, late maturing (Chianina, Charolais, Brown Swiss and Limousin crosses) and 120 small, early maturing (Hereford, Angus and Red Poll crosses) steers were evaluated. At approximately 250 days of age, steers were assigned to one of five feeding regimes:

A. Winter growing ration of 48% corn silage, 50% alfalfa haylage and 2% supplement (metabolizable energy (ME) = 2.18 Mcal/kg) for 134 days followed by 133 days grazing on cool and warm season grasses and then 98 days on a 60% corn silage ration (ME = 2.84 Mcal/kg).
B. Same as regime A, except a 20% corn silage ration (ME = 3.11 Mcal/kg) was fed for the last 98 days.
C. Complete corn silage, except a 1.75% supplement and 1.60% soybean meal (ME = 2.40 Mcal/kg) was fed for 315 days.
D. Same as regime C, except a 60% corn silage ration (ME = 2.84 Mcal/kg) was fed the last 105 days.
E. A 60% corn silage ration for 266 days (ME = 2.84 Mcal/kg).

Serial slaughter techniques were used. Steers were killed at about 90 and 105% of the approximate mature weights for females of these biological types (small: 475 kg; large: 550 kg). An additional slaughter group was slaughtered at the beginning of the higher concentrate feeding periods in regimes A, B and D. The experimental design is summarized in Figure 1. The effects of genotype and feeding regime on feed efficiency, growth rate and carcass characteristics have been presented by Smith et al. (1977).

Slaughter and carcass evaluation

All steers were slaughtered by a commercial packer. Carcasses were evaluated and quality graded by USDA (1965) (QG 65) and by USDA (1976a) (QG 76) standards after a 24-hr chill at 2°C. Traits evaluated and scoring systems used are shown in Table 1. The 6th to 12th rib section of the right side was removed according to procedures described by Hankins and Howe (1946), shipped to the University of Nebraska Meat Laboratory and placed in a 1.7°C cooler for a total chill period of 5 days. After chilling, ribs were frozen at −32°C for subsequent chemical and taste panel analyses.

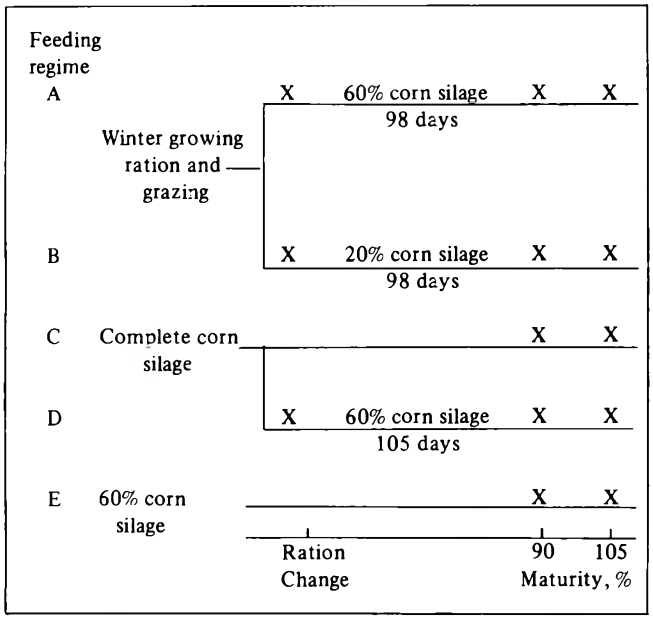

Fig. 1—Experimental design. Slaughter points (X) at ration changes and at 90 and 105% approximate mature weights for females.

Chemical and taste panel analyses

Soft tissues from the 9-10-11th rib section were thoroughly ground and mixed and analyzed for moisture, fat (ether extract), protein and ash by AOAC (1970) procedures. The other part of LM corresponding to the 12th rib was trimmed and analyzed for intramuscular fat by similar procedures.

A 5-cm section corresponding to the eighth rib was removed for taste panel evaluation. Warner-Bratzler shear determinations were made on a 3.8-cm thick section taken at the seventh rib. All rib sections for sensory panel and shear evaluations were placed on a metal rack (5-cm ht) in a 2.5-cm deep broiling pan and cooked in an oven roasting unit preheated to 163°C. Steaks were cooked to an internal temperature of 71°C. Five 1.9-cm cores from each eighth rib section were halved for independent sensory evaluation by 10 panelists. Thus, each panelist received an internal and an external surface of the cooked sample. Panelists evaluated steaks for tenderness, juiciness, flavor and general acceptability. All traits were evaluated on a 7-point hedonic scale: 1 was extremely undesirable and 7 was extremely desirable. Three 2.54-cm cores were removed from the cooked seventh rib section, allowed to cool at room temperature for 30 min and sheared in triplicate by a Warner-Bratzler shear apparatus.

Data analyses

Data were analyzed by correlation and regression techniques. Computations were made on variation resulting from deviations about the overall mean or residual variation. Residual variation was that variation observed after removal of variation for the overall mean, biological type of steer, feeding regime, type by regime interaction; and linear covariates for steer starting age, deviations of initial steer weight from respective biological type subclass mean and the interaction between the latter covariate and breed type.

RESULTS & DISCUSSION

Means and variation

Unadjusted means and standard deviations (SD) for quantitative, qualitative and organoleptic traits of the 240 steer carcasses are given in Table 1. The overall SD includes variation due to treatment subclass means and covariates.

Steers were fed an average of 281.8 days, gained an average of 0.886 kg/day, and had an averaged weight of 505 kg at slaughter. Average hot carcass weight was 300.9 kg.

The unadjusted mean for QG 65 was 9.08 (high good), with a standard deviation (SD) of 1.74 (58% of a quality grade) and a coefficient of variation (CV) of 19%. Mean marbling score 9.53 (high slight) was lower than the average small reported by Campion et al. (1976a) in a study of palatability of steak meat of carcasses obtained from a diverse population of breeds.

Table 1—Overall means and standard deviations

Trait	Mean	Overall SD	Residual SD
Conformation[a]	11.48	1.26	1.20
Muscling score[b]	5.05	1.75	1.60
Lean color[c]	3.70	1.04	0.97
Lean texture[d]	3.21	0.81	0.78
Lean maturity[e]	2.28	0.52	0.50
Skeletal maturity[e]	2.00	0.50	0.46
Final maturity[e]	2.05	0.51	0.46
Days on feed	281.8	58.7	3.9
Marbling score[f]	9.53	3.99	3.56
Longissimus fat, %	4.11	2.60	2.14
Quality grade 65[a]	9.08	1.74	1.52
Quality grade 76[a]	8.88	2.25	2.00
Hot carcass weight	300.9	43.6	35.57
Fat thickness, 12th rib, mm	8.42	4.63	3.64
Adjusted fat thickness, 12th rib, mm	8.58	4.60	3.67
Longissimus area, cm^2	79.2	10.8	8.2
Kidney, pelvic and heart fat, %	2.54	0.82	0.70
Fat color[g]	2.58	0.64	0.60
Rib fat, %	30.11	8.54	6.38
Rib protein, %	15.78	1.84	1.32
Moisture, %	53.35	6.53	5.03
Taste panel tenderness[h]	4.90	0.76	0.74
Taste panel juiciness[h]	4.75	0.63	0.61
Taste panel flavor[h]	5.15	0.39	0.37
Taste panel acceptability[h]	5.01	0.53	0.50
Warner-Bratzler shear, kg	7.50	1.99	1.86
Retail product, %[i]	74.55	5.32	3.89
Slaughter weight	505	68	54
Average daily gain, kg/day	0.886	0.151	0.12
Dressing percentage	59.48	2.11	1.86

[a] Scored: 10 = low choice, 11 = average choice, 12 = high choice, etc.
[b] Scored: 1 = extremely heavily muscled to 10 = extremely light muscled.
[c] Scored: 1 = very light cherry red to 7 = black.
[d] Scored: 1 = very fine to 7 = very coarse.
[e] 1 = A^-, 2 = A^0, 3 = A^+, etc.
[f] Scored: 10 = $small^-$, 11 = $small^0$, 12 = $small^+$, etc.
[g] Scored: 1 = extremely white to 5 = extremely yellow.
[h] 1 = extreme dislike to 7 = extremely acceptable.
[i] Estimated percentage retail product = 87.0 − 8.01 (adj. FT, in) +0.33 (longissimus area, in^2) −0.70 (Est. % K & P fat) −0.399 (% rib chemical fat), Crouse and Dikeman (1976).

The amount of variation in organoleptic traits was greatest in taste panel (TP) tenderness (CV = 16%) and least in TP flavor (CV = 8%). Similar rankings in variability of organoleptic traits were observed by Campion et al. (1976a); however, the degree of variation was somewhat less in their study than in the present study.

Correlations

Correlations among selected carcass traits are presented in Table 2. Correlations above the diagonal were computed from deviations from the overall mean, and correlations below the diagonal were computed from residual variation after fitting the least-squares model.

Residual correlations among TP response variables are of the same magnitude as overall correlations; thus subclass means for biological types and feeding regimes and the covariates had little effect on relationships among these traits. Subclass means and covariates also had little effect on correlations of conformation, lean color, lean texture or final maturity with the TP traits. The very low correlation between final

Table 2—Simple correlations among selected carcass traits and palatability traits[a,b,c]

Trait No.	Trait	Trait number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Conformation	–	−0.17	−0.05	0.20	0.17	0.17	0.12	0.18	0.24	0.43	0.14	0.03	0.06	0.06	0.06	−0.03
2	Lean color	−0.15	–	0.33	0.09	−0.15	−0.22	−0.18	−0.24	−0.18	0.05	−0.20	−0.18	−0.01	−0.14	−0.19	0.16
3	Lean texture	−0.14	0.35	–	0.44	0.13	−0.12	−0.15	−0.10	−0.15	0.11	−0.13	−0.07	−0.03	−0.08	−0.08	0.11
4	Final maturity	0.24	0.17	0.09	–	0.47	0.25	0.17	0.26	0.22	0.15	0.19	0.02	−0.07	0.08	0.04	0.04
5	Days on feed	0.36	−0.08	0.02	0.50	–	0.21	0.04	0.22	0.08	0.26	0.34	−0.06	−0.02	0.09	0.04	0.21
6	Marbling	0.17	−0.18	−0.02	0.27	0.54	–	0.72	0.96	0.66	−0.21	0.73	0.22	0.24	0.32	0.33	0.15
7	Longissimus fat, %	0.08	−0.19	−0.04	0.23	0.55	0.69	–	0.69	0.70	−0.18	0.74	0.22	0.34	0.38	0.37	0.11
8	Quality grade 76	0.17	−0.18	0.00	0.28	0.55	0.95	0.64	–	0.66	−0.19	0.74	0.22	0.24	0.33	0.34	0.14
9	Adj. fat thickness	0.25	−0.14	−0.02	0.30	0.58	0.58	0.54	0.58	–	−0.21	0.70	0.27	0.29	0.38	0.38	0.12
10	Longissimus area	0.44	0.03	0.01	0.23	0.32	−0.02	−0.01	0.00	0.03	–	−0.28	−0.07	−0.08	−0.11	−0.08	0.10
11	Rib fat, %	0.12	−0.16	0.01	0.29	0.62	0.68	0.64	0.70	0.76	−0.05	–	0.25	0.32	0.39	0.39	0.12
12	Taste panel tenderness	−0.01	−0.17	−0.03	0.04	0.08	0.16	0.14	0.17	0.16	−0.03	0.15	–	0.32	0.55	0.78	0.63
13	Taste panel juiciness	0.04	0.02	0.03	−0.08	0.10	0.16	0.26	0.17	0.19	0.02	0.25	0.29	–	0.64	0.66	0.05
14	Taste panel flavor	0.03	−0.12	−0.03	0.07	0.26	0.23	0.29	0.24	0.27	−0.04	0.31	0.54	0.61	–	0.84	0.23
15	Taste panel accept.	0.02	−0.18	−0.02	0.03	0.21	0.25	0.28	0.26	0.26	−0.02	0.32	0.78	0.65	0.88	–	0.39
16	Warner-Bratzler shear	0.00	0.24	0.07	−0.02	−0.06	−0.11	0.00	−0.10	−0.01	0.07	0.02	−0.62	0.11	−0.20	−0.37	–

[a] N = 240.
[b] Correlations > 0.13, P < 0.05; Correlations > 0.17, P < 0.01.
[c] Overall correlations are above the diagonal and residual correlations are below the diagonal.

maturity and TP traits in these A maturity carcasses agrees with previous observations (Campion et al., 1976a; Berry et al., 1974) and supports recent modifications of maturity in the USDA (1976a) grade standards.

Residual correlations (Table 2) among TP traits were 0.29–0.83. Variation in TP general acceptability was highly associated with TP tenderness (r = 0.78), TP juiciness (r = 0.65) and TP flavor (r = 0.88). The lowest correlation among TP traits studied was between tenderness and juiciness (r = 0.29). The correlation between TP tenderness and Warner-Bratzler shear was −0.62, similar to the −0.58 reported by Campion et al. (1976a). TP juiciness, flavor and acceptability were correlated less with Warner-Bratzler shear than with TP tenderness. This result indicates that taste panelists may be influenced by organoleptic characteristics other than tenderness in determining meat tenderness. Berry et al. (1974), in a study of maturity and palatability, used a tenderness profile described by Cover et al. (1962) to measure components of tenderness. This procedure could possibly remove some of the covariance between TP tenderness and other TP organoleptic traits studied.

Correlations (Table 2) of conformation, lean color and lean texture with TP traits were generally very low and nonsignificant ($P > 0.05$). Lean color, however, was correlated with TP tenderness (r = 0.17) and acceptability (r = 0.18) and thus indicates that lighter color longissimus muscles tend to be more acceptable. These observations agree with those of Tuma et al. (1962b) who indicated that texture, within A maturity carcasses, was not associated with TP traits but that darker colored meat from more mature animals was less tender than lighter colored meat from less mature animals (Tuma et al., 1962a). Nonetheless, very low correlation between final maturity and TP traits in these A maturity carcasses agrees with correlations in previous studies (Campion et al., 1976a; Berry et al., 1974).

Residual correlations (Table 2) indicate that variation in days on feed was more highly associated with TP traits than was carcass maturity. This was not the case, however, with the overall correlations in which the diverse feeding regimes obscured any relationship between days on feed and TP traits. Examination of feeding regime means revealed that those that involved longer feeding periods were negatively associated with TP acceptability. However, length of time on feed within a feeding regime was positively associated with TP acceptability. The effect of feeding regimes was similar on the correlations between days on feed and other TP response variables. Feeding regimes affected the relation of physiological maturity to chronological maturity. Analysis of variance of effects of feeding regime on final maturity holding days on feed constant was highly significant ($P < 0.01$) and suggests that steers on a low plane of nutrition do not mature physiologically as rapidly as those on a higher plane of nutrition. Mean final maturity scores at constant days on feed for the five feeding regimes were; A = 1.73, B = 1.78, C = 2.07, D = 2.09, and E = 2.17. Biological type of steers also significantly affected final maturity ($P < 0.05$). The observed effects of biological type on physiological maturity agree with data of Koch et al. (1976) in a study of diverse sources of germ plasm for beef production.

Carcass traits most highly associated with TP traits were measures of fatness. Positive correlations between marbling, percentage of LM fat and percentage of rib fat with TP traits were low. A low positive correlation between marbling and TP traits was also observed by McBee and Wiles (1967) and Wellington and Stouffer (1959); however, the amount of variability in TP tenderness accounted for by marbling (3%) was similar to those reported by Campion et al. (1976a), Dikeman and Crouse (1975), Berry et al. (1974), Huffman (1974), Parrish et al. (1973), Dryden and Marchello (1970), Breidenstein et al. (1968), Romans et al. (1965), Walter et al. (1965), Blumer (1963), Tuma et al. (1962a), Naumann et al. (1961), Alsmeyer et al. (1959), Wellington and Stouffer (1959), Cover et al. (1958) and Palmer et al. (1958).

Interestingly, adjusted fat thickness (AFT) and actual fat thickness (not reported) were as highly correlated to TP traits as measures of intramuscular fat content. This relationship was not appreciably affected by treatment subclass means. The covariance between marbling and AFT (r = 0.58) would partly account for the relation of AFT to the TP traits. Partial correlations between AFT and TP items holding marbling constant (Table 3) were low but significant ($P < 0.05$) for TP flavor and acceptability. Partial correlations between marbling and TP items holding AFT constant were lower than the former correlations. Partial correlations between LM fat and TP traits holding AFT constant were similar to correlations between AFT and TP traits holding LM fat constant. These observations indicate that AFT and marbling would be of similar value in estimating TP panel evaluations of organoleptic traits. Studies (Marsh, 1972; McCrae et al., 1971; Marsh and Leet, 1966; and Locker and Hagyard, 1963) in ovine muscle indicate that increased fat cover protects against toughening due to the cold shortening phenomenon. The results of the present study indicating that AFT was as important as intramuscular fat in explaining variation in TP traits support previous observations and suggest that the insulation properties of external fat increase tenderness.

Holding maturity constant had little effect on correlations between marbling and TP traits (Table 3). However, variation in marbling appears to be slightly less associated with TP traits at a constant time on feed than when time on feed is allowed

Table 3—Partial correlations between carcass traits and panel traits holding selected traits constant[a,b,c]

Carcass traits	Carcass traits held constant	Palatability trait			
		Tenderness	Juiciness	Flavor	Acceptability
Marbling	Maturity	0.18	0.19	0.22	0.25
Maturity	Marbling	0.00	−0.13	0.01	−0.04
Marbling	Days on feed	0.14	0.13	0.11	0.17
Days on feed	Marbling	−0.01	0.02	0.17	0.09
Marbling	Adj. fat thickness	0.08	0.06	0.09	0.11
Adj. fat thickness	Marbling	0.08	0.12	0.17	0.17
Marbling	Longissimus area	0.16	0.16	0.23	0.25
Adj. fat thickness	Longissimus area	−0.17	0.19	0.27	0.28
Longissimus fat	Adj. fat thickness	0.07	0.19	0.18	0.16
Adj. fat thickness	Longissimus fat	0.10	0.06	0.14	0.16

[a] N = 240.
[b] Residual correlation matrix used to calculate partial correlations.
[c] Correlations > 0.13, P < 0.05; correlations > 0.17, P < 0.01.

to vary within subclass treatments. The reduced correlation is the result of the covariance (r = 0.54) between marbling and days on feed. The magnitude of the correlation between marbling and TP acceptability was greatly reduced when computed at constant time on feed. However, overall partial correlation (partial r = 0.33) between marbling and TP acceptability were not reduced. On an overall subclass basis, the covariance between marbling and days on feed was low (r = 0.21). This can be interpreted to mean that marbling is relatively less important, at constant lengths of time on feed, within a feeding regime than on different feeding regimes.

Regressions

Simple regressions of TP tenderness and TP acceptability on carcass traits computed from residual sums of squares and cross products matrix are shown in Table 4. Regression curves of TP traits on carcass traits were flat. Quadratic terms for TP tenderness and TP acceptability on percentage LM fat or marbling were not significant (P > 0.10). A change of 30 degrees in marbling (scored 0 = devoid to 30 = very abundant) was required to make a one-unit increase in TP tenderness values. Percentage LM fat had to increase 20% to make a one-unit change in TP tenderness and adjusted fat thickness was required to increase by 26 mm to make a similar change.

Marbling, percentage LM fat, QG 76 and AFT independently accounted for 2–3% of the variation in TP tenderness and 6–8% of the variation in TP acceptability. Equations with independent variables, conformation, marbling and overall maturity (Table 5, Eq 1 and 9) accounted for 2.7 and 6.4% of the variation in TP tenderness and TP acceptability, respectively. Substituting days on feed for maturity increased the percentage of variation accounted for in TP acceptability to 7.3 (Eq 10). When percentage LM fat replaced marbling (Eq 3 and 11), percentage of variation accounted for in TP tenderness decreased to 2.8% and increased to 8% for TP acceptability.

Equations including marbling and AFT accounted for 3.2 and 9.0% of the variation in TP tenderness and TP acceptability (Eq 5 and 13). Magnitude of standard partial regression coefficients for marbling and AFT shows that they were of equal importance in explaining variation in TP tenderness. However, the standard partial regression coefficient of 0.203 for AFT explained 61% of the total 9% (R^2 = 0.090) variation accounted for by regression equation for TP acceptability and the standard partial regression coefficient of 0.132 for marbling explains 39% of the total variation. These observations indicate that AFT was more important than marbling in describing variation in TP acceptability when used simultaneously. The addition of days on feed made no practical improvement in Eq 5 or 13. Equations involving Warner-Bratzler shear and AFT accounted for 40.8% of the variation in TP tenderness and 21.3% of the variation in TP acceptability. No practical improvement in these equations was made with the addition of marbling.

Frequency distributions

Tables 6 and 7 give frequency distributions of TP tenderness scores and acceptability scores for each marbling score. The percentage of samples with or above a given level of desirability for each marbling score are shown. At a level of TP

Table 4—Regression equations for taste panel dependent variables on selected carcass traits[a]

Dependent variable	R^2	SE	Intercept	Carcass traits and coefficients			
				Marbling[b]	LM fat[c]	QG 76[d]	AFT[e]
TP tenderness	0.03	0.753	4.60	0.033 ± 0.014			
	0.02	0.756	4.70	—	0.049 ± 0.023		
	0.03	0.753	4.39	—	—	0.06 ± 0.024	
	0.03	0.753	4.57	—	—	—	0.039 ± 0.013
TP acceptability	0.06	0.506	4.68	0.036 ± 0.009			
	0.08	0.501	4.73	—	0.067 ± 0.015		
	0.07	0.504	4.46	—	—	0.066 ± 0.016	
	0.08	0.501	4.65	—	—	—	0.042 ± 0.009

[a] Regression coefficients computed from residual sums of squares and cross products matrix.
[b] Marbling scored: 10 = small$^-$, 12 = small$^+$, etc.
[c] Percentage longissimus muscle fat.
[d] USDA quality grade (1976) scored: 10 = choice$^-$, 11 = choice^0, 12 = choice$^+$, etc.
[e] Adjusted fat thickness, mm.

Table 5—Standard partial regressions of taste panel traits on carcass traits[a]

Dependent variable and Eq no.	R^2	Regression coefficients						
		Conformation	Marbling	LM fat[b]	AFT[c]	Maturity	D on Fd	W-B shear
Tenderness								
1	0.027	−0.039	0.165	–	–	0.005	–	–
2	0.027	−0.040	0.164	–	–	–	0.006	–
3	0.020	−0.024	–	0.139	–	0.014	–	–
4	0.026	–	–	–	0.171	–	−0.019	–
5	0.032	–	0.101	–	0.101	–	–	–
6	0.034	–	0.118	–	0.124	–	−0.056	–
7	0.408	–	–	–	0.154	–	–	−0.618
8	0.408	–	0.004	–	0.151	–	–	−0.618
TP acceptability								
9	0.064	−0.016	0.263	–	–	−0.037	–	–
10	0.073	−0.059	0.191	–	–	–	0.128	–
11	0.080	0.006	–	0.288	–	−0.038	–	–
12	0.082	–	–	–	0.238	–	0.072	–
13	0.090	–	0.132	–	0.203	–	–	–
14	0.091	–	0.121	–	0.190	–	0.034	–
15	0.213	–	–	–	0.276	–	–	−0.367
16	0.217	–	0.076	–	0.233	–	–	−0.359

[a] Analysis was based on residual correlation matrix.
[b] Percentage longissimus muscle fat.
[c] Adjusted fat thickness.
[d] Days on feed.

Table 6—Frequency distribution of taste panel tenderness scores

Marbling score	No. of samples	Taste panel tenderness scores											
		3 or greater			4 or greater			5 or greater			6 or greater		
		No.	%	Est. %[a]	No.	%	Est. %	No.	%	Est. %	No.	%	Est. %
P devoid	17	17	100	99 ± 1.2	13	76	74 ± 6.2	6	35	35 ± 7.1	1	6	3 ± 8.4
Traces	47	46	98	99 ± 1.1	37	79	82 ± 5.6	20	43	42 ± 6.6	4	9	6 ± 7.6
Slight	83	83	100	99 ± 1.0	72	87	87 ± 5.5	41	49	49 ± 6.3	4	5	8 ± 7.3
Small	54	53	98	99 ± 1.0	50	93	92 ± 5.4	27	50	56 ± 6.2	4	7	10 ± 7.3
Modest	26	26	100	100 ± 1.0	26	100	95 ± 5.5	19	73	63 ± 6.3	1	4	13 ± 7.6
Moderate	8	8	100	100 ± 1.1	7	88	96 ± 5.6	5	63	70 ± 6.6	2	25	15 ± 8.4
⩾ S abundant	5	5	100	100 ± 1.2	5	100	97 ± 6.2	4	80	78 ± 7.1	0	0	
Number/score			28			88			106			16	
Regression coefficient[b]			98.86 + 0.14(X)			66.71 + 8.73(X) − 0.63(X^2)			27.71 + 7.12(X)			1.13 + 2.34(X)	
R^2			.10			.78			.88			.31	
$SE\hat{y}$(%)			1.0			5.4			6.2			7.2	

[a] Estimated percentage scored equal to or greater than given TP response level based on computed regressions plus or minus standard error of estimate.
[b] X = marbling (scored P devoid = 1 to > S abundant = 7).

Table 7—Frequency distribution of taste panel overall acceptability scores

Marbling score	No. of samples	Taste panel acceptability scores										
		3 or greater			4 or greater			5 or greater			6 or greater	
		No.	%	Est. %[a]	No.	%	Est. %	No.	%	Est. %	No.	%
P devoid	17	17	100	100	15	88	88 ± 4.8	3	18	21 ± 13.1	0	0
Traces	47	47	100	100	43	91	93 ± 4.5	17	36	34 ± 12.2	2	4
Slight	83	83	100	100	82	99	97 ± 4.3	48	58	46 ± 11.6	3	4
Small	54	54	100	100	54	100	99 ± 4.2	34	41	58 ± 11.4	1	1
Modest	26	26	100	100	26	100	101 ± 4.3	21	81	71 ± 11.6	0	0
Moderate	8	8	100	100	8	100	100 ± 4.5	6	75	83 ± 12.2	0	0
⩾ S abundant	5	5	100	100	5	100	99 ± 4.8	5	100	96 ± 13.1	0	0
Number/score			7			99			128			6
Regression coefficient[b]			100 + 0.00(X)			80.86 + 7.34(X) − 0.68(X^2)			8.86 + 12.39(X)			1.50 + .30(X)
R^2			1.00			.94			.87			.04
$SE\hat{y}$ (%)			0			4.2			11.4			1.7

[a] Estimated percentage scored equal to or greater than given TP response level based on computed regressions plus or minus standard error of estimate.
[b] X = marbling (scored P devoid = 1 to > S abundant = 7).

satisfaction for tenderness of three or over, the probability of attaining this level of satisfaction would be 100% at the practically devoid level of marbling. However, the probability of attaining a higher level of satisfaction, say 5, would only be 35% at the practically devoid level of marbling. To attain a TP tenderness score of four or greater with an 87% probability, slight amounts of marbling would have been required.

Percentage of samples with or above a given level of desirability was regressed on marbling score to estimate minimum level of acceptability associated with degrees of marbling. Regression coefficients, R^2 and standard errors are reported in Tables 6 and 7. Estimated percentage levels of desirability were estimated by these equations for each minimum degree of satisfaction and reported with associated standard errors of estimate. The level predicted by regression was preferred to the point estimates because corresponding confidence intervals have been assigned. The equation estimating TP overall acceptability of six or greater was considered not valid because of the small number in each subclass and estimated percentages were not reported. Quadratic terms for equations estimated TP responses of four or greater were considered real ($P < 0.05$). The regression coefficient of 12.39 for TP overall acceptability within the five or greater level of desirability is very steep (Table 7) in contrast to the regression at a level of four or more. The curve indicates that with each increase in one degree of marbling an associated 12.39% ± SE increase in probability of exceeding the TP score of five can be expected. At modest amounts of marbling, 71 ± 11.6% of samples would have been expected to have had a score of five or greater for TP acceptability.

In the present study and in previous studies cited, the relationship existed between carcass quality indicating criteria and TP traits was very low. For example, marbling accounted for only 6% of the variation in TP acceptability, and a 30-fold increase in marbling would be required to yield a one-unit change in TP responses. However, before final conclusions are drawn on the usefulness of carcass criteria studied for estimating carcass palatability, the reader should remember that in the present study, a very palatable cut of meat was cooked under excellent conditions. Important sources of variation may exist in interactions of body composition, maturity, location of cut and cooking method. These sources of variation need a thorough examination before new or different criteria to estimate carcass beef palatability are established.

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B. S. EMSWILER, C. J. PIERSON, A. W. KOTULA and H. R. CROSS
USDA Meat Science Research Lab., ARS
Beltsville, MD 20705

MICROBIOLOGICAL EVALUATION OF GROUND BEEF CONTAINING MECHANICALLY DEBONED BEEF

ABSTRACT

Ground beef chub packs containing 0, 5, 10, 15, 20, 25 or 30% mechanically deboned beef (MDB) were examined for aerobic plate counts (5°C and 35°C), coliforms, *Escherichia coli, Staphylococcus aureus, Clostridium perfringens* and salmonellae after storage at two temperatures for various times. Chub packs were stored at −1.1°C for 0, 6, 12, 18 and 24 days and at −23.3°C for 2, 4 and 6 months. Isolation of *Salmonella enteritidis* serotype *rubislaw* from the pure MDB used in product formulation and from two (1%) of the chub packs containing MDB indicated that a large number of commercial samples of MDB from various sources should be evaluated. However, the aerobic plate counts, coliform, *E. coli, S. aureus* and *C. perfringens* counts in the seven formulations indicated that the addition of MDB to ground beef at levels up to 30% would not affect microbiological safety or shelf-life if the product were formulated and stored according to the procedures outlined in this study.

INTRODUCTION

MECHANICALLY DEBONED MEAT is a promising innovation for a world facing a protein crisis. Studies on the production, characterization and utilization of mechanically deboned red meat show that the potential of this technology to increase the world's protein supply is great (Field, 1974, 1976; Field and Riley, 1974; Field et al., 1974; Meiburg et al., 1976). At least one billion pounds of red meat can be added to the nation's food supply each year through mechanical deboning (Fried, 1976). Mechanically deboned beef can be added to ground beef at levels up to 20% without adversely affecting palatability (Cross et al., 1977). Mechanically deboned poultry is being used in such processed products as bologna and frankfurters (Froning, 1976; Ostovar et al., 1971).

However, further studies are needed to determine whether the use of mechanically deboned red meat poses a health hazard. Mechanically deboned meat produced by the extrusion process is very finely minced and is an ideal medium for microbial growth. USDA MPI Bulletin 865 (USDA, APHIS, 1974) specified requirements for the preparation, labeling and distribution of mechanically deboned red meat. APHIS (USDA, 1976) issued an interim regulation for the use of mechanically deboned meat in processed meat products. This regulation did not allow mechanically deboned meat in hamburger, ground beef or fabricated steaks. In September 1976, a Federal judge ruled that bone particles in mechanically deboned meat are not a part of "meat" and must be considered an additive and adulterant whose effect on health is undetermined. The decision forced the USDA to stop commercial firms from manufacturing and using mechanically deboned red meat.

The purpose of this study was to evaluate the microbiological quality of ground beef containing various levels of mechanically deboned beef after storage at two temperatures for various times.

EXPERIMENTAL

Product formulation and storage

Seven batches of ground beef that contained either 0, 5, 10, 15, 20, 25 or 30% mechanically deboned beef (MDB) were prepared. The ground beef was manufactured from USDA Utility triangles (chuck, foreshank, brisket and plate) and USDA Choice plates that were fabricated for the Schedule AA USDA School Lunch Program under the supervision of a USDA meat grader. The hand boned meat was ground through a Weiler grinder (1.90-cm plate). Coarsely ground meat samples were randomly selected throughout the daily production (150,000 lb) until the quantity was sufficient for the seven formulations.

The MDB was prepared from necks, backs, ribs and pelvic girdles of USDA Utility carcasses that had been hand boned. The bones were passed through a Weiler bone cutter into a model AU 4171 Beehive boning machine with 0.46 mm diameter holes in the cylinder. The MDB was then boxed, quick frozen, and stored at −34°C for subsequent product formulation prior to formulation, the MDB was coarsley ground through a Weiler grinder (1.90-cm plate) and stored at −20°C.

The coarsely ground portions of beef and MDB were then mixed in a Chemetron mixer for 3–4 min. Carbon dioxide snow was injected twice during mixing to maintain an internal temperature of 7°C or less. Fat content of the mixture was determined with a Hobart Commercial Fat Tester (Hobart Manufacturing Company, Troy, OH). Fat or lean from USDA Choice plates was added to adjust the fat content to 23 ± 1%. Each of the seven batches was mixed, then finely ground through a Weiler grinder (0.32-cm plate). The product was then stuffed into 2-lb Cryovac Keeper Casings via a Model 43 VeMag Pump. The chub packs were boxed, blast frozen at −45°C for 48 hr, and stored at −30°C until shipment. The frozen chub packs and a package of frozen MDB were shipped from the meat processing plant to the Meat Science Research Laboratory at Beltsville, MD, via truck at −20°C; transport time was approximately 20 hr.

Chub packs from each formulation were then stored at −1.1°C for 0, 6, 12, 18 and 24 days and at −23.3°C for 2, 4 and 6 months. Three chub packs from each of the seven formulations were evaluated for each storage time and temperature. In addition, the microbiological quality of the frozen MDB used in product formulation was evaluated.

Microbiological analyses

Three locations within each chub pack were sampled aseptically to obtain a 25-g sample that was blended 2 min in 225 ml of sterile Butterfield's phosphate diluent (USDA, 1974b). Serial dilutions of the samples were plated in duplicate on plate count agar (Difco Laboratories, Detroit, MI) in cluster dishes. Aerobic plate counts were determined after the plates were incubated 48 hr at 35°C or 10 days at 5°C.

Coliforms, *Escherichia coli* and *Staphylococcus aureus* were enumerated according to the methods in the *Microbiology Laboratory Guidebook* (USDA, 1974b). All EC broth (Baltimore Biological Laboratory, Cockeysville, MD) tubes showing gas after 24 hr at 45.5°C were streaked onto Levine eosin methylene blue agar (BBL) for detection of typical *E. coli* colonies.

Clostridium perfringens was isolated by two methods: (1) direct pour plating with tryptose-sulfite-cycloserine (TSC) agar (Harmon, 1976) and (2) selective enrichment in liquid sulfite medium prior to streaking on TSC agar with egg yolk (Emswiler et al., 1977). Suspect *C. perfringens* colonies from both the TSC agar pour plates and the TSC agar with egg yolk streak plates were confirmed by inoculation of tubes of buffered motility-nitrite medium and lactose-gelatin medium (Harmon, 1976). Tubes were incubated at 35°C for 24 hr (negatives incubated an additional 24 hr). Nonmotile, gram-positive bacilli that reduced nitrates to nitrites, produced acid and gas from lactose and liquified gelatin within 48 hr were identified as *C. perfringens*.

The salmonellae analysis was performed by adding 25g of meat to sterile Mason jars each containing 225 ml of lactose broth (Difco). The samples were blended for 2 min and incubated at 35°C for 24 hr. Inoculum (0.5 ml) from the lactose broth pre-enrichment was then transferred to 10-ml tubes of selenite cystine broth (BBL) which were incubated at 35°C for 24 hr. Plates of bismuth sulfite (BBL), XLD (Difco), and brilliant green-phenol red (Moats and Kinner, 1974) agars were streaked with inoculum from the selenite cystine broth enrich-

Table 1—Aerobic plate counts (log_{10}) in ground beef containing MDB after storage at −1.1° C[a,b]

	Days of storage	%MDB						
		0	5	10	15	20	25	30
Aerobic plate count (35°C)	0	4.59pq	4.89j-q	4.87k-q	4.96i-p	5.01h-o	4.95i-p	5.02h-o
	6	4.69nopq	4.68nopq	4.78l-q	–	4.89j-q	4.91j-q	4.98i-p
	12	4.54q	4.82l-q	4.89j-q	4 87k-q	4.85k-q	4.77l-q	4.89j-q
	18	4.98i-p	5.30d-i	5.46cdef	5 41defg	5.28d-j	5.06g-n	5.37d-h
	24	6.25a	6.27a	6.30a	6 34a	6.29a	5.81bc	5.58bcd
Aerobic plate count (5°C)	0	4.34	4.20	4.30	4 44	4.37	4.42	4.64
	6	4.36	4.07	4.13	4.32	4.30	4.28	4.43
	12	4.12	4.01	4.27	4 50	4.38	4.17	4.38
	18	4.51	5.52	4.65	4 74	4.93	4.55	4.57
	24	5.97	5.67	6.00	6 10	6.19	5.85	5.71

[a] Each value is the mean log_{10} count/g of three chub packs.
[b] Values in the same column or row followed by the same letter(s) are not significantly different ($P < 0.05$) according to Duncan's (1955) multiple range test.

ment and incubated at 35°C for 24 and 48 hr. The suspected salmonellae colonies were picked and stab-streaked on triple sugar iron agar (Difco) and lysine iron agar (Difco) slants. Tubes showing typical reactions for salmonellae were confirmed by selective biochemical tests (indole, methyl red, Voges-Proskauer, citrate, urease, lysine decarboxylase, malonate, lactose, sucrose, dulcitol) and serotyping (Center for Disease Control, Atlanta, GA).

The logarithms (base 10) of the bacterial counts were analyzed statistically by analysis of variance (ANOVA) and Duncan's (1955) multiple range test.

RESULTS & DISCUSSION

THE FROZEN MDB used in product formulation had 35°C and 5°C aerobic plate counts of 3.4×10^5 and 3.0×10^4/g, respectively. The one log difference between these counts was probably due to a greater number of mesophiles than psychrotrophs in the original unfrozen MDB product. Most Probable Numbers (MPN's) of coliforms, *E. coli* and *S. aureus* were 100, 10 and 10/g, respectively. *C. perfringens* was not detected in the MDB. *Salmonella enteritidis* serotype *rubislaw* was isolated from the MDB and from two (1%) of the chub packs. The two salmonellae-positive chub packs included one 20% and one 30% MDB formulation stored at −23.3°C. The pure MDB probably contained very few salmonellae which were further diluted during formulation. No salmonellae were isolated from chub packs containing ground beef without MDB.

Table 1 presents the 35°C and 5°C aerobic plate counts of the chub packs stored at −1.1°C. The ANOVA of the 35°C aerobic plate counts showed that the counts were significantly affected by product formulation ($P < 0.01$), days of storage ($P < 0.01$), days × days ($P < 0.01$) and days × days × formulation ($P < 0.05$). At day 0, the counts were significantly higher in the 20 and 30% MDB formulations than in the ground beef without added MDB, but the magnitude of the differences was of questionable importance. Initial counts in the 5, 10, 15 and 25% MDB formulations were not significantly different from counts in the regular ground beef (0% MDB). After 6 and 12 days, counts did not differ significantly among the seven formulations. After 24 days, counts were lower for the 25 and 30% than for the 0, 5, 10, 15 or 20% MDB formulations, but the magnitude of the differences was also of questionable importance. The 35°C aerobic plate counts for all formulations gradually increased during storage and after 24 days were significantly higher than the initial counts. However, the higher the percentage of MDB, the lower the log increase in counts after 24 days. For example, there was a 1.66 log increase in counts from the 0% MDB after 24 days as compared to 0.86 and 0.56 log increases in the 25 and 30% MDB formulations, respectively. Counts did not differ significantly among the 0, 5, 10, 15 and 20% formulations after 24 days of storage. The inverse relation between the increase in the 35°C aerobic plate counts during storage and the percentage of MDB in the formulation is unexplained.

The ANOVA of the 5°C aerobic plate counts showed that neither formulation (% MDB), days × formulation, nor days × days × formulation significantly affected the counts. The counts were significantly affected by days of storage ($P < 0.01$). The counts gradually increased during storage, but did not differ significantly among the seven formulations for any storage time. Log increases in counts after 24 days of storage ranged from 1.07/g for the 30% MDB formulation to 1.82/g for the 20% formulation.

MPN's of coliforms, *E. coli* and *S. aureus* in the chub packs stored at −1.1°C are presented in Table 2. MPN values did not differ significantly for either coliforms or *E. coli* among the seven formulations for any of the storage days. Coliform counts ranged from 10–464/g and *E. coli* counts ranged from 0–215/g. The low numbers of viable coliforms and *E. coli* indicate that most of those cold-sensitive bacteria were probably killed when the chub packs were frozen after formulation. The ANOVA of the *S. aureus* counts showed that formulation ($P < 0.01$), days × formulation ($P < 0.01$), and days × days × formulation ($P < 0.01$) were significant. MPN's of *S. aureus* were very low and ranged from 0–215/g. Some differences among the counts were significant but were of unimportant size.

Clostridium perfringens counts of the chub packs stored at −1.1°C were all <10/g. This organism is very cold-sensitive and seldom survives frozen storage.

The aerobic plate counts in the ground beef with and without MDB after frozen storage are shown in Table 3. Both the 35°C and 5°C aerobic plate counts decreased slightly from the initial values during 6 months of storage at −23.3°C. However, the counts did not differ significantly among formulations or days of storage.

MPN's of coliforms, *E. coli* and *S. aureus* after frozen storage appear in Table 4. Mean coliform counts ranged from 10–464/g; mean *E. coli* counts, from 2–215/g; and mean *S. aureus* counts, from 2–100/g. Differences were not important in counts of those microorganisms among formulations or days of storage. The *C. perfringens* counts were very low (<10/g).

CONCLUSION

THE AEROBIC PLATE COUNTS, coliform, *E. coli*, *S. aureus* and *C. perfringens* counts in the seven MDB formulations we evaluated indicate that MDB could be added to ground beef at

Table 2—Most Probable Numbers of coliforms, Escherichia coli and Staphylococcus aureus in ground beef containing MDB after storage at −1.1°C[a,b]

Bacteria	Days of storage	%MDB 0	5	10	15	20	25	30
	0	22	100	215	46	46	100	100
	6	46	100	215	22	10	46	22
Coliforms	12	10	464	215	10	22	46	22
	18	46	100	100	100	46	46	22
	24	46	46	464	100	100	215	22
	0	10	22	215	46	22	10	100
Escherichia	6	22	22	46	22	2	5	5
coli	12	10	22	10	5	22	5	0
	18	10	22	10	10	5	2	2
	24	22	10	10	22	5	0	2
	0	5cd	22abcd	10bcd	22abcd	2d	100ab	46abc
Staphylococcus	6	10bcd	22abcd	100ab	22abcd	2d	100ab	100ab
aureus	12	22abcd	10bcd	46abc	2d	10bcd	22abcd	10bcd
	18	2d	46abc	22abcd	2d	5cd	46abc	46abc
	24	0d	22abcd	10bcd	46abc	2d	22abcd	215a

[a] Each value is the mean count/g of three chub packs.

[b] Staphylococcus aureus counts in the same column or row followed by the same letter(s) are not significantly different ($P < 0.05$) according to Duncan's (1955) multiple range test.

Table 3—Aerobic plate counts (log_{10}) in ground beef containing MDB after storage at −23.3°C[a]

	Days of storage	%MDB 0	5	10	15	20	25	30
	0	4.59	4.89	4.87	4.96	5.01	4.95	5.02
Aerobic plate	56	4.76	4.78	4.95	4.82	4.86	4.86	4.99
count (35°C)	112	4.52	4.73	4.83	4.96	4.87	4.89	4.94
	168	4.56	4.85	4.82	4.74	4.77	4.85	4.89
	0	4.34	4.20	4.30	4.44	4.37	4.42	4.64
Aerobic plate	56	3.99	3.98	3.90	3.94	4.17	4.09	3.98
count (5°C)	112	4.19	4.19	4.57	4.77	4.57	4.04	4.05
	168	3.82	3.80	3.81	3.70	3.80	3.63	3.66

[a] Each value is the mean log_{10} count/g of three chub packs.

Table 4—Most Probable Numbers of coliforms, Escherichia coli and Staphylococcus aureus in ground beef containing MDB after storage at −23.3°C[a]

Bacteria	Days of storage	%MDB 0	5	10	15	20	25	30
	0	22	100	215	46	46	100	100
Coliforms	56	100	100	100	22	46	46	100
	112	10	100	464	46	22	22	100
	168	46	46	100	10	10	22	22
	0	10	22	215	46	22	10	100
Escherichia	56	100	22	22	10	22	10	46
coli	112	10	10	46	10	10	22	22
	168	10	5	22	2	5	5	22
	0	5	22	10	22	2	100	46
Staphylococcus	56	10	22	22	5	5	46	46
aureus	112	5	46	10	10	5	100	100
	168	10	22	22	5	5	46	46

[a] Each value is the mean count/g of three chub packs.

levels up to 30% without affecting microbiological safety or shelf-life if the product is formulated and stored according to our procedures. It is not yet possible to commercially market ground beef that is completely free of pathogens, and no immediate practical solution is in sight. The levels of *E. coli, S. aureus* and *C. perfringens* in the MDB formulations in this study are normally found in commercial ground beef. The incidence of salmonellae in ground beef is very low. Surkiewicz et al. (1975) isolated salmonellae from only three (0.4%) of 735 beef patties and from only one (0.1%) of 690 production line samples collected from 42 federally inspected plants. Although there is no evidence that the incidence of salmonellae would be higher in MDB than in ground beef, a large number of MDB samples from various sources should be evaluated if commercial MDB again becomes available. Mechanically deboned beef is an ideal medium for microbial growth, but the conditions we specified limited the growth of the microorganisms to levels that would not affect the wholesomeness of the product.

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M. A. THOMAS, R. McBRIDE,[1] A. D. TURNER and G. H. ABAD
Dairy Research Centre, P.O. Box 217, Richmond, N.S.W. 2753, Australia

EFFECT OF EXTENDING BEEF PATTIES WITH MILK CO-PRECIPITATES

ABSTRACT

Granulated type calcium co-precipitates (CCP) with and without wheat flour were used to extend ground beef with fat levels of 20 and 30%. The hydrated CCP was added at the level of 30% by weight and the physical, chemical and sensory properties of the mixture were evaluated along with those of all-meat patties and patties extended with texturized soya protein. Results of physical and chemical analyses were variable and showed no definite trend. All-meat patties gave the highest yield, lowest shrinkage and highest fat content after cooking. Patties extended with CCP and wheat flour had the highest moisture content and lowest penetrometer values at both fat levels. Sensory evaluation revealed that patties extended with CCP and wheat flour had the best appearance, flavor and texture and were generally the most acceptable.

INTRODUCTION

THE EFFECTS of extending ground meat with soya protein in both texturized and flour form have been extensively evaluated, especially in the United States. Such investigations have included: the effect of varying the level of soya protein, effect on consumer acceptability, the effect on fat and water binding and shrinkage after cooking. The findings of these investigations often varied.

Cross et al., (1975) reported that addition of texturized soya protein (TSP) to the formulation of meat patties did not have an adverse effect on consumer preferences. In most instances, patties with 12.5 or 25% TSP received equivalent or better palatability ratings than the all-beef patties. Kotula et al., (1974) reported that patties containing 20% TSP were scored about equal to all-beef patties, whereas patties containing TSP at 30% tended to be scored lower than the all-meat patties. Drake et al., (1975) reported that all-meat patties were rated significantly higher than patties containing 15 and 20% TSP. Increasing the TSP level to 25% resulted in a further significant decrease in flavor ratings.

Judge et al., (1974) suggested that cook shrinkage (decrease in diameter of a patty) should be a criterion for the quality of ground beef and reported that soya additives substantially reduced shrinkage in samples containing 20 and 30% fat. Bowers and Engler (1975) found all-meat patties did not differ significantly in diameter after cooking from patties containing 15 and 30% TSP.

Addition of TSP to meat patties decreases cooking losses (Anderson and Lind, 1975; Bowers and Engler, 1975; Drake et al., 1975). Although Anderson and Lind (1975) and Drake et al., (1975) reported that addition of TSP decreased moisture loss during cooking, Bowers and Engler (1975) reported that the amount of added TSP had no influence on the moisture content of patties after cooking. Anderson and Lind (1975) reported that, irrespective of fat content, retention of fat in cooked all-beef patties is greater than in TSP-extended beef patties. The binding effect of TSP on fat content remained unchanged when TSP was increased from 0–25% (Drake et al., 1975).

[1] C.S.I.R.O. Food Research Laboratory, North Ryde. N.S.W. 2113, Australia

Neither the New South Wales Pure Food Act (1908) nor the Commonwealth Food Specification (1971) define or specify the addition of other food ingredients or the composition of meat patties. However, companies manufacturing meat patties advertise their products as minced meat containing both soya flour and TSP.

In Australia, consideration has also been given to the use of other materials as meat extenders, especially calcium co-precipitates (CCP). CCP is a precipitate of casein and whey proteins, in which 95–97% of the milk proteins are recovered (Buchanan et al., 1965; Muller et al., 1967). By varying the calcium content, it is possible to produce several types of co-precipitates with various functional properties (Muller et al., 1967). Milk co-precipitates were successfully used in the manufacture of comminuted meat products (Thomas et al., 1973) and simulated meat products (Thomas et al., 1976). Their functional properties are reported by Thomas et al., 1974).

This paper reports on shrinkage, moisture and fat binding, penetrometer values and eating quality of minced beef patties extended with granular type milk co-precipitates. To allow comparison, all-meat patties and patties extended with TSP were also evaluated.

EXPERIMENTAL

TWO MEDIUM TYPE granulated CCP (87% protein) were used; these were (1) fine type of granule size 0.5–0.6 mm (30–35 mesh); (2) a coarse type with granule size of 1–4 mm (5–18 mesh). Both types were obtained from Drouin Co-operative Butter Factory Co. Ltd., Drouin, Victoria.

The commercially prepared, unflavored TSP used was Maxten CS (52% protein); granule size ranged from 1–4 mm (5–18 mesh). It is distributed by Miles Laboratories Inc., Elkhart, IN.

Flour

Plain wheat flour (WF), 12% protein, was used.

Preparation of patties

Ground beef (9.3% fat) and tallow (92% fat) obtained from a local butcher, were used in the preparation of the patties. The seven samples prepared comprised an all-meat control (mince and tallow) and six beef-protein blends. The blends consisted of a mixture of 30% hydrated proteins and 70% beef plus tallow by weight. Each of the above treatments consisted of two levels of fat, 20 and 30% in the final mix. Composition of the patties is reported in Table 1. Samples 1–7 had 20% fat while samples 8–14 had 30% fat. Although the intention was to keep the fat content exactly at the level reported in Table 1, actual fat contents approximated these levels (see Table 2).

Meat, tallow and protein mixture were mixed thoroughly, ground twice through a 5 mm plate and sampled for fat and moisture. Patties (100 g, 10 cm diameter and 1.3 cm thick) were formed using a K-tel Pattie Stacker (Le Mark Industries Inc., St. Paul, MN). Patties were stacked in groups of four, separated by plastic sheets and snap-frozen at −30°C.

Flavorings

The following flavor composition was found to resemble the flavor of commercial patties; 20% beef spice powder, 19% hamburger flavor, 38% barbecue flavor; 20% onion powder and 3% monosodium glutamate. (Flavors were supplied by Allied Falvors, Smithfield, N.S.W. and International Flavors and Fragrances (Aust.) Pty. Limited, Dee Why, N.S.W.). Flavors were added uniformly to all treatments at the rate of 1.5%. Also added were 1% cooking salt and 0.2% tetra sodium diphosphate.

Table 1—Formulation of patties used for chemical, physical and sensory evaluation

Fat %	Treatment no.	Ingredients % Meat	CCP[b] Fine	CCP[b] Coarse	TSP[c]	Wheat flour	Added H_2O
	1[a]	80					
	2	50	10				20
	3[a]	50		10			20
20	4[a]	50			10		20
	5	50	7.5				22.5
	6	50		7.5			22.5
	7[a]	50		5.0		2.5	22.5
	8	70					
	9	40	10				20
	10	40		10			20
30	11	40			10		20
	12	40	7.5				22.5
	13	40		7.5			22.5
	14	40		5.0		2.5	22.5

[a] Treatments used for sensory evaluation
[b] Calcium co-precipitate
[c] Texturized soya protein

Patties were cooked from the frozen state in a convection oven (General Electric Topchoice) at 200°C for 15 min. Total cooking loss of the patties (hence yield) was determined by weight difference. Moisture and fat loss were determined on the patties after cooking using the AOAC (1970) method. The values for percent retention of fat and moisture were derived (Anderson and Lind, 1975) from each of the average runs by multiplying the percent yield by percentage of fat or moisture in cooked patties and dividing the resulting product by percentage of fat or moisture in the raw patties. Changes in the physical dimensions of the patties were measured at room temperature 1 hr after cooking using a vernier caliper. A representative diameter and height of each patty was determined by averaging the maximum and minimum readings. Four readings were taken on each of three replicated patties for each treatment.

Physical measurements of firmness of patties were determined 1 hr after cooking using a single-pin penetrometer (Huntington and Rutledge, 1974) with a 3 mm diameter blunt pin. Maximum resistance to penetration was recorded in grams weight and is referred to in this article as penetrometer value. Three patties in each trial were used and 10 readings on each patty were taken and the results averaged.

For sensory evaluation, four treatments (1, 3, 4, and 7, Table 1) were selected. A balanced incomplete block design was used for the sensory evaluation. Each of the 30 tasters on the panel received two samples at each session, for two sessions per day for three days. Each of the four treatments was presented with every other treatment giving three replications per treatment.

Standard taste testing conditions prevailed. Each member of the panel received hot, two half patties, one from each treatment. Tasters were required to assess the samples for appearance, flavor, texture and general acceptability using standard nine-point hedonic scales (9 = like extremely, 1 = dislike extremely). Separate analyses of variance were done for each of the four sensory variates assessed.

RESULTS & DISCUSSION

Physical and chemical evaluation

Mean values for percent yield, shrinkage, fat and water binding and penetrometer values of three replications and 14 treatments are reported in Table 2.

The all-meat patties (treatment 1) gave the highest yield, at 20 and 30% fat level, higher in fact than the TSP extended patties (treatments 4 and 11), a finding which is contrary to that of other workers (Anderson and Lind, 1975; Bowers and Engler, 1975; Drake et al., 1975). The yields for patties extended with CCP were generally lower, although the values for one CCP treatment (5.0% coarse CCP and 2.5% WF) were close to those obtained for the TSP treatment. The patties which initially contained 20% fat had a higher average yield (70.80%) than those containing originally 30% fat (66.61%) and this is in accord with the findings of Drake et al. (1975), who showed that as the amount of fat was reduced, total cooking losses were also reduced.

Changes in diameter of the patties during cooking showed some degree of negative correlation with % yield. The treatments with the highest yield (all-meat patties and patties extended with TSP) showed the smallest decrease in diameter. The TSP extended patties (treatments 4 and 11) demonstrate the least increae in height during cooking.

It is interesting to note that the difference in fat content between patties initially containing 20 and 30% fat disappears after cooking. In fact, the average fat content of the cooked patties was lower if they had originally contained a higher level of fat (22.48% for the 30% fat level, 23.64% for the 20% fat

Table 2—Average values of yield, fat and moisture in raw and cooked patties, percent retention of fat and moisture, shrinkage and penetrometer values in cooked patties

Treatments no.	% Yield	% Shrinkage Diameter decrease	% Shrinkage Height increase	% Fat Raw	% Fat Cooked	% Moisture Raw	% Moisture Cooked	% Retention Fat	% Retention Water	Penetrometer value (g)
1[a]	83.09	14.61	26.46	23.95	27.51	62.91	54.03	95.44	71.36	763
2	68.86	23.34	28.08	18.82	20.59	61.86	54.01	75.34	60.12	688
3[a]	66.00	25.55	41.92	18.71	21.19	62.24	52.72	74.75	55.90	800
4[a]	74.02	19.73	19.46	20.63	22.64	62.57	56.43	81.23	66.76	783
5	63.98	23.51	28.23	20.93	26.78	63.20	53.92	81.86	54.59	677
6	66.73	25.28	19.77	21.45	21.50	63.57	55.49	66.89	58.25	639
7[a]	72.89	24.28	29.46	21.68	25.30	62.96	57.02	85.06	66.01	578
Average	70.80	22.33	27.63	20.88	23.64	62.76	54.80	80.08	61.86	704
8	72.63	24.68	31.69	29.05	29.18	56.21	52.34	72.96	67.63	760
9	67.78	28.19	32.31	27.19	17.73	55.37	53.40	44.19	60.55	792
10	65.85	26.95	27.15	28.59	23.92	54.36	53.13	55.09	64.36	761
11	70.89	23.01	21.85	28.34	20.08	54.64	55.09	50.23	71.47	730
12	58.70	29.36	28.54	30.67	22.18	56.67	53.20	42.45	55.11	707
13	66.58	28.51	28.69	28.04	22.49	57.10	52.18	53.40	60.84	705
14	68.84	26.28	42.92	29.84	21.79	56.62	55.11	50.27	67.00	570
Average	66.61	26.71	30.45	28.82	22.48	55.85	53.49	52.66	63.85	718

[a] Treatments used for sensory evaluation

level). These results are in agreement with those of Drake et al. (1975) who showed that the amount of fat lost during cooking depended upon the level of fat originally incorporated into raw ground beef patties before cooking.

The penetrometer values indicate that the substitution of meat by TSP or CCP alone did little to systematically change the firmness of patties, at least at the level of substitution used in this experiment. However, the addition of WF did affect the treatments 7 and 14 (5.0% coarse CCP, 2.5% WF) quite substantially. These treatments showed much less resistance to penetrometer puncture and is reflected in the lower penetrometer values.

Sensory evaluation

Results of sensory evaluation are summarized in Table 3.

Table 3—Mean sensory ratings of cooked patties for appearance, flavor, texture and general acceptability for each of the four treatments

Treatments	Appearance[c]	Flavor N.S.	Texture[c]	General acceptability
1	6.1a	5.3	3.4a	4.3a
3	6.7b	5.6	5.1b	5.2bc
4	6.8bc	5.2	5.0b	5.0b
7	7.1c	5.7	5.6c	5.5c
L.S.D.	0.33	–	0.45	0.41

[a] Treatments significantly different ($P < 0.001$); N.S.—Treatments not significantly different and L.S.D. omitted; L.S.D.—Least significant difference. Any pair of treatments withing a column without a letter in common are significantly different ($P < 0.05$).

Appearance

Panelists rated the appearance of all three extended treatments (3, 4 and 7) significantly higher ($P < 0.05$) than that of the all-meat patties (treatment 1). Comments from the panel about the extended treatments included "more even surface," "more attractive," "better color." The appearance of the patties extended with 5.0% coarse CCP and 2.5% WF were liked most overall.

Flavor

There was no significant difference in flavor ratings between any of the samples. This lack of difference was probably due to the high level of flavoring used which was common to all treatments, i.e., the flavoring material masked any changes in flavor which might have arisen from the use of extenders. Other workers (Kotula et al, 1974; Bowers and Engler, 1975; Drake et al., 1975) reported flavor changes due to addition of TSP, but only with unseasoned material.

Texture

Differences between treatments were most pronounced on the texture variate. Patties extended with 5.0% CCP and 2.5% WF (treatment 7) were significantly ($P < 0.05$) preferred by the panel because they were considered to be "softer" and more "tender." This is also in accord with the observed lower penetrometer values. There was no significant difference between patties extended with TSP (treatment 4) and those extended with 10% CCP (treatment 3); however, all extended treatments were rated higher than the all-meat patties (treatment 1). These results are in agreement with earlier findings (Huffman and Powell, 1970; Kotula et al., 1974; Cross et al., 1975). The panel disliked the texture of the all-meat patties because it was considered to be "too tough" and "rubbery." Cross et al., (1975) state that factors responsible for toughness in ground beef have been related to the myofibriller and stromal proteins. Extending the ground beef with CCP or TSP would, apart from any other effect, have a "diluting" influence on the role of these proteins and this provides a possible explanation for the increased tenderness.

General acceptability

The acceptability ratings followed much the same pattern as the ratings for appearance and texture. All extended treatments (3, 4 and 7) were significantly preferred ($P < 0.05$) to the all-meat patties (treatment 1); the 5.0% coarse CCP plus 2.5% WF treatment being the most liked.

CONCLUSION

IT IS EVIDENT from the sensory testing that CCP does have potential as a meat extender. Patties containing CCP were found to be much more acceptable than all-meat patties and slightly more acceptable than patties containing TSP, a meat extender which is already widely used and accepted. The patties containing CCP and a low level of WF were rated the highest on all sensory variates, but the advantages of this treatment were not restricted to sensory qualities. It also had the highest yield of all the CCP treatments and would be the cheapest to manufacture, both important economic considerations. The viability of using CCP commercially as a meat extender is open to speculation, but these results do suggest that the possibility warrants further investigation.

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K. L. NEER,[1] J. F. CAMPBELL[2] and R.W. MANDIGO
Animal Science Dept., University of Nebraska, Lincoln, NE 68583
F. CAPORASO
Dept. of Food Science & Technology, University of Nebraska, Lincoln, NE 68583
and G. M. SMITH
U. S. Meat Animal Research Center, North Central Region, ARS, Clay Center, NE 68933

PRESS/CLEVE PORTIONING AND MECHANICAL TENDERIZATION OF BEEF TOP LOINS AS AFFECTED BY NUTRITIONAL REGIME AND BIOLOGICAL TYPE

ABSTRACT

Eighty steers, forty each of two biological types (large, late maturing, and small, early maturing) were placed on nutritional regimes containing four levels of roughage. After slaughter, 40 top loins were press/cleave portioned and mechanically tenderized, 40 top loins were press/cleave portioned only, 40 top loins were mechanically tenderized only and 40 top loins remained untreated to serve as controls. Each nutritional regime and biological type was equally represented in each of the processing treatments. Taste panel evaluations indicated a preference for tenderized steaks ($P < 0.05$) over those not tenderized. Press/cleave portioning had very little effect on the steaks. The combination of press/cleave portioning and mechanical tenderization appeared to be an acceptable industry practice.

INTRODUCTION

OVER THE LAST FEW YEARS the growth in the number of large, late maturing "grass finished" cattle appearing in the market place has been substantial. This growth has been accompanied by a concern for the tenderness and overall consumer acceptability of the beef from these cattle. With this in mind, work at the University of Nebraska has been initiated to evaluate the effects of mechanical tenderization, mechanical pressing and cleaving and the combined effects of these treatments on beef top loins.

According to May et al. (1975), various types of cattle that have been maintained on similar feeding rations and slaughtered at equal physiological ages have varying muscle fiber characteristics. Johnston et al. (1975) reported that longissimus muscle fibers were larger in diameter in Charolais than in Angus steers. Earlier work by Berry et al. (1974) had correlated tenderness with muscle fiber size. They showed that larger muscle fibers tended to be less tender than smaller fibers.

Miller (1975) stated that if a general dissipation of connective and muscle tissue can be accomplished, such as through mechanical tenderization, an increase in tenderness will be perceived. Work by Hinnergardt et al. (1975) substantiated this finding by showing that mechanical tenderization increased tenderness of beef inside rounds. Work by Schwartz and Mandigo (1974) and Campbell et al. (1977) also reported similar findings.

Goldner et al. (1974) reported only small changes in the tenderness of pork loins due to mechanical tenderization. However, very significant increases in tenderness have been associated with ram lamb and goat (Bowling et al., 1975). Bowling et al. (1975) further stated that little or no increase in tenderness was accomplished by more than one pass through the tenderizer. Therefore, mechanical tenderization will not greatly improve the tenderness of meat cuts which already possess acceptable tenderness (Huffman, 1975).

It has been shown that mechanically tenderized cuts cook in a shorter time than do untenderized cuts (Schwartz and Mandigo, 1974; Goldner et al., 1974; Goldner, 1975). For this reason increased cooking losses have been associated with mechanically tenderized meat (Davis et al., 1975). However, Schwartz and Mandigo (1974) reported no difference in cooking loss between tenderized and untenderized inside round steaks.

Although very little work has been done which pertains to the cooking atributes of press/cleave portioned meat cuts, some work has been done which pertains to the system itself. Projects at the University of Nebraska by Cheseney (1973), Goldner et al. (1974) and Schwartz (1975) all have pointed out the desirability of forming a uniform log of meat that can, in turn, be cleaved into uniform chops or steaks. Such a system is being used extensively by the industry and is quite well adapted to such cuts as boneless pork loins, boneless beef strip loins and boneless beef ribs.

The objective of this study was to evaluate the effects of press/cleave portioning and mechanical tenderization on beef top loins from cattle of large and small body types which had been fed various feeding regimes.

EXPERIMENTAL

Source of meat

Forty large, late maturing steers and 40 small, early maturing steers were randomly assigned within type to one of four feeding regimes at approximately 205 days of age (Fig. 1A).

All steers were slaughtered by a commercial packer when they were between 90 and 105% of the mature dam's weight. The carcasses were then chilled in a 2°C cooler. After 24 hr of chilling the carcasses were quartered between the 12th and 13th ribs, graded by a USDA grader (quality and yield) and both hindquarters were shipped to the University of Nebraska Meat Laboratory where they were placed in a 1.7°C cooler for further processing. The boneless beef top loins (longissimus) (two per animal) were removed 5 days postmortem, double wrapped in freezer paper and frozen in a -32°C freezer. Following 14 days of storage the beef top loins were tempered in a -2°C cooler for 48 hr. One loin from each animal was mechanically tenderized, the other loin from the animal was not. Loins from five animals within a ration-body type class were press/cleave portioned; loins from the other five animals of each class were not press/cleave portioned (Fig. 1B).

Top loins were mechanically tenderized with a Bettcher TR-2 reciprocating blade mechanical tenderizer set at a conveyor speed of 7.62 cm/penetration. The top loins were passed through the TR-2 one time with the external surface of the top loin toward the conveyor. For press/cleave portioning, top loins were pressed with a Bettcher Model 70 Hydraulic press that exerted 28 kg/cm² (400 psi) to all surfaces of the top loin. The pressed loins were then sliced in a Bettcher power cleaver to a uniform thickness of 2.5 cm.

Those top loins that were to be both tenderized and press/cleave portioned were mechanical tenderized immediately prior to the pressing operation. Five top loins from each feeding regime-biological type subclass served as controls and were never tenderized nor press/cleaved.

[1] Present address: Dept. of Animal Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061
[2] Present address: Dept. of Food Science & Human Nutrition, Michigan State University, East Lansing, MI 48824

A. Number of cattle per ration - body type class

Body Type	Ration[a] A	B	C	D
Large	10	10	10	10
Small	10	10	10	10

B. Treatment of boneless top loins within any ration - body type class.

5 animals		5 animals	
MT[b] loin 1	NMT[b] loin 2	MT[b] loin 1	NMT[b] loin 2
PC[c]		NPC[c]	

a A = A winter growing ration of 48% corn silage, 50% alfalfa haylage and 2% supplement (metabolizable energy (M.E.) = 2.18 Mcal/kg) for 134 days followed by 133 days grazing on cool and warm season grasses. B = The same as regime A with the addition of 98 days on a high concentrate ration (M.E. = 3.11 Mcal/kg) after grazing. C = A complete forage ration except for 3.35% supplement and soybean meal (M.E. = 2.40 Mcal/kg) for 315 days. D = A 60% forage ration for 260 days (M.E. = 2.84 Mcal/kg).

b MT = Mechanically tenderized, NMT = not mechanically tenderized

c PC = Press/cleaved, NPC = not press/cleaved

Fig. 1—Experimental design

Table 1—Least squares means for quality and yield grades by nutritional regime

Traits	Nutritional regime A	B	C	D	S.E.
Quality grade[d]	6.90[a]	9.25[b]	9.65[b]	9.90[b]	0.399
Yield grade	1.23[a]	2.10[b]	2.35[b]	2.80[c]	0.107

a,b,c, Means with different superscripts in same row are significantly different ($P < 0.05$).

d Low Choice = 10; High Good = 9; Average Good = 8; Low Good = 7.

Table 2—Least squares means for quality and yield grades by biological type

Trait	Biological type Large	Small	S.E.
Quality grade[a,**]	8.10	9.75	0.282
Yield grade**	1.78	2.46	0.076

a Low Choice = 10; High Good = 9; Average Good = 8; Low Good = 7.

** Means significantly different ($P < 0.01$).

Table 3—Least squares means for physical properties by nutritional regime

Trait	Nutritional regime A	B	C	D	S.E.
Shear force (kg)	1.49[a]	1.74[b]	1.34[c]	1.14[d]	0.059
Thawing loss (%)	4.54[a]	2.90[b]	2.93[b]	2.52[b]	0.184
Cooking loss (%)	16.88[a,b]	18.63[a]	16.30[b]	15.22[b]	0.657
Expressed fluids (%)	57.08[a]	54.43[b]	44.36[c]	52.78[b,c]	0.983

a,b,c,d, Means with different superscripts in the same row are significantly different ($P < 0.05$).

One slice (approximately 0.83 cm) was removed from the anterior surface of all top loins to attain a flat surface, and the rest of the top loin was cut either by hand or by the power cleaver into 2.5 cm thick steaks. The first steak was used for raw proximate analysis and water-holding capacity (WHC) determinations. The second steak was used for organoleptic evaluation, cooking measurements, shear force determinations and cooked proximate analysis. Both steaks were vacuum packaged and heat sealed at -686 mm of Hg and frozen in a -32°C freezer.

Cooking

After 1 wk of storage at -32°C, the steaks were weighed and placed in a 1.7°C cooler to thaw. The steaks were reweighed 24 hr later to determine thawing loss. Each steak was placed on a metal rack (1.27 cm high) in a 5.0-cm-deep broiling pan and cooked in a Westinghouse oven roasting unit that had been preheated to 163°C. All samples were cooked to an internal temperature of 71°C as determined by a thermometer inserted into the center of each steak. All steaks were blotted and weighed to determine cooking loss and cored with a 2.5 cm core. Five cores from each steak were cut in two, so each panelist received an external and an internal surface from each sample. The three other cores were sheared with a Warner-Bratzler shear to obtain an indication of tenderness. The rest of the longissimus muscle was vacuum packaged and frozen as previously described until analysis for moisture, fat, protein and ash content (AOAC, 1970) could be accomplished.

Organoleptic evaluation

Ten semi-trained panelists evaluated one sample from each steak for tenderness, juiciness, flavor and general acceptability. All traits were evaluated on a 7-point scale, 1 was extremly undesirable, and 7 was extremly desirable. The panelists evaluated the samples in a room lighted by red lights in an attempt to eliminate possible biases due to color of the samples.

Chemical analysis

All steaks, both raw and cooked, which were designated for chemical evaluation were analyzed for moisture, fat, ash and protein as described by AOAC (1970). Water-holding capacity (WHC) of the raw steaks was determined as the percentage of expressed fluids when the press method described by Wierbicki and Deatherage (1958) was used.

Statistical analyses

This study was a split plot arrangement of treatments. The main plot treatments, nutritional regime and biological type, were in a randomized complete block design, and the sub-plot treatments, tenderization and press/cleave portioning, were in an incomplete block design. Data were analyzed by analysis of variance procedures as described by Harvey (1960). Correlation analyses were according to Snedecor and Cochran (1967). Differences were tested for significance using Duncan's new multiple range test (Duncan, 1955).

RESULTS & DISCUSSION

Effects of nutritional regime and biological type

The effects of nutritional regime on top loin steaks are shown in Table 1. Cattle receiving feeding regime A produced carcasses that were significantly lower concerning quality grade than those from cattle which received feeding regimes B, C and D. As one might expect regime A carcasses, having the lowest quality, produced the most acceptable yield grades ($P < 0.05$) also the highest quality grade (regime D) had the poorest yield grades. These results were expected due to the relative energy levels associated with each nutritional regime.

The effects of body type are contained in Table 2. The small, early maturing steers produced higher quality, but lower yielding carcasses than the large, late-maturing steers produced, as was expected.

The least squares means for physical properties by nutritional regime are presented in Table 3. All regimes produced beef top loin steaks with significantly ($P < 0.05$) different shear force values. Steaks from regime B produced the highest value and those from regime A the next highest value. Thaw loss for steaks from regime A cattle was significantly higher ($P < 0.05$) than the other regimes. Cooking losses were greatest ($P < 0.05$) for steaks from regime B cattle which may partially explain the higher shear values. Steaks from regime A cattle

also had high cooking losses and expressed the highest percentage of fluids ($P < 0.05$). From these results it is apparent that regimes A and B produced the least desirable steaks.

Effects of mechanical tenderization

Table 4 shows the effects of mechanical tenderization of beef top loin steaks. Product tenderness, as evaluated by the taste panel, was not affected by mechanical tenderization. However, product juiciness was superior ($P < 0.01$) for those steaks which had been tenderized. These results concerning juiciness are in agreement with those of Hinnergardt et al. (1975). The failure of mechanical tenderization to produce an increase in tenderness may be due to the muscle which was utilized in this study. This theory is in agreement with Huffman (1975).

Tenderization also resulted in increased general acceptability and improved flavor ($P < 0.01$). Cooking loss was the only other trait of those measured which was affected by tenderization. Cooking loss values were significantly higher ($P < 0.01$) for the tenderized steaks which is believed to be related to the general cell disruption caused by mechanical tenderization (Miller, 1975).

Table 5 shows the least squares means for chemical composition as affected by mechanical tenderization. None of the chemical components, either raw or cooked, were changed by mechanical tenderization.

Effects of press/cleave portioning

Table 6 contains the least squares means for taste panel evaluations and physical properties as affected by press/cleave portioning. None of the parameters evaluated by the taste panel was affected by press/cleave portioning. The only physical properties which were affected by press/cleave portioning were thawing loss and cooking loss. However, when thawing loss and cooking loss are combined, as has been done in Table 6, little difference between the treated and control products exists. This leads one to conclude that there are very little if any, practical differences in the data.

The least squares means for chemical composition as affected by press/cleave portioning are presented in Table 7. Of the four chemical components measured, only cooked protein and cooked moisture were affected by the portioning system. These differences do exist but are probably of little or no economic importance. The feeding regime and biological type X portioning method and mechanical tenderization interactions were not significant.

CONCLUSIONS

THESE RESULTS showed beef top loin steaks from nutritional regimes B, C and D to have higher quality grades and to be more tender than steaks from cattle fed regime A, low quality forage followed by grazing. The data also showed that small, early maturing cattle produced higher quality but lower cutability carcasses than did large, late maturing cattle.

Mechanical tenderization significantly improved product tenderness, juiciness and general acceptability. In addition, mechanical tenderization did not affect the chemical composition of beef top loin steaks.

Press/cleave portioning, a procedure by which uniform logs of meat are formed and mechanically sliced, did not affect product tenderness, flavor, juiciness, general acceptability or chemical composition.

Through the combination of mechanical tenderization and press/cleave portioning, uniform meat products, with enhanced palatability traits can be achieved with only minor changes in chemical composition.

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Table 4—Least squares means for taste panel evaluation and physical properties by mechanical tenderization

Trait	Tenderization		S.E.
	Tenderized	Control	
Tenderness	4.91	4.81	0.087
Juiciness**	5.09	4.66	0.082
Flavor**	4.92	4.72	0.048
General acceptability**	4.74	4.44	0.054
Cooking loss** (%)	17.86	15.66	0.409

** Means significantly different ($P < 0.01$)

Table 5—Least squares means for chemical composition by mechanical tenderization

Trait	Tenderization		S.E.
	Tenderized	Control	
Raw ash (%)	0.97	0.95	0.016
Raw fat (%)	4.52	4.62	0.083
Raw protein (%)	22.03	22.15	0.062
Raw moisture* (%)	71.81	71.53	0.082
Cooked ash (%)	1.03	1.00	0.010
Cooked fat* (%)	5.80	6.30	0.135
Cooked protein (%)	28.49	28.30	0.141
Cooked moisture (%)	64.15	63.95	0.160

* Means significantly different ($P < 0.05$).

Table 6—Least squares means for taste panel evaluations and physical properties by portioning method

Traits	Portioning method		S.E.
	Press/Cleave	Control	
Tenderness	4.89	4.82	0.061
Juiciness	4.93	4.82	0.082
Flavor	4.81	4.83	0.048
General acceptability	4.63	4.56	0.055
Thawing loss**	3.60	2.85	0.138
Cooking loss	16.25	17.26	0.409
Total loss	19.75	20.11	N/A

** Means significantly different ($P < 0.01$).

Table 7—Least squares means for chemical composition by portioning method

Trait	Portioning method		S.E.
	Press/Cleave	Control	
Raw ash (%)	0.98	0.94	0.016
Raw fat (%)	4.53	4.62	0.083
Raw protein (%)	22.03	22.15	0.063
Raw moisture (%)	71.56	71.78	0.061
Cooked ash (%)	1.01	1.02	0.011
Cooked fat (%)	6.06	6.06	0.135
Cooked protein* (%)	28.60	28.19	0.141
Cooked moisture* (%)	63.78	64.31	0.160

* Means significantly different ($P < 0.05$).

T. C. LANIER[1], J. A. CARPENTER, R. T. TOLEDO and J. O. REAGAN
Dept. of Food Science, University of Georgia, Athens, GA 30602

TRANSPORTATION AND COLOR MAINTENANCE OF HANGING BEEF

ABSTRACT

Environmental conditions (temperature, humidity and air velocity) were monitored inside refrigerated trailers loaded with beef carcasses during stationary and in-transit trials. The effects on meat color maintenance were followed on meat samples located throughout the trucks. The effect of initial carcass heat load on color maintenance during transport was studied utilizing a simulated trailer environment. Results show that an excellent environment is maintained in refrigerated trailers for beef color maintenance in-transit despite fluctuations in outside temperature and over-the-road conditions. Results from tests in which carcasses with elevated internal round temperature (15°C) were held in a simulated trailer environment suggests that shipping of such carcasses in refrigerated trailers could lead to bacterial growth and discoloration in-transit.

INTRODUCTION

DARKENING OR DISCOLORATION of beef quarters is sometimes encountered during transportation in refrigerated trailers. To accurately assess the cause of such discoloration, it is necessary to investigate the carcass handling procedures and their effect on environmental conditions during cooling and shipping.

In a previous study (Lanier et al., 1977), the effects of temperature, air velocity and relative humidity on color maintenance of beef slices were examined. Results indicated that moisture as well as temperature in the immediate environment of the beef surface were important in color maintenance and that bacterial growth was the probable causative agent of accelerated discoloration at low temperature. It should be noted that bacterial growth sufficient to cause discoloration need not result in concurrent production of slime or off-odors (Butler et al., 1953). Bacteria as the cause of discoloration during transport of hanging beef may thus be overlooked in the absence of such signs of microbial growth.

Usually, beef holding coolers in packing plants are designed such that carcass spacing, air movement, and temperature are optimal for heat removal. Relative humidity is also regulated at a level that minimizes shrink yet is low enough to minimize bacterial growth also. However, when carcasses are transferred to tractor trailers for transport, radical changes in the storage environment may occur. Data on environmental conditions in refrigerated trailers during shipment of hanging beef other than some temperature monitoring are not available in the literature. Assuming that the design of most conventional refrigerated trailers and their cooling systems are similar, it might be expected that the conditions inside a trailer would be affected only by outside ambient conditions and the initial heat (and moisture) load of carcasses at the time of loading.

EXPERIMENTAL

Stationary trailer studies

Three separate part-loads of beef carcasses were monitored over 4-day periods in a refrigerated trailer at Greenwood Packing Co., Greenwood, SC. The trailer, a Trailmobile trailer designed for the shipping of hanging beef, was loaded with 60 quarters of beef packed tightly into three sections of the trailer; front, middle and rear, to simulate actual packing conditions as closely as possible within these three sections.

The trailer was cooled by a Carrier Transicold refrigeration unit mounted on the front. Cool air was directed from the unit directly toward the top of the truck from the front with no ductwork to conduct air to the back. After loading, air velocity was measured at various points within the truck, including the three positions in which test meat samples were located. Temperature and humidity were continuously monitored throughout each trial. A Honeywell multipoint potentiometer and thermocouples were used for temperature measurements while relative humidity was monitored with a Hygrodynamics hygrometer with recorder. Measurements of weight loss and pigment oxidation were made on meat samples placed in the trailer and held 1–4 days. These samples, 1.3 cm thick and 5 cm in diameter, were obtained with a sterile knife from beef M. semitendinosus previously rinsed in 70% ethanol. The samples were then placed in aluminum dishes and suspended in a wire basket in the center of each group of carcasses. Pigment oxidation on the surface of meat samples was estimated by reflectance spectrophotometry using a modification (Lanier et al., 1977) of the method of Franke and Solberg (1971).

In-transit trailer studies

In-transit environmental studies were made on trailers carrying full loads (ca 60 head of cattle) of quartered hanging beef. Two loads were monitored during the entire trips from the Iowa Beef Processor's Plant in Amarillo, TX, to either Raleigh, NC, or Atlanta, GA. Monitoring equipment included temperature and humidity recorders, as previously described, powered by a DC-AC inverter. Temperature was measured inside and outside the trailer, on the walls, floor, and at different points within the interior space of the trailer. The humidity sensor was located near the ceiling directly in front of the air entrance duct. Air velocity measurements were made prior to loading at different points within the trailer. Cooling was provided by a standard ThermoKing unit. Air movement differed in these trailers from that in the stationary trailer in that canvas ductwork conducted air to the back of the trailer, causing the air velocity downward through the carcasses to be less.

Simulated loading of carcasses with elevated internal temperature

As industrial practices did not permit loading "hot" beef onto trailers for study, an experiment was devised to study the effects of such a loading practice on carcass cooling rates and bacterial growth. A small number of carcasses were cooled in a chill cooler maintained at 4–5°C, with high air velocity, overnight to an internal round temperature of 15°C, then transferred to a holding cooler maintained at the same temperature with low air velocity. The carcasses were packed together tightly with both meat surfaces of adjacent pairs in contact to simulate actual loading patterns in trailers. Temperature at the exposed surface, deep center, and at the interface of contacting carcasses in the round area were monitored using thermocouples and a recorder. Swabs of each interface area, where the rounds had been in contact with one another, were taken each day until the carcasses were completely chilled and total aerobic plate counts were determined in plate count agar (Difco) using an incubation temperature of 37°C for 48 hr.

RESULTS & DISCUSSION

Environmental conditions and beef quality maintenance in a stationary trailer

Figure 1 shows the relationship between outside ambient temperature and the temperature, average relative humidity, and low heat/cool cycling time inside a partially loaded stationary truck for a typical 24-hr period. Cycling time is the time period between successive temperature maxima as the temperature fluctuated due to alternating heat and cool cycles. Thus, cycle time is the inverse of the frequency with which the cooling unit switched to low heat operation. The greatest influence of outside temperature was on the cycling time and thus on the average relative humidity. Average relative humidity decreased slightly as the outside temperature rose and cycling time increased. Inside temperature remained fairly

[1] Present address: Dept. of Food Science, North Carolina State Univ., Raleigh, NC 27607

constant with a slight increase accompanying a large increase in the outside temperature.

Temperature remained fairly uniform throughout the length of the trailer, cycling continuously (±2°C) at any point, due to alternating heat and cool cycles. Increased operation on the high speed cool cycle was noted during the first 2 days of the initial run. Outside temperature was higher during this time period than at any other time during the experiment and may have been the cause for the increased cooling.

Inside the trailer tested, the relative humidity of the air cycled between 88 to 94 and 100% (±3%), probably resulting in an intermittent drying and wetting of the meat surface. During the cooling cycle, air was partially dried as it passed through the cooling coils. This drier air would evaporate moisture from the surface of the meat. At each heating and defrosting cycle of the refrigeration unit, water and ice on the coils were vaporized and humidified the warm air stream, which then allowed condensation of water on the relatively cooler meat surface. The overall cycle, however, resulted in a net loss of water from the meat.

Air velocity vertically downward through each group of carcasses was 0.1–0.2 meters per second (mps) at the low fan cycle and 0.2–0.3 mps at the high fan (high cool) cycle, with the exception of the midpoint of the trailer, where air flow was approximately twice that of the other two sections. The cooling unit of the trailer in which these studies were conducted forced air from the front blower horizontally along the ceiling at a velocity of 15–25 mps. A downward flow occurred through the hanging carcasses as the air stream progressed the length of the trailer, the greatest downward air velocity occurring at the midpoint of the trailer. Air was eventually recycled through an intake port located at the front and near the floor of the trailer.

Weight loss and extent of pigment oxidation in meat samples placed in each of three locations within the three stationary truck trials are given in Tables 1 and 2. Except for run 1, weight loss was significantly greater ($p < 0.05$) for samples placed within the group of carcasses at the middle of the trailer due to higher air flow in this region. However, contrary to the findings of our previous study of a model system (Lanier et al., 1977), these samples exposed to the higher air flow did not have significantly higher ($p < 0.05$) pigment oxidation compared to samples in the front and rear of the trailer where air velocity was lower.

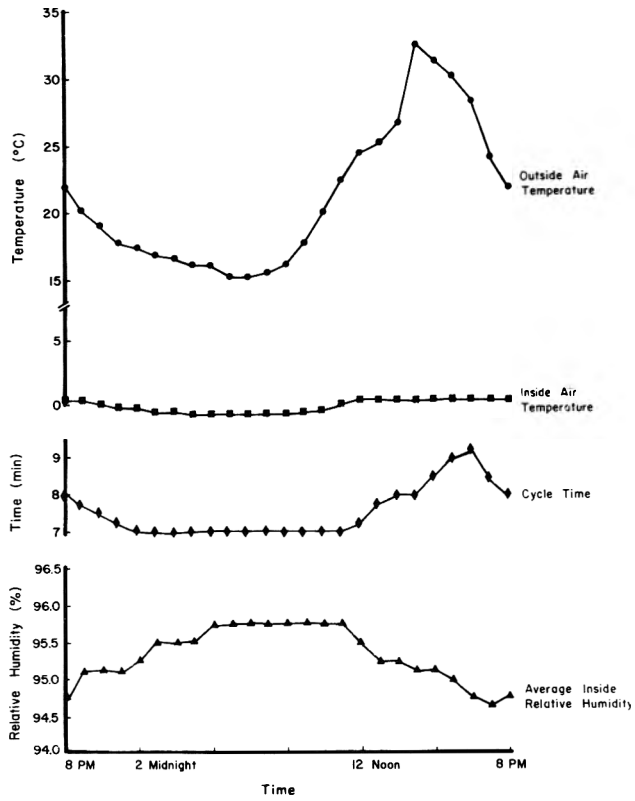

Fig. 1—Relationship between outside temperature and inside trailer environment for typical 24-hr time period in a stationary trailer.

The degree of shrinkage occurring in samples from runs 1 and 3 was greater than that which occurred in samples from run 2. Trailer inside temperature was 1°C in run 3 and 3°C in runs 1 and 2. The colder temperature of run 3 lowered the average relative humidity during the trial because of increased operation in the low speed cool mode. Increased operation of the cooling unit in the high speed cool mode which occurred during run 1 undoubtably contributed to increased desiccation in these samples.

Table 1—Percent water loss of samples from stationary truck trials

Day	Run	Sample location[a] Front	Middle	Rear
1	1	*	1.82a	1.67a
	2	0.83a	1.25b	1.61a
	3	0.95a	1.99c	1.71a
2	1	*	2.62a	2.66a
	2	1.40a	2.41a	1.85b
	3	1.45a	3.30b	2.90a
3	1	*	3.65a	4.09a
	2	1.79a	3.10a	2.42b
	3	1.94a	4.67b	3.60c
4	1	*	2.86a	5.38a
	2	2.45a	4.26a	3.40b
	3	2.43a	6.20b	4.15c

[a] All means within a day and location having different letters are significantly different ($P < 0.05$) from each other. n = 3 for each mean.
* Data not available for front location, Run 1.

Table 2—Pigment oxidation (percent metmyoglobin) of samples from stationary truck trials

Day	Run	Sample location[a] Front	Middle	Rear
1	1	*	19a	23a
	2	9a	4b	9b
	3	2a	0b	5b
2	1	*	18a	23a
	2	16a	11a	15b
	3	4a	5a	5c
3	1	*	30a	39a
	2	32a	25a	17b
	3	7a	3b	5c
4	1	*	39a	46a
	2	21a	23b	27b
	3	14a	18c	11c

[a] All means within a day and location having different letters are significantly different ($p < 0.05$) from each other. n = 3 for each mean.
* Data not available for front location, Run 1.

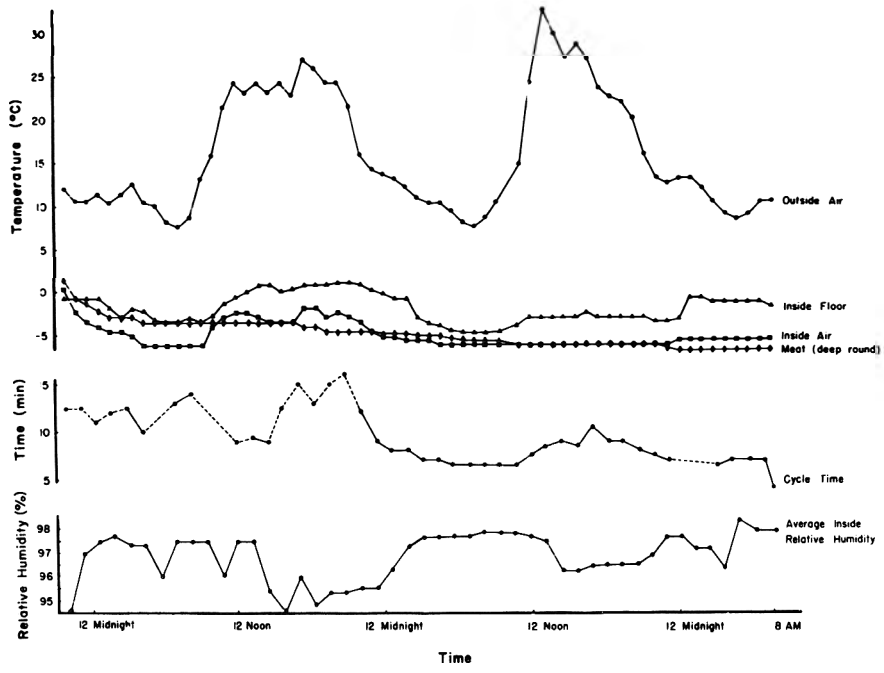

Fig. 2—Relationship between outside temperature and inside trailer environment during a typical in-transit run. Dotted lines indicate irregular cycling during that period, the cycle times indicated for that period being mode values.

The cool, dry conditions to which the meat was exposed in run 3 resulted in the lowest amount of pigment oxidation, although the color was a deeper red due to desiccation. The conditions of run 2 resulted in the most desirable meat color after 4 days storage; however, it was subjectively noted that carcasses from this run deteriorated more rapidly in color than carcasses from the other two runs upon further holding in a storage cooler for 3 days at 1°C. This was assumed to be due to bacterial growth stimulated by the warmer, more moist environment as suggested by results of a previous study (Lanier et al., 1977). Samples from run 1 sustained the greatest color deterioration although the color still appeared quite acceptable after 4 days. Subjective observations revealed that the desiccation which occurred in samples from runs 1 and 3 appeared to increase the stability of the carcass meat color upon further holding at 1°C, probably by reducing bacterial growth. There appears to be no evidence of a correlation between drying of the meat surface and increased pigment oxidation, as reported by Ledward (1971).

Environmental conditions inside refrigerated trailers in-transit

Conditions monitored in the meat trailers for a period of 2–3 days in-transit were very similar to conditions monitored inside stationary trailers over the same time period (Fig. 2). This similarity indicates that the differences in design of the refrigeration units on these trailers did not have a significant effect on the actual conditions maintained within the trailers. Increasing outside temperature resulted in little or no increase in the inside air temperature in-transit. Temperature probes placed deep inside the round revealed continued cooling for the first 8 hr followed by a steady temperature throughout the remainder of the trip. The floor temperature increased more than 5°C during the day, a factor which should be considered if products were packed tightly against the floor without adequate air circulation. The relative humidity again showed a cycling between approximately 90 and 100% ± 3% relative humidity, the higher humidity resulting from the heating and defrosting cycles. Therefore, an average relative humidity of 94–98% was maintained. As in the stationary trailer, cycle time in a moving trailer also had an inverse effect on average relative humidity. The low heat cycle occurred less often during the day, due to the need for more cool air to maintain the temperature. Therefore, due to the dryness of the cool air, the average relative humidity during a hot day was lower than during a cool day or night. A colder air temperature was maintained near the floor, as expected; however, this difference was never more than 1–2°C. Air velocity through the meat was reduced due to ductwork which conducted air further to the rear of the trailer. Average air velocity down through the carcasses in the center region of the truck was 0.1–0.2 mps, measured in a loaded trailer.

Cooling of beef in a simulated trailer environment

It has been reported that an internal round temperature of 15°C might be desirable at time of loading to support evaporative cooling, resulting in a dryer meat surface during shipment (Robertson, 1975). Robertson (1975) stated that the overall heat transfer coefficient is a constant for the cooling of beef. Actually, the overall heat transfer coefficient is a function of the surface film heat transfer coefficient which depends greatly on air movement at the surface of the carcass.

Air movement through tightly packed carcasses in a trailer is much less than that in a well-designed chilling room. Furthermore, tight packing of carcasses in a trailer reduces surface area available for transferring heat to air because of tight contact between meat interfaces. Figure 3 shows a representative cooling curve from an average size carcass (ca 270 kg) in one of four separate storage trials in a simulated trailer environment, involving 3–4 carcasses in each trail. When transferred to the simulated trailer conditions, the exposed surface of carcasses at the round increased several degrees in temperature. The interface temperature rose sharply, eventually equalling the internal round temperature. At this point, cooling in the interface region evidently proceeded more slowly than for either adjacent internal round region. This pattern of cooling was observed for all carcasses in the experiment with weights exceeding about 250 kg. Total aerobic plate counts increased up to four or more log phases in the interface region (Table 3), the larger carcasses (carcasses b and c of each run in Table 3, 250–350 kg) showing greater bacterial growth due to slower cooling. The interface of these carcasses became moist and tacky and sometimes developed an

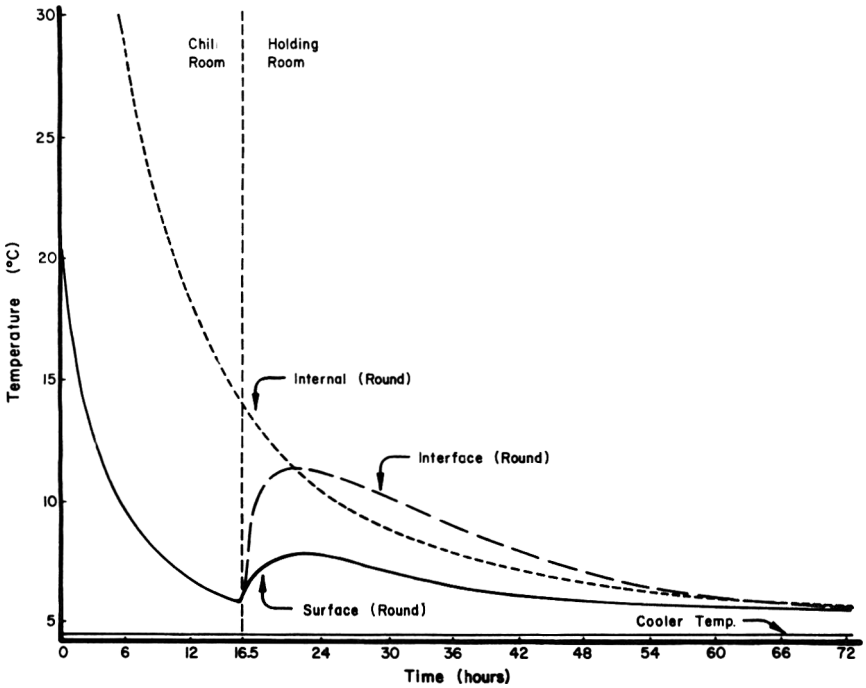

Fig. 3—Typical cooling curve for large carcass (>250 kg) transferred from high air velocity area (chill cooler) to low air velocity area (holding cooler) and packed tightly in a meat-to-meat fashion.

Table 3—Log total aerobic bacteria (per cm²) from the interface area between adjacent carcass sides which were insufficiently chilled and transferred to a simulated trailer environment[a]

Storage time (days)[b]	Run 1 carcasses			Run 2 carcasses		
	a	b	c	a	b	c
0	<2.0	<2.0	<2.0	<2.0	2.26	<2.0
1	<2.0	5.11	4.48	<2.0	6.22	5.99
2	2.98	5.87	2.21	<2.0	6.33	5.23

[a] Reliable bacteriological data from Runs 3 and 4 were not available.
[b] Beginning at the time of transfer of carcasses to simulated trailer environment.

off-odor. Actual trailer conditions are more severe than those employed in the above experiment since air movement is more restricted and more carcass surfaces are in contact. Heat transfer from hanging beef inside trailers would be substantially slower, surface temperatures higher, and the air, as well as the carcass surface, would carry a higher moisture load. All these conditions would promote deterioration of the beef color and quality, primarily due to increased bacterial growth.

CONCLUSION

THE RESULTS of this study indicate that the environment inside conventional refrigerated trailers is excellent for maintenance of beef color in high-quality carcasses when the initial carcass temperature is low and the refrigeration unit is operating properly and maintaining a temperature near 0°C. When loaded with thoroughly chilled carcasses, conditions within a properly operating refrigerated trailer are maintained at optimum conditions for beef color maintenance (Lanier et al., 1977) despite fluctuations in outside temperature and over-the-road conditions. This study suggests, however, that the normal tight packing of carcasses in these trailers does not allow adequate air circulation to all meat surfaces to permit extensive cooling of meat having higher internal temperatures (≥15°C) when loaded. Loading of carcasses with elevated internal temperature risks the promotion of a warm, high moisture environment in the immediate vicinity of the meat surface which is conducive to bacterial growth and surface discoloration.

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L. G. LANE,[1] G. R. AMMERMAN and R. H. LANE[2]
Dept. of Horticulture, Mississippi State University, Mississippi State, MS 39762

INFLUENCE OF THERMAL PROCESSING AND COOKING METHODS ON NATURALLY OCCURRING RESIDUES OF DDT AND ITS METABOLITES IN BEEF

ABSTRACT

The effects of two thermal processes and two cooking methods (broiling and microwave) on natural residues of DDT and its metabolites in beef were studied. Residue analysis was performed by aluminum oxide cleanup of fat and electron capture gas chromatography. Similar residue losses resulted from broiling, microwave preparation, and processing beef at 104°C for 342 min. Less residue loss occurred when beef was processed at 127°C for 66 min.

INTRODUCTION

THERMAL DEGRADATION of DDT during food processing is well known. Tressler (1947) monitored the partial destruction of DDT during canning of various foods using the colorimetric method of Schechter and Haller (1944). Farrow et al. (1966), employing electron capture gas chromatography, demonstrated the dehalogenation of DDT to DDD during thermal processing of spinach. At 121°C, results showed a linear decline in p,p^1-DDT concentration with time and a concomitant increase in amount of p,p^1-DDD. As processing continued, levels of p,p^1-DDT and p,p^1-DDD decreased. These data indicated that p,p^1-DDT was converted to p,p^1-DDD and the latter underwent further degradation to compounds that were not detected by electron capture gas chromatography. Carter et al. (1948) studied the effects of five cooking methods on the DDT content of beef. Frying and pressure cooking removed 35 and 50%, respectively, of the residues present; but roasting, broiling and braising caused insignificant losses. It was speculated that higher temperatures associated with frying and pressure cooking caused greater losses by rendering larger amounts of fat. Liska et al. (1967) found that simmering hens in water for 3 hr removed up to 90% of the chlorinated hydrocarbons initially present, while autoclaving removed essentially all residues except heptachlor.

Ritchey et al. (1969) investigated the thermal stability of DDT during baking, frying and pressure cooking of chicken as well as heating in closed containers. All methods caused a decrease in DDT levels with a concomitant increase in DDD. DDE levels remained unchanged, indicating no thermal conversion of DDT to DDE. Ritchey et al. (1967) found that baking and frying resulted in decreases in both DDT and DDE residues. Hemphill et al. (1967) evaluated the effects of home preparation on residues of DDT and its metabolites in green beans. Methods employed were pressure cooking, microwave cooking and boiling which resulted in reduction in DDT residue levels of 62.9, 48.6 and 47.1%, respectively. Boiling and pressure-cooking partially degraded DDT to DDD. For the pressure cooking methods there was a mean increase in DDD of 11.8% over the level in the raw control with a corresponding decrease of 54.7% in DDT. Similar results were reported by Elkins et al. (1972) concerning thermal processing and storage of apricots and spinach containing DDT among other pesticides.

Raw agricultural commodities containing pesticide residues above the FDA tolerances have been seized and destroyed. If processing and preparation of foods could be relied upon to reduce amounts of residues to levels below the FDA tolerance, significant quantities of these contaminated foods could be salvaged. This study was undertaken to investigate the effect of thermal processing, cooking by microwave, and cooking by broiling on residues of DDT and its metabolites in beef when present at levels above the FDA tolerance.

EXPERIMENTAL

Materials

Experimental material for this study was obtained from beef cattle fed a daily ration for 216 days consisting of gin trash containing high levels of p,p,^{1}-DDE, p,p^1-DDD and p,p^1-DDT residues (Martin, 1974). After a 56-day waiting period, during which the depletion rates of the pesticides were monitored, two animals were slaughtered, deboned, and the total edible portion from each, consisting of fat and lean tissue, was composited and ground. The beef was frozen and held in storage at −23°C. The two lots of ground beef were chosen for this study on the basis of pesticide residue analyses of fat from each composite. Samples from these two lots were designated Low (5.02 ppm) and High (8.11 ppm), representing total residue levels (DDE + DDD + DDT) in the fat.

Analytical method

Raw beef samples and cooked samples were lyophilized for 72 hr to remove moisture and were then ground with granular anhydrous sodium sulfate using a mortar and pestle. The mixture was packed lightly in 25 × 100 mm Whatman cellulose extraction thimbles, covered with a plug of glass wool and extracted with petroleum ether (distilled in glass) by Soxhlet reflux for 24 hr. The petroleum ether was allowed to evaporate at ambient temperature and the extraction flasks were weighed until a constant weight was reached. Three grams of extracted fat were weighed into a 10 ml graduated cylinder fitted with a ground glass stopper. The cylinder was made to volume with petroleum ether and a 1-ml aliquot was pipetted directly onto a chromatographic column (Kontes, 37.1 × 1.3 cm) containing 5g aluminum oxide (woelm neutral grade for column chromatography, activity grade IV) deactivated by the addition of 10% water by weight and shaken until free flowing. The column was eluted with 50 ml petroleum ether into a 100 ml beaker and the eluate was concentrated with a Kuderna-Danish concentration (Kontes) to the appropriate volume for gas chromatographic analysis.

All gas chromatographic data were obtained from a Barber Coleman Model 5360 Pesticide Analyzer equipped with an electron capture, Ni^{63} concentric-type detector. A column, 6 ft × 1/4 in., containing a 1:1 mixture of 10% DC-200 and 15% OF-1 on Gas Chrom Q was maintained at an operating temperature of 200°C for separation of residues. Levels of residues were based on peak height. Results were expressed in parts per million (ppm) on a fat basis. The identity of the residues in the raw beef were confirmed on a second gas chromatographic column (10% DC-200).

Heat penetration studies were conducted on six samples of raw ground beef to determine process times for equivalent lethal cooks at 104°C and 127°C (F_o = 6; Z = 18). For each temperature studied, six 303 × 406 cans were fitted with thermocouples at the coldspot in the cans and filled at room temperature to 95% capacity with 400g ground beef. The cans were closed with an American Can Company Steam-Flo closing machine and placed in an FMC Steritort. Heat penetration curves were plotted based on data obtained by measurement of temperatures which were recorded at one minute intervals with a strip chart recorder. The slowest heating and fastest cooling curves were selected for calculation of process times at the two temperatures.

[1] Present address: Mississippi State Chemical Laboratory, Mississippi State, MS 39762
[2] Present address: Dept. of Nutrition & Food Science, University of Kentucky, Lexington, KY 40502

Analyses of raw samples

Each of the two lots of beef was mixed thoroughly to obtain homogeneity. Each lot was quartered and three portions discarded until a quantity of beef was obtained which could be handled easily. From these portions, four samples were chosen at random for residue analysis of raw beef; and subsequent samples were chosen at random for the replications of each treatment.

Steritort processing study

For each of the two levels of pesticide (Low and High), four 303 × 406 tin cans were filled at room temperature with 400g of ground beef and closed with a Steam-Flo closing machine. The cans were processed in the Steritort at 4 rpm for the calculated time at each temperature (342 min at 104°C and 66 min at 127°C. Each can was opened and the contents transferred quantitatively to 800 ml beakers. The beakers were placed on a steam bath and stirred until a homogeneous mixture was obtained. Aliquots were taken from each beaker and analyzed by the previously described method.

Microwave cooking study

Four 25-g patties for each of the two levels of pesticide were cooked by microwave energy (1560 watts, 14.5 amps). Grills consisting of four 15 mm glass rods were placed over tared 94 mm glass petri dishes and the four patties were cooked simultaneously for 2 min. The drip from each patty was collected in a separate petri dish. After microwave cooking, the glass rods were rinsed with petroleum ether into the dishes which were covered with aluminum foil and stored at −18°C until analyzed. Cooked patties were weighed to obtain cooking losses (volatile, drip and total) and analyzed as described above.

The petri dishes containing the drip were alternately warmed to 65°C in an oven and cooled until they reached constant weight. Samples of rendered fat were weighed into 10 ml graduated cylinders, made to volume with pet ether, and stoppered. Aliquots were pipetted onto an aluminum oxide column, eluted with pet ether, and residues were determined by electron capture gas chromatography.

Broiling study

Four 25-g beef patties from both levels of pesticide were cooked by broiling. The electric oven was preheated for 30 min at the "Broil" setting. Each patty was placed on a metal grill (4 in. from coil) over a tared aluminum pan to collect the drip and cooked 4 min per side. The patties and drippings were weighed and analyzed for residues by the aluminum oxide method described earlier.

RESULTS & DISCUSSION

THERMAL PROCESSES for ground beef at two temperatures were calculated from heat penetration data. Calculated process times of 342 min at 104°C and 66 min at 127°C represented equivalent lethal cooks with each process the equal of F_o = 6.0.

Recoveries greater than 90% were obtained by the aluminum oxide method for fats spiked at 0.5 ppm. A standard deviation of 0.07 ppm and a coefficient of variation of 13% were calculated from a set of 14 results.

Results from the residue analyses, in terms of ppm, fat basis, of raw and cooked samples for Low and High levels of the p,p[1] isomers of DDE, DDD, DDT and Total Residues (DDE + DDD + DDT) are presented in Table 1. Total levels for DDT plus its metabolites for Low and High samples of the raw product were 5.02 and 8.11 ppm, respectively (means of four replicates for each of the two levels). The mean fat percent levels for Low and High samples were 28.4% and 29.92%, respectively.

Analysis of variance of the DDE means for both pesticide levels and the four methods of preparation indicated there was an interaction between pesticide levels and cooking methods. Separation of DDE means by Duncan's New Multiple Range Test (DNMRT) indicated for both Low and High levels of pesticide there was no difference ($P < 0.01$) between microwave cooking and broiling (Table 2). For each level of pesticide the mean was significantly greater ($P < 0.01$) when thermally processed at 127°C than when processed at 104°C. DDE means for each Steritort process were greater ($P < 0.01$) than means for microwave cooking and broiling.

Table 1—Pesticide levels of beef before and after processing and cooking

Method of preparation	Pesticide level	Pesticides found, ppm, fat basis			
		DDE	DDD	DDT	Total
Raw product	Low	3.21	0.27	1.54	5.02
104° C Steritort	Low	2.63	0.94	0.09	3.66
127° C Steritort	Low	3.09	0.60	0.47	4.15
Microwave	Low	2.23	0.31	1.22	3.76
Broil	Low	2.13	0.43	1.03	3.58
Raw product	High	5.56	0.74	1.81	8.11
104° C Steritort	High	4.52	1.36	0.10	5.97
127° C Steritort	High	4.92	1.27	0.91	7.10
Microwave	High	3.85	0.76	1.35	5.96
Broil	High	3.74	0.90	1.22	5.86

Table 2—Means of DDE as influenced by pesticide level and method of processing and cooking[a]

Method of preparation	Pesticide level (ppm)			
	Low	% Reduction	High	% Reduction
104° C Steritort	2.63 b A	18	4.52 b B	19
127° C Steritort	3.09 a A	4	4.92 a B	12
Microwave	2.23 c A	30	3.85 c B	31
Broil	2.13 c A	34	3.74 c B	33
Raw product	3.21		5.56	

[a] Means followed by the same lower case letters in a column and capital letters in a row are not different ($P < 0.01$) by DNMRT.

Table 3—DDD means as influenced by pesticide level and method of processing and cooking[a]

Method of preparation	Pesticide level (ppm)			
	Low	% Gain	High	% Gain
104° C Steritort	0.94 d A	248	1.36 d B	84
127° C Steritort	0.60 c A	122	1.27 c B	72
Microwave	0.31 a A	15	0.76 a B	3
Broil	0.43 b A	59	0.90 b B	22
Raw product	0.27		0.74	

[a] Means followed by the same lower case letter in a column and capital letter in a row are not different ($P < 0.01$) by DNMRT.

Both Steritort processes and cooking methods produced some loss of DDE in contrast to reports by Ritchey et al. (1969) and Farrow et al. (1966). Broiling and cooking by microwave produced the greatest loss of DDE, while the 127°C Steritort process had the least effect of DDE loss, as shown in Table 2.

A significant interaction between pesticide levels and methods of preparation was indicated for DDD means for both levels of pesticide. Separation of DDD means by DNMRT showed all means were different ($P < 0.01$) for the four processes and for each pesticide level (Table 3).

Table 4—Means of DDT as influenced by level of pesticide and method of processing and cooking[a]

Method of preparation	Pesticide level (ppm)			
	Low	% Reduction	High	% Reduction
104° C Steritort	0.09 a A	94	0.10 a A	95
127° C Steritort	0.46 b A	70	0.91 b B	50
Microwave	1.22 d A	21	1.35 d B	25
Broil	1.03 c A	33	1.22 c B	33
Raw product	1.54		1.81	

[a] Means followed by the same lower case letters in a column or capital letters in a row are not different ($P < 0.01$) by DNMRT.

Table 5—Total residue means as influenced by level of pesticide and method of processing and cooking[a]

Method of preparation	Pesticide level (ppm)			
	Low	% Reduction	High	% Reduction
104° C Steritort	3.66 a A	27	5.97 a B	26
127° C Steritort	4.15 b A	17	7.10 b B	12
Microwave	3.76 a A	25	5.97 a B	27
Broil	3.58 a A	29	5.86 a B	28
Raw product	5.02		8.11	

[a] Means followed by the same lower case letters in a column and capital letters in a row are not different ($P < 0.01$) by DNMRT.

Table 6—Total pesticide concentration of raw and cooked beef

Cooking method	Pesticide level	Wt of beef patty (g)	Wt of fat (g)	Total pesticide conc (μg)
Raw product	Low	25.00	7.10	35.64
Microwave	Low	13.79	3.34	12.56
Broil	Low	11.51	2.80	10.02
Raw product	High	25.00	7.48	60.66
Microwave	High	13.57	3.19	19.01
Broil	High	11.75	2.54	14.88

Table 7—Mean residue levels and total pesticide concentration in cooking drip

Cooking method	Pesticide level	Pesticides in fat drip (pm)				Wt of drip (g)	Total pesticide conc (μg)
		DDE	DDD	DDT	Total residues		
Microwave	Low	2.15	0.28	1.10	2.52	3.64	12.81
Broil	Low	0.71	0.16	0.38	1.23	1.29	1.59
Microwave	High	3.86	0.89	1.39	6.14	4.33	26.59
Broil	High	0.76	0.24	0.25	1.25	0.90	1.13

DDD levels increased during each Steritort process and cook for both levels of pesticide indicating dechlorination of DDT to DDD. A similar transformation has been reported by Hemphill et al. (1967), Ott and Gunther (1964), Thornberg (1963), Elkins et al. (1972), Langlois et al. (1964), Ritchey et al. (1967, 1969). The greatest conversion of DDT to DDD appeared to occur during the Steritort processes which indicated that reductive dechlorination is a function of time rather than temperatures in the range of 104°C to 127°C (Table 3).

Analysis of variance of DDT data indicated an interaction between level of pesticide and method of preparation. Separation of DDT means by DNMRT (Table 4) revealed there was a difference ($P < 0.01$) in all DDT means within each pesticide level for both Steritort processes and cooks. There was no difference in DDT means ($P < 0.01$) for Low and High levels of pesticide for the 104°C Steritort process. The greatest reduction in DDT (94%, Low level; 95%, High level) occurred during the 104°C Steritort process while the least reduction (21%, Low level; 25%, High level) resulted from microwave treatment. The higher percent loss during the 104°C Steritort process was in agreement with the report of Maul et al. (1971) that longer heating times were the most effective in reducing pesticide levels.

Analysis of variance of means for Total Residues indicated there was an interaction between pesticide levels and methods of preparation. Separation of the Total Residue means by DNMRT showed that for each level of pesticide, there was no difference ($P < 0.01$) in reduction of total residues by steritort processing at 104°C, cooking by microwave or by broiling (Table 5). For each pesticide level, these three methods of preparation were more effective ($P < 0.01$) in reducing total residues than processing at 127°C. These data showed that when thermal processing methods of equal lethality were employed, greatest maximum removal of total pesticides occurred at low temperature-long time, rather than at high temperature-short time processing.

Total concentration of pesticides in beef patties before and after cooking by broiling and microwave energy is shown in Table 6. There was no difference ($P < 0.01$) in Total Residue levels in fat from patties cooked by either of these methods; however, greater loss of fat during broiling was reflected by lower levels of total residues (μg) in broiled patties.

For both Low and High Level samples, approximately the same level of DDE, DDD, DDT and Total Residues was found in the fat drip from beef cooked by microwave energy (Table 7) as was found in the fat from the beef patties (Tables 1, 2, 3 and 4) cooked by the same methods. This trend was not seen in residue analysis of drip from cooking by broiling. These results were considerably lower, suggesting that the more intense heat encountered in broiling caused greater pesticide destruction. Moreover, total concentration of residues in raw samples of Low and High level beef were not accounted for by adding the levels found in cooked patties and collected drip. It appeared that some thermal destruction of residues occurred and possibly co-distillation of pesticides with moisture during cooking (Tables 6 and 7). Similar findings have been reported by Ritchey et al. (1967), Maul et al. (1971), Funk et al. (1971) and Yadrich et al. (1971).

Preparation by all methods caused reduction of pesticide residues in beef. Cooking by microwave, broiling and thermal processing at 104°C were more effective in reducing pesticide residue levels than thermal processing at 127°C.

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P.E. BOUTON, P.V. HARRIS and J.McK. SNOWDEN
CSIRO Division of Food Research, P.O. Box 12
Cannon Hill, Australia 4170

EFFECTS OF RESTRAINT DURING COOKING ON THE SHEAR PROPERTIES OF COOKED MEAT

ABSTRACT

Stretched muscle samples restrained from thermal shortening along the muscle fiber length, when cooked at 80°C, have significantly ($P < 0.001$) greater peak shear force values than those obtained for samples free to shorten. Restraint during cooking increased peak shear force values in samples subjected to a pressure-heat treatment, known to substantially reduce myofibrillar strength, to the same extent as in control samples. The magnitude of the restraint effect increased with animal age. As cooking time at 90°C was increased, in order to reduce the connective tissue contribution to peak shear force values, the effect of restraint during cooking was reduced and after 8 hr of cooking was neglible. The results were interpreted as showing that restraint during cooking increased the connective tissue contribution to peak shear force values possibly by changing the stress-strain characteristics of the collagen fibers.

INTRODUCTION

POST-RIGOR BOVINE MUSCLE samples decrease in length when cooked at temperatures greater than 60°C (Hostetler and Landmann, 1968; Giles, 1969; Dubé et al., 1972; Bouton et al., 1974a; Hegarty and Allen, 1975). Samples with long sarcomeres decrease more in length during cooking at 80°C than those with short sarcomeres (Dubé et al., 1972; Bouton et al., 1974a; Hegarty and Allen, 1975). It has been found that samples with long sarcomeres, when restrained during cooking (and thus prevented from shortening along the direction of the muscle fibers), had significantly ($P < 0.001$) larger peak shear force values than those obtained for samples allowed to contract freely during cooking (Bouton et al., 1976a). Restrained samples shrank more perpendicular to muscle fiber direction so the fiber packing density was increased by restraint (Bouton et al., 1976a). The increased fiber packing density in the restrained samples was, however, not entirely responsible for the increase in peak shear force value because an increase in peak shear force values, albeit reduced, was still observed even when the restrained and unrestrained samples had equal muscle fiber packing density (Bouton et al., 1976a).

Since the increase in fiber packing density produced by restraint does not account for the increase in peak shear force values, the explanation must be sought elsewhere. It is known that the collagen fibers in stretched meat are closely aligned with the main muscle fiber axis (Rowe, 1974; Swatland, 1975) so that restraining samples of streched meat, from thermal shortening, should prevent or reduce the shortening of both muscle and collagen fibers. Recently it was shown that isolated collagen fibers restrained from shortening when heated to 80°C had markedly different stress-strain properties from those allowed to contract i.e. the greater the contraction the greater the extension for a given load (Snowden et al., 1977). There is thus a possibility that restraint during cooking could cause changes in the collagen fibers in situ which increases the effective contribution of the connective tissue to peak shear force values.

This present work describes experiments carried out to determine whether restraint during the cooking of samples of stretched meat could affect the connective tissue contribution to the shear strength of the cooked meat. In the first experiment use has been made of the fact that pressure-heat treatment weakens the myofibrillar structure with negligible effect on the connective tissue (Bouton et al., 1977; Ratcliff et al., 1977). The effect of restraint during cooking was thus investigated in samples with low myofibrillar strength. In the second experiment the effects of restraint parallel or perpendicular to the muscle fiber direction on shear force values were investigated. The third experiment compared results obtained with samples from animals of widely different ages to determine whether age-related changes in the connective tissue influenced the extent of the increase in shear force produced by restraint. In the final experiment, prolonged cooking at 90°C was used to weaken the connective tissue and, hence, reduce its possible contribution to the restraint effect.

MATERIALS & METHODS

Selection and treatment of muscles

Eight pre-rigor semitendinosus (ST) muscles from eight steers, aged 2–4 yr, were obtained, within 1 hr of slaughter, for Experiment 1. Each muscle was stretched by about 50% and the ends nailed to a plastic-covered wooden board. These stretched samples were then stored in polyethylene bags at 0–1°C until 2 days after slaughter. Each stretched sample was then cut into four sub-samples. The four sub-samples were then randomly assigned to four treatments. Two of each set of four sub-samples were pressurized (Bouton et al., 1977) at 150 MNm^{-2} for ½ hr at 60°C after preheating for 1 hr at 45°C. The other two were left unpressurized as controls. One pressurized and one control sample, selected at random, were cooked, at 80°C for 90 min, restrained on racks designed to prevent thermal shortening of the muscle fibers (Bouton et al., 1976b) while the others, also cooked at 80°C for 90 min, were free to contract during cooking. The samples were cooked by total immersion in a water bath controlled at 80° ± 0.5°C.

For Experiment 2, eight cows 8–10 yr old were used. A side from each animal was hung from the aitch bone within 1 hr of slaughter and the semitendinosus (ST) muscles removed from the carcass 48 hr post slaughter, after storage at 0–1°C. Each muscle was divided into three sub-samples. Two sub-samples measuring about 4 × 4 × 12 cm (with muscle fibers 12 cm in length) were randomly assigned to cooking (at 80°C for 90 min) either with or without restraint as described earlier. The remaining sub-sample was taken across the entire cross section of the muscle (ca 10 cm wide) and was about 8 cm in muscle fiber length. This sample was nailed around the circumference to restrain shrinkage perpendicular to the muscle fiber direction, i.e. perpendicular restraint. Unless otherwise stated when 'restraint' is mentioned in the text it means 'parallel restraint'.

The effect of restraint on the shear properties of meat from animals of widely different age groups was investigated using deep pectoral (DP) muscles because (a) they were known to have sarcomere lengths near 3 μm, when removed from the post-rigor carcass, and (b) the DP muscle samples from the very young animals were long enough to fit the restraining racks. Six animals were used in each age group i.e. calves (3–4 months), steers (2–4 yr) and old cows (8–10 yr). Both DP muscles were removed from each of the calves and one DP muscle from each of the steers and old cows after the carcasses had been chilled at 0–1°C for 2 days. All muscles were trimmed of extraneous fat and tissue before cutting into rectangular blocks measuring approximately 3 × 2 cm in cross section and *10–12* cm along muscle fiber direction. The muscles from the calves were, in some cases, slightly thinner than 2 cm

Table 1—Effects of restraint during cooking and/or pressure-heat treatment on the shear force properties of stretched ST muscles cooked at 80°C for 90 min

Parameter measured	Control[a]		Pressure-heat treated[b]		LSD[c]
	Free	Restrained	Free	Restrained	
Initial yield force (kg)	4.70	4.68	2.20	2.35	0.59
Initial yield distance (cm)	0.53	0.41	0.47	0.36	0.03
Peak force (kg)	6.60	9.55	4.93	7.93	1.34
Final yield distance (cm)	0.77	0.83	0.83	0.86	0.03
Slope at yield (kg/cm)	21.3	25.3	9.4	13.2	0.8

[a] Control sample i.e. not pressurized
[b] Pressure-heat treatment viz pre-heat at 45°C for 60 min then pressurized at 150 MNm^{-2} for 30 min at 60°C
[c] Same LSD (least significant difference at $P < 0.05$) is valid for pressure-heat and restraint effects.

Table 2—Changes in parameters of the shear force-deformation curves obtained for stretched ST muscles (from 8–10 yr old cows hung from aitch bone) cooked at 80°C for 90 min either free to shorten or restrained either parallel to the muscle fibers or perpendicular to the muscle fibers

Parameter measured	Restraint during cooking			LSD[a]
	Free	Parallel	Perpendicular	
Initial yield force (kg)	7.10	7.68	6.10	1.03
Initial yield cistance (cm)	0.54	0.42	0.60	0.03
Peak force (kg)	10.53	15.73	8.50	2.45
Final yield cistance (cm)	0.80	0.84	0.75	0.06
Slope at yield (kg/cm)	31.9	36.9	32.3	3.8
Sarcomere length of cooked meat (μm)	1.98	2.61	1.75	0.20

[a] Least significant difference at $P < 0.05$ level.

but the time taken for their internal temperature during cooking to reach the temperature of the water bath was only about 10 min less than the times for the samples from the older animals. One of each pair of calf DP muscles was cooked unrestrained and the other restrained. For the older animals each DP muscle yielded two samples, one cooked restrained and the other unrestrained.

For Experiment 4 a DP muscle was removed from the carcass of each of 12 old cows (8–10 yr) after 2 days storage at 0–1°C. Four rectangular shaped samples measuring approximately 3 x 2 cm in cross section and 10–12 cm along muscle fiber direction were cut from each muscle. Two of the four samples from each animal were restrained on racks and the others were left unrestrained. One pair i.e. a restrained and an unrestrained sample from each animal was then assigned to one of four cooking treatments i.e. 1, 2, 4 or 8 hr at 90°C; each animal being thus represented by a block in a balanced incomplete block design (Cochran and Cox, 1957, Plan 11.1).

Pins were inserted in the post-rigor samples before mounting on the racks and cooking. Changes in the distance between pins inserted in the samples were measured in all experiments after the restraint and/or cooking of the sample. These measurements were necessary to ensure that the samples had been sucessfully restrained.

Measurement of pH and sarcomere length

A Philips PW 9405 digital pH meter equipped with a Philips C64/1 probe-type combined electrode was used to measure pH on the raw meat before cooking to ensure only samples of normal pH (5.4–5.8) were used. Sarcomere lengths were measured on the cooked meat samples, used for the shear force measurements, using a light diffraction method (Bouton et al., 1974b).

Shear force measurements

Five-to-six samples measuring 1.5 x 0.67 x 4-6 cm with fibers lying along the 4–6 cm length were prepared from each cooked sample for shear force measurements. The parameters measured from the force-deformation curves have been described elsewhere (Bouton et al., 1975a, b).

Statistical methods

Analysis of variance has been used to ascertain treatment effects and to calculate standard errors and, where appropriate, least significance difference (LSD) values at $P < 0.05$ level between treatement means.

RESULTS & DISCUSSION

Experiment 1

The results in Table 1 confirm that the pressure-heat treatment has reduced initial yield force and peak force values. Restraint had no significant effect on the initial yield force values obtained for either the pressure-treated or control. Peak shear force values for both pressure-heat treated and control samples were, however, significantly increased by restraint.

The connective tissue should be equally restrained for both the pressure-heat treated and control samples so that any increase, attribuable to restraint, in the connective tissue contribution to peak shear force values should be the same for both. The increases in peak shear force values produced by restraint were the same for both the control and the pressure-heat treated samples (2.95 and 3.00 kg respectively). This result thus accords with the suggestion that restraint increases the connective tissue contribution.

Initial yield distrance values are significantly reduced by restraint. This could be due to restraint removing the kinks which occur during pre-rigor shortening, during the release of tension when the raw stretched samples were cut from the boards, and during cooking. There is little variation in final yield distance values and the peak force values occur when the shear blade is part way through the slit of the shearing device.

Experiment 2

The different methods of restraint were intended to study the effects on shear force values of stressing meat samples, during cooking, in directions parallel or perpendicular to the main muscle fiber axis. In stretched meat the collagen fibers lie almost parallel to the muscle fiber axis (Rowe, 1974; Swatland, 1975) so that prevention of shortening along that axis (i.e. parallel restraint) should have a greater restraining effect on the collagen fibers than either the samples cooked without restraint or those prevented from contraction perpendicular to the muscle fiber axis (i.e. perpendicular restraint). For the latter samples the material, close to the nails maintaining the perpendicular restraint, was obviously partially restrained from contraction along the muscle fiber axis by the nails themselves, which were not perfectly smooth. However, the samples for shear force measurement were taken only from the central section to avoid those edge effects. The results in Table 2 showed that the perpendicularly restrained samples had significantly shorter sarcomeres after cooking than the unrestrained samples. Thses results thus show that perpendicular restraint increased thermal contraction along the muscle fiber axis and might thus have allowed greater contraction of the collagen fibers than would occur in samples cooked without restraint.

Perpendicular restraint reduced initial yield and peak force values (close to significance at $P < 0.05$ level) while parallel restraint significantly ($P < 0.001$) increased peak shear force

values. Peak shear force values appeared to increase with increase in sarcomere length—the reverse of the normal shear force-sarcomere length relationship found for cooked meat. Initial yield distance values decreased significantly ($P < 0.001$) with increase in sarcomere length, although final yield distance values were not greatly affected. Parallel restraint increased 'slope at yield' values.

Experiment 3

The results obtained for the DP muscles from the three age groups are shown in Table 3.

It should be noted that the restraint effect, already shown to occur for stretched ST muscles (Tables 1 and 2), also occurs for DP muscles (Tables 3 and 4). It also occurs for stretched semimembranosus (SM) and, albeit much reduced, for psoas major (PM) muscles (P.E. Bouton and P.V. Harris, unpublished work). The effect is, thus not confined to just the 'stretched' ST muscles but also occurs in other 'stretched' muscles. Initial yield force values are not significantly affected by either animal age or by restraint. Peak shear force and the difference between peak and initial yield values all increased with animal age. Restraint increased these values significantly ($P < 0.05$) for each age group. These results also show that restraint produces the largest increase in peak-shear force values when the connective tissue contribution was greatest i.e. for the older animals.

Experiment 4

The results obtained with prolonged cooking at 90°C are listed in Table 4. Initial yield force values were not significantly affected by restraint but were reduced by cooking time—the reduction being slightly greater for the restrained samples. The differences between peak and initial yield force values decreased with cooking time for both restrained and unrestrained samples. However, although restrained samples had increased values at the shorter cooking times, the effect of restraint was gradually reduced until at 8 hr it had been eliminated. Similarly peak shear force values decreased with cooking time and the restraint effect was eliminated by prolonged cooking. The results thus show that, when the connective tissue contribution has been reduced by prolonged cooking, restraint does not significantly affect shear force values.

Table 3—Means of the shear force parameters, and their standard errors, for DP muscle samples obtained from animals of different ages (3–4 months, 2–4 yr and ca 10 yr) with six animals in each age group. The samples were cooked, both unrestrained (F) and restrained (R), at 80°C for 90 min

Parameter measured	Cooking method	Animal age		
		3–4 month	2–4 yr	10 yr
Peak shear force (kg)	F	5.55	7.47	12.96
	R	6.79	9.56	20.73
	S.E.[a]	0.15	0.45	1.90
Initial yield force (kg)	F	4.77	4.56	4.81
	R	5.03	4.49	4.32
	S.E.[a]	0.21	0.28	0.36
Peak-initial yield force (kg)	F	0.78	2.91	8.15
	R	1.76	5.07	16.41
	S.E.[a]	0.19	0.32	2.10

[a] Standard errors

Table 4—Shear force parameters obtained for DP muscle samples from 8–10 yr old cows and cooked, unrestrained (F) and restrained (R), at 90°C for 1, 2, 4 or 8 hr

Parameter measured	Cooking method	Cooking time (hr)				
		1	2	4	8	S.E.[a]
Peak shear force (kg)	F	8.40	7.45	4.16	3.15	0.39
	R	14.85	11.33	7.08	2.64	1.34
Initial yield force (kg)	F	5.49	4.79	3.61	3.07	0.29
	R	5.64	4.95	3.47	2.54	0.66
Peak-initial yield force (kg)	F	2.91	2.66	0.55	0.08	0.41
	R	9.20	6.38	3.60	0.10	1.50

[a] Standard error as obtained by analysis of variance of the balance incomplete block design of Plan 11.1 in Cochran and Cox (1957).

CONCLUSIONS

IN THE FIRST EXPERIMENT it was shown that, whether or not myofibrillar strength was minimised with a pressure-heat treatment, restraint increased peak shear force values (Table 1). The increase in peak shear force values produced by restraint for the pressure-heat treated samples of stretched meat was almost the same as that produced in the controls. This result indicated that myofibrillar structure per se was not responsible for the increase in peak shear force values produced by restraint and supports the suggestion that restraint increased the connective tissue contribution. In Experiment 2 it was shown (Table 2) that restraint had to be parallel to the muscle fiber axis (and hence to the predominant collagen fiber direction) before peak shear force values were increased.

The third experiment, using DP instead of ST muscles, showed (Table 3) that increasing animal age substantially increased the effect of restraint on peak shear force values. It was also shown that increasing animal age had little effect on initial yield force values, but substantially increased the differences between initial yield and peak force values.

In the last experiment (i.e. 4) prolonged cooking at 90°C was used to reduce the connective tissue contribution. The results (Table 4) showed that the restraint effect was reduced by cooking time until, after 8 hr, there was no difference in peak shear force values obtained for restrained or unrestrained samples.

The results from all four experiments thus provided evidence that the restraint effect was not affecting the mechanical properties of the myofibrillar component. The evidence supported the suggestion that the connective tissue contribution was affected by restraint since the effect increased with animal age and decreased on prolonged cooking. The increase in peak shear force values was not entirely due to changes in muscle fiber packing density (and the concomitant increase in number of connective tissue sheaths) since the effect still occurred for equal muscle fiber packing densities (Bouton et al., 1976a). It seems probable that changes in the stress-strain properties of the collagen fibers of the connective tissue network, when heated under restraint, could have greatly increased the connective tissue contribution to peak shear force values.

It is unlikely, even when cooking a joint where the muscles are still attched to the bone, that the restraint will be sufficient to affect the tenderness of the cooked meat. However, any treatment which affects one component of meat structure, such as the connective tissue, to a much greater extent than the other main structural component, is a potentially useful experimental tool. Pressure-heat treatment of post-rigor meat offers a method of altering the myofibrillar contribution. Restraint of samples during cooking would appear to offer the opportunity of altering the connective tissue contribution albeit only for stretched meat. Restraint has little effect on the shear properties of cold-shortened meat (Bouton et al., 1976a)

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J. McK. SNOWDEN, P. E. BOUTON and P. V. HARRIS
CSIRO, Division of Food Research, Meat Research Laboratory
P.O. Box 12, Cannon Hill, Queensland, Australia, 4170

INFLUENCE OF ANIMAL AGE ON THE MECHANICAL PROPERTIES OF RESTRAINED, HEAT-TREATED COLLAGENOUS TISSUE

ABSTRACT

It has been shown that restraining collagenous tissue from contraction during heating influences elasticity and final yield strain but has little effect on tensile yield strength or relative yield length. The tension developed in collagenous tissue from old animals heated at temperatures above 60°C was linearly dependent on temperature. The tension developed during heating of collagenous tissue from young animals reached a peak and then rapidly declined. The temperature at which the peak occurred was dependent on both restrained length and animal age. The loss of tension was greater when the amount the samples were allowed to contract during heating was increased.

INTRODUCTION

THE EFFECT OF HEATING, at temperatures greater than the shrinkage temperature, on the mechanical properties of collagenous tissue has been the subject of a recent investigation (Snowden et al., 1977). It was shown (Snowden et al., 1977) that the stress-strain characteristics of collagenous tissue, measured after heating and cooling, were markedly influenced by the constraint that the tissue was under during heat treatment, i.e., restraint from contraction increased the stress required to produce a given strain. Collagen fibers, in situ, in meat are partially restrained during the cooking of the meat by the presence of the interstitial myofibrillar structure so that their mechanical properties after cooking should be different from those of unrestrained fibers cooked and measured ex situ. It thus appears that collagen in meat is not as easily converted to gelatin in situ as it would ex situ. It might consequently retain sufficient mechanical strength to make a significant contribution to the strength of the cooked meat.

It has been shown (Bouton et al., 1976a) that restraining stretched meat (i.e., meat with long sarcomeres > c2.5 mμ) from contraction during cooking increased peak shear force values. In a further investigation (Bouton et al., 1978) it was shown that the increase in peak shear force values of restrained samples was greater as animal age increased. It appeared likely that the increase produced by restraint was due to changes in the stress-strain characteristics of the collagenous connective tissue (Bouton et al., 1978).

The earlier study (Snowden et al., 1977) was concerned with the effects of restraint during heating (at temperatures > shrinkage temperature) on the mechanical properties of collagen from mature animals. There is, however, more than adequate evidence that animal age affects the physical properties of collagen fibers (Verzar, 1964; Boros-Farkas and Everitt, 1967; Diamant et al., 1972; Vidiik 1967a, b). However, none of these authors studied the changes which occur at the temperatures likely to be encountered during the cooking of meat and with the collagen fibers under the restraints which occur in meat.

In this present work the effect of restraint and animal age on the yield strength of collagenous tissues has been investigated.

MATERIALS & METHODS

Experimental material

Strips of collagenous tissue have been obtained from epimysial/tendinous tissue of the longissimus dorsi muscle from young (9–12 months) and mature (6–8 yr) sheep and from beef animals of different ages (1, 10, 16, 27, 42 and 120 months). The tissue used, although often called epimysial, is actually tendon and is easily separated into strips, consisting of bundles of parallel fibers, suitable for mechanical measurements. These strips were equilibrated in buffer (0.14M NaCl, 0.01M $PO_4^{\equiv}$ adjusted to pH 5.5 with lactic acid) for 16–24 hr at 5°C prior to use. A pH of 5.5 was selected because it is within the pH range of 'normal' meat i.e., 5.4–5.6. The strips were mopped dry to remove free moisture, cut into samples 5 cm long, weighed and samples with a weight of between 9 and 11 mg/cm were then selected for examination. As shown later the cross sectional area of the collagenous tissue could then be calculated from the mopped weight per unit length (g/cm), the known density of collagen and the estimated collagen content.

Experimental procedures and measurements

Samples were heated at 2°C/min to a pre-selected temperature i.e., 65, 70, 75, 80 or 90°C using the apparatus, described by Bouton et al., 1976b, mounted on an Instron Universal Testing Machine (Type TM-M). This equipment allowed measurements of length and applied tensile load to be carried out with the samples completely immersed in buffer solution. Temperature was measured with a copper/constantan thermocouple adjacent to the samples.

When required some samples were extended, before heating, by 3% of their natural (i.e., unstressed) length L_o, at a cross head speed of 0.5 cm/min, so that their original, untreated, load-deformation curves could be obtained. In most instances, however, the initial, unstressed length L_o was measured then the Instron controls were set so that, during subsequent heating, the sample could contract to any length L_T which was a desired fraction R (0.6–1.03) of the initial, unstressed length L_o. After reaching the desired temperature the sample was rapidly cooled to about 5°C by circulating iced water round the system. When the sample was at 5°C the load was reduced to zero and the new, unstressed sample length L_f determined. After the heating and cooling treatment some samples were extended at 0.5 cm/min until they yielded. The yield length L_y and yield strain ($L_y - L_f$) could then be determined.

Essentially four separate groups of experiments were carried out. They investigated (1) the effect of animal age on the tension developed during the heating of collagenous tissue, (2) the effect of restraint on yield stress values, (3) the tensile properties of collagenous tissue from animals of different ages and (4) the changes in elasticity produced by the maximum temperature of heating and by animal age.

Parameters measured

Stress has been calculated in N/mm² according to the equation Stress = F_A/A_o where F_A is the applied load (kg) and A_o is the cross-sectional area of the collagenous tissue as calculated from the mopped weight of the unheated sample per unit length (g/cm) assuming a density of 1.4 g/cm³ for collagen and a collagen content of 30% of the wet weight of the collagenous tissue.

Moduli of elasticity have been calculated using the equation (Elden, 1966) Stress = $K_{Lf}(\Delta_{Lf}/L_f)^2$ where K_{Lf} is a constant for a particular sample. The corresponding value for the raw, untreated samples was K_o. This equation was only used for strains < 10%.

Yield stress was defined as stress at which the sample yielded and corresponded to the maximum stress. The tensile stress T developed during heating has been expressed as $T = F_M/A_o$ where F_M – maximum force developed during heating and A_o was as defined earlier.

Statistical treatment of results

Unless otherwise stated all sample means quoted are the means of at least three replicates. Analysis of variance has been used where appropriate to work out the significance of treatment effects and to calculate least significant differences (LSD) at $P < 0.05$ level.

RESULTS & DISCUSSION

Tension developed during heating

The tensile stress developed during the heating of collagenous samples from beef animals of 1, 10, 16, 27, 42 and c 120 months is shown in Figure 1. The samples were heated while restrained either at the original length (R = 1.0) or at 80% of this length (R = 0.8). For the oldest animals tension increased almost linearly with temperature up to over 85°C. The tension developed during heating of samples from the younger animals reached a peak then declined. The temperature at which this peak tension occurred was dependant on animal age and on restraint. Over the age range 1–42 months the temperature at which the peak occurred increased from 71°C to 81°C at R = 1.0 and from 62 to 76°C respectively for R = 0.8.

A more detailed comparison of the effect of age on the mechanical properties of the collagenous tissue has been carried out using material from ten sheep, five about 9–12 months and five 6–8 yr of age. After heating to 80°C the samples from the younger animals did not retain sufficient mechanical strength to allow satisfactory characterization. At 70°C the loss of mechanical strength was less drastic so samples from young and old sheep were compared at 70°C for different levels of restraint. The results (in Table 1) show that the maximum tension developed in young and old samples was not affected by restraint. The maximum tension, however, developed in the samples from the older animals was nearly 2½ times that obtained for the younger animals (Table 1).

In the material from the older animals once maximum tension was achieved it remained constant for the 15 min during which observation was maintained but for the younger animals there was a rapid decrease of the maximum tension with time. The rate of this tension decrease was highly dependent on restraint as the results in Figure 2, obtained from two different sheep (<1 yr), show. This decrease in tension could be taken as an indication that there is a decrease in the amount of mechanically effective collagen in the sample. This loss in mechanical effectiveness could be due to breakages occurring within individual collagen fibrils and/or dissolution of collagen per se.

Effect of restraint on yield stress values

The stress vs extension curves obtained for samples of collagenous tissue from a mature beef animal heated to 70 or 80°C, and subsequently cooled, are shown in Figure 3. The samples were restrained during heat treatment at 0.7, 0.8, 0.9 and 1.0 of their initial length (L_o). The samples extensions

Table 1—Mean maximum tension developed during heating to 70° C of samples of collagenous tissue, from five mature (6–8 yr) and five young (<1 yr) sheep, while being restrained at different values of L_r/L_o

Restraint L_r/L_o	Maximum tension developed (N/mm²)		
	Mature	Young	LSD[a]
1.03	2.0	0.9	0.3
1.0	2.2	0.9	
0.9	1.9	0.9	
0.8	2.1	0.8	

[a] Least significant difference at $P < 0.05$ level.

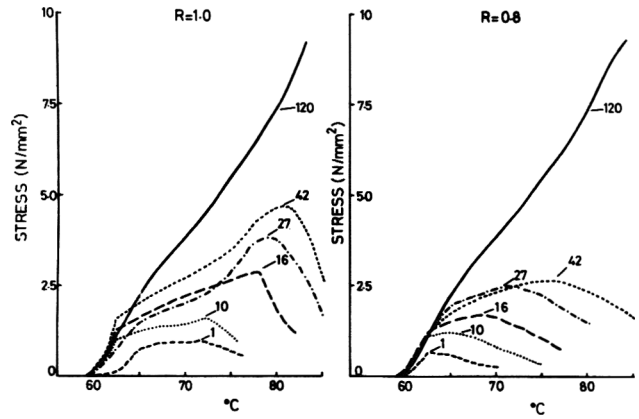

Fig. 1—Tension (N/mm²) developed with increasing temperature in collagenous tissue from beef animals aged from 1, 10, 16, 27, 42 or 120 months restrained at different fractions R (i.e., 0.8–1.0) of initial, unstressed length L_o.

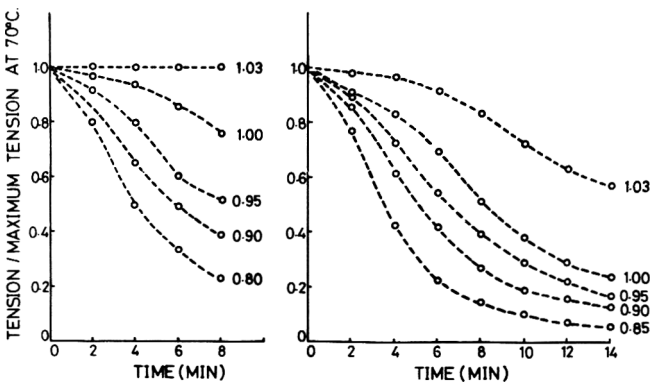

Fig. 2—Dependence of tension developed with heating up to 70° C on time at 70° C for collagenous samples from young sheep (<1 yr) restrained at different fractional values (R) of initial, unstressed length (L_o). The separate sets represent the results obtained for two different animals.

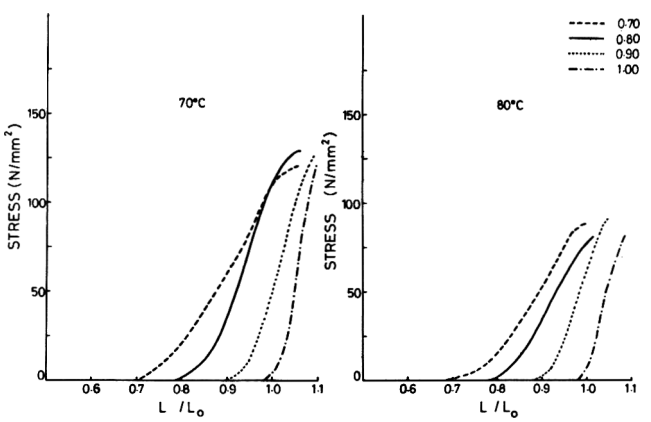

Fig. 3—Stress (N/mm²) vs L/L_o values obtained for samples of old (8–10 yr) bovine collagenous tissue heated to 70° or 80° C while restrained at different fractions R (i.e., 0.7, 0.8, 0.9, 1.0), relative to the initial, unstressed length L_o. The curves obtained are shown up to the point of failure i.e., final yield.

(mean of three replicates at each restraint) have been plotted as L/L_o (i.e., actual length L/natural unstressed (or initial) length L_o) rather than 'true' strain $(L - L_f)/L_f$ in order to show that relative yield length values L_y/L_o were not greatly affected by restraint. The results (Fig. 3) show that, while restraint markedly affects the stress-strain characteristics of the collagenous tissue, restraint has little effect (for samples restrained at lengths $< L_o$) on the values obtained for yield

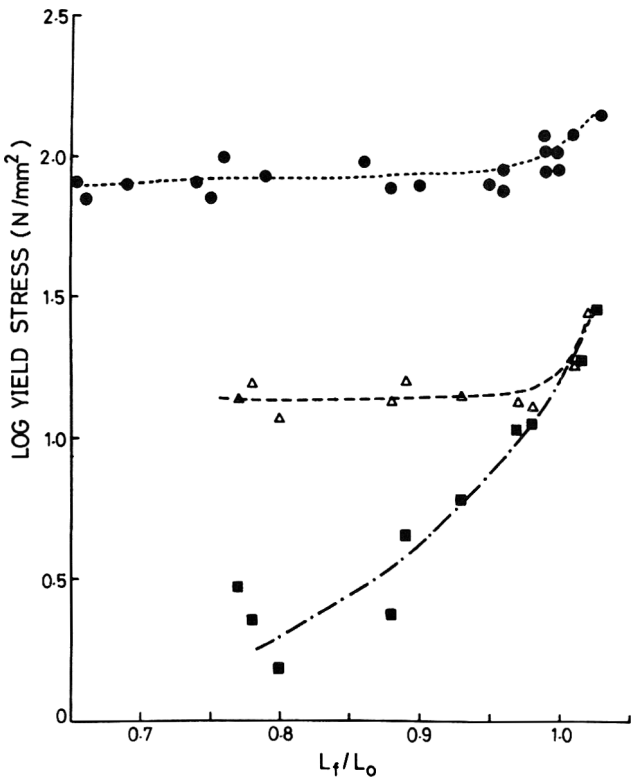

Fig. 4—Dependence of log (yield stress) in N/mm² on L_f/L_o values obtained for samples of collagenous tissue from young sheep (■), mature sheep and old cow (●) heated to 70°C or 80°C. The values for young sheep (△) have been corrected for collagen dissolution as described in text.

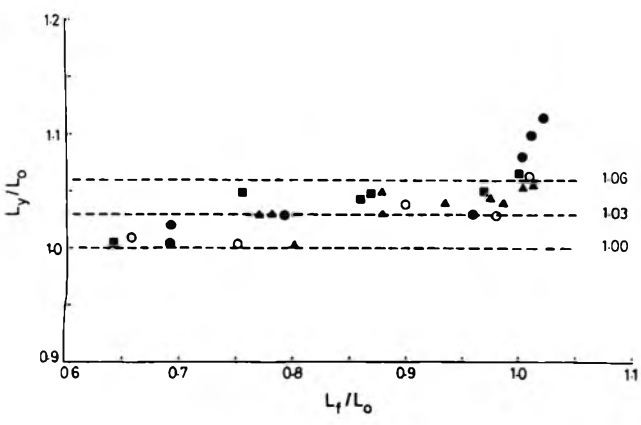

Fig. 5—Dependence of relative yield length (L_y/L_o) on L_f/L_o values obtained for samples of collagenous tissue from young sheep (▲), mature sheep (●), old cow (■), all heated to 70°C and for mature sheep samples heated to 80°C (○).

stress. In a separate experiment samples of collagenous tissue from mature beef were restrained at 0.8 and 1.0 L_o and subjected to the same heating-cooling cycle (up to 70°C) as those shown in Figure 3. The yield stress values obtained (± standard deviation and n = 6) were 117 ± 8 and 130 ± 16 N/mm² respectively. There was thus no significant difference due to restraint.

The similarity of the yield stress values is consistent with a model proposed (Snowden et al., 1977) to account for the elastic behavior of partially heat-shrunken collagenous tissue. In this model it was suggested that each collagen fibril in partially heat-shrunken tissue was composed of regions of melted and unmelted collagen with the ratio of melted to unmelted collagen increasing with increased shrinkage. For such a system the strength of the weaker region (whether it be the melted or the unmelted) would determine the yield strength of the sample, rather than the relative quantities of the different regions. It has also been suggested (Snowden and Wiedemann, 1977) that once sufficient thermal energy is supplied to initiate melting (contraction) in a collagen sample then melting will continue until sufficient tension is developed within the collagen fibers to prevent further melting.

Effect of age on the tensile properties of collagenous tissue

The stress vs L_f/L_o curves obtained for replicate samples from young and old animals are markedly different, as are shown in the plots of the log of yield stress against L_f/L_o (Fig. 4). The samples from the older animals showed that yield stress values were independent of L_f/L_o for values of $L_f/L_o < 1$. This result was consistent with the model proposed for partially heat shrunken collagenous tissue (Snowden et al., 1977).

The yield stress values obtained for the samples from the young sheep were (a) very much lower than those obtained from the older animals and (b) decreased with decrease in values for L_f/L_o. If the tension developed at all times is dependent on the amount of mechanically effective collagen present then the yield stress values obtained for samples from young animals could be adjusted for the decrease in tension (see Fig. 2) that occurred before cooling (i.e., after having achieved maximum tension) by multiplying the experimentally determined values by the ratio of peak (maximum) tension developed i.e., T_p to the final tension T_F i.e., the tension value to which it had decreased prior to cooling down. Using this correction factor the derived values for the young animals showed very similar behavior to that observed for the older animals (see Fig. 4). The corrected yield stress values obtained for the young animals at L_f/L_o values < 1 were less than 20% of the equivalent values obtained for the older animals.

Surprisingly little variation was found for the values of relative yield length obtained for the heat-treated samples from both young and old animals (i.e., L_y/L_o) (see Fig. 5). All samples with values of $L_f/L_o < 1$ had values of L_y/L_o in the range 1.00–1.06. For values of $L_f/L_o > 1$ the L_y/L_o values were closer to 1.10. There was considerable variation in the L_y/L_o values obtained for untreated samples and these values were in the range 1.10–1.30.

The yield strain i.e., $(L_y - L_f)/L_f$, which represents the amount of deformation the heated and cooled tissue undergoes before yielding, was linearly dependant on L_o/L_f since L_y/L_o was effectively constant. Since $L_y/L_o = C$, where C is a constant, then substituting for L_y in yield strain = $(L_y - L_f)/L_f$ gives yield strain = $C(L_o/L_f) - 1$.

The experimental results show such a linear relationship exists (Fig. 6).

Changes in elasticity produced by temperature and by animal age

In a previous study (Snowden et al., 1977) it was found that the elasticity of samples subjected to the same heating-cooling cycle was dependent on the relative restrained length

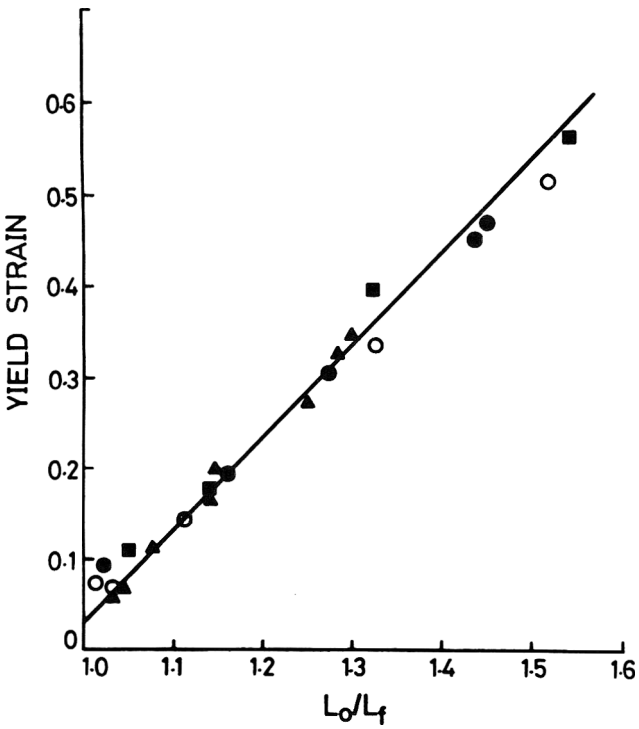

Fig. 6—Yield strain ($L_y - L_f)/L_y$ vs L_o/L_f for samples of collagenous tissue. The symbols used are young sheep (▲), mature sheep (●) and old cow (■) heated to 70°C and mature sheep (○) heated to 80°C.

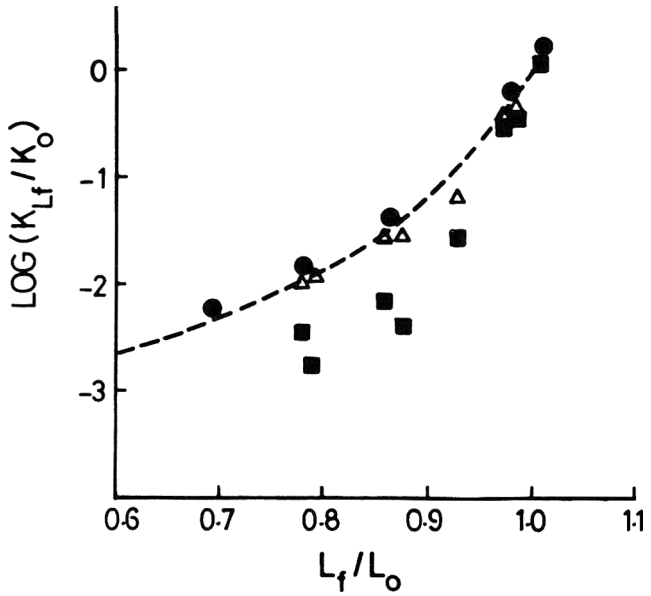

Fig. 7—Dependence of log (K_{Lf}/Ko) on L_f/L_o values obtained for collagenous samples from different sources, i.e., mature sheep and old cows (●), young sheep (■) and corrected values for young sheep (△). Each experimental point represents the means of two to three replicates. The curve shown is the theoretical curve derived elsewhere (Snowden et al., 1977).

Table 2—The influence of maximum heating temperature (65, 70, 75 or 80° C) on the elastic properties and L_f/L_o values obtained for samples of collagenous tissue, restrained at R = 0.8 of original length L_o, from four mature sheep (6–8 yr)

Measured parameter	Heating temperature (°C)				
	65	70	75	80	LSD[a]
L_f/L_o	0.79	0.78	0.77	0.77	0.2
K_{Lf}/K_o	2.3×10^{-2}	1.6×10^{-2}	1.2×10^{-2}	0.7×10^{-2}	0.9×10^{-2}

[a] Least significant difference at $P < 0.05$ level

(for restrained lengths > 0.6 L_o). The present study has investigated the effects of heating replicate samples from four mature sheep (6–8 yr) to maximum temperatures of 65, 70, 75 or 80°C whilst restrained at a length of 0.8 L_o (Table 2). The decrease in K_{Lf}/K_o values obtained with increase in temperature (Table 2) was similar to the decrease in K_{Lf}/K_o values produced when samples, which had been subjected to heating to 70 or 80°C and then cooled, were allowed to contract to restrained lengths > 0.6 L_o during heating (Snowden et al., 1977). The L_f/L_o values obtained appeared to be independent of heat treatment temperature.

The samples from the older animals heated to 70°C showed a very similar dependence of K_{Lf}/K_o on L_f/L_o (Fig. 7) as that found for similar samples heated to 80°C. For the samples from the younger animals the K_{Lf}/K_o values were dependent on L_f/L_o but the K_{Lf}/K_o values were substantially less than the K_{Lf}/K_o values obtained for samples from the older animals with comparable L_f/L_o values (Fig. 7). As previously discussed a fraction of the collagen in the samples from the young animals might be regarded as rendered mechanically ineffective by heating. Allowance for this loss has been made by using the correction factor T_P/T_F suggested earlier. The corrected K_{Lf}/K_o values, found by multiplying the experimental values by T_P/T_F, are similar to those found for the samples from the older animals (Fig. 7).

Discussion of the possible significance of the results to the mechanical properties of meat.

Where there is no loss of mechanically effective collagen the relative restrained length (L_f/L_o) influences the elasticity of the sample (K_{Lf}), the relative yield strain ($L_y - L_f)/L_f$ and, hence, the stress-strain characteristics of the heat treated collagenous tissue up to where it yields. However the final length, after heat treatment, relative to the initial, unstressed length (L_f/L_o) has little influence on the tensile yield strength or relative yield length (L_y/L_o) of samples in which some shrinkage has occurred i.e., $L_f/L_o < 1$. For samples where a loss of mechanically effective collagen occurs during heating the restrained length L_r relative to the initial unstressed length (L_r/L_o) has the additional effect of influencing the rate of loss of mechanically effective collagen.

In order to explain the yield strengths and elastic properties of the samples of heat treated collagenous tissues examined in these studies it is necessary to invoke a spectrum of collagen states. The observed behavior is consistent with changes from State 1 (unheated) → State 2 (heated but unmelted) → State 3 (melted) → State 4 (mechanically ineffective). The mechanical strength would be reduced as a result of dissolution or breakage of the fibril. States 2, 3 and 4 can all co-exist (Snowden and Weidemann, 1977) and the mechanical properties of the heat-treated samples would be determined by the relative proportions of these states i.e., 2, 3 and 4. The yield stress results indicate that the properties of states 2 and 3 are age-dependent for a given tissue. This age-dependence may simply reflect differences in the degree of crosslinking of the collagen present in

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M. W. VAUGHN, D. P. WALLACE and B. W. FORSTER
University of Maryland, Eastern Shore, Princess Anne, MD 21853

YIELD AND COMPARISON OF NUTRITIVE AND ENERGY VALUES, FATTY ACID AND CHOLESTEROL CONTENT OF RAW AND COOKED CHITTERLINGS

ABSTRACT

Three brands of frozen raw chitterlings were used to determine yield, total waste and distribution of waste in preparation and cooking based on purchased weights. Raw and cooked tissue were compared for proximate composition, minerals, vitamins, amino acids, estimated protein efficiency ratios (Est. PER), saturated and polyunsaturated fats and cholesterol. The yield was 37.8%, while the total wastage was 62.2%. The moisture content was significantly lower for the cooked tissue. Cooking produced significant losses of protein and fat. The caloric content for the cooked tissue was significantly higher than for the raw tissue. There were some changes in minerals, vitamins, amino acids, saturated fats and cholesterol content due to cooking. The raw and cooked tissue revealed no significant difference for riboflavin and niacin. The estimated PER's Alsmeyer et al. (1974) [Food Technol. 28(7): 34] using Eq 3 indicated lower values than Eq 1 and 2. There was no significant difference in C18:2 in raw and cooked tissue. There was a significant decrease in cholesterol content in the cooked tissue.

INTRODUCTION

SINCE VERY LITTLE has been reported on the nutritive value of selected pork by-products, this study is the first in a series to determine the yield as well as the nutritive value of chitterlings as the first selected pork by-product.

As described by Levie (1970), chitterlings are made from a hog's large and small intestines, which are completely emptied, rinsed thoroughly, and are cooked in this form. Our best estimates are that chitterlings are eaten by about 5–10% of the U.S. population. Currently the documented statistics on the consumption of chitterlings in the United States are very sparse and were taken prior to the marketing of the frozen product. However, chitterlings are listed as a frozen variety meat and priced in "Carlot Beef and Pork Prices," National Provisioner (see March 26, 1977 page 30).

According to a personal communication from the Consumer and Food Economics Institute, ARS (Watt, 1972), numerous requests have come from dietitians and welfare and public health workers conducting dietary surveys for data on the composition and nutritive value of a variety of pork by-products, such as chitterlings.

Agriculture Handbook, No. 102 (Pecot and Watt, 1956) reports that until recently little work has been reported on yields of edible cooked or prepared foods obtained under practical working conditions, and that for many of the foods listed the number of samples is totally inadequate for developing reliable averages. Conspicuous among such items are bone, skin, and other losses.

According to *Agriculture Handbook, No. 8* (Watt and Merrill, 1963), no data are included on the composition values of pork chitterlings. *The American Meat Institute Foundation Bulletin* (1964) reported the proximate composition and caloric content of raw chitterlings: moisture, 69.2%; protein, 9.9%; fat, 20.3%; ash, 0.5%; and 223 calories per 100-g sample. The same publication reported data for one mineral and one vitamin but no data for the amino acid profile for chitterling tissues. No information was given in reference to the number of samples analyzed.

A preliminary mineral study on chitterling tissue showed that cooked chitterlings contained 12 times as much sodium as the raw tissue. This was due to the added salt during cooking.

Feeley et al. (1972) suggested that studies to determine the cholesterol content of paired raw and cooked meats are badly needed. In this study all composition values will be determined on paired raw and cooked chitterling tissue. This data could be useful for the nutritive data bank for *Agriculture Handbook, No. 8*, Composition of Foods.

EXPERIMENTAL

Preliminary

After a determination of the proximate composition, minerals and vitamins from ten different frozen raw chitterling samples the standard deviation of the most variable component value (fat) was used to calculate the number of sample analyses to be used. Using standard statistical procedures with a 95% level of confidence, four analysis values were needed to assure the precision desired.

Three brands of frozen raw chitterlings purchased from four different sources of the Lower Eastern Shore of Maryland were used to determine yield, total waste and distribution of waste in preparation and cooking on the basis of purchased weights. Four composite raw and cooked chitterling tissue samples were analyzed for proximate composition, minerals, vitamins, amino acids, saturated and polyunsaturated fats, and cholesterol. Food energy was calculated from the proximate composition. The estimated protein efficiency ratio (Est. PER) was calculated for each replication of raw and cooked products from amino acid data using Eq No. 3 of Alsmeyer et al. (1974).

Preparation of samples

There were three available sources of frozen raw chitterlings based on brand name. The A, B and C brands were available in various locations. A sample of each brand was purchased from four different locations, giving a total of 12 different purchased samples in 5- or 10-lb plastic buckets.

Yield

Samples were coded with an equal number of raw samples for cooking purposes. The weight of the chitterlings as purchased was ascertained. Thawing was initiated in the refrigerator at 1.1°C during a period of 48 hr, and, when necessary, completed in cold water used for the first washing. Only large pieces of fat were trimmed from the chitterlings during washing. Although the chitterlings were purchased as cleaned, constant inspection was desirable. Occasionally fiber was removed. With the use of double stainless steel sinks and cold water, the chitterlings were washed through five different changes of water. As the chitterlings were removed from one wash to another, draining was accomplished by squeezing the water from the product. At the end of the fifth washing, the chitterlings were drained in a colander for 5 min. The drained, washed, trimmed chitterlings were weighed in order to determine the raw edible weight and to calculate the thawed and trimmed losses.

A 12-qt stainless steel covered pot was used for the cooking process. After cold water was added to cover the chitterlings, they were simmered for 3 hr or until tender, removed from heat, and drained in a colander for 5 min. Afterwards the cooked weight and the losses during cooking were calculated, (Table 1).

Preparation for chemical analyses

The cooled, drained cooked chitterlings were stored in polyethylene freezer bags, air-exhausted, sealed, and chilled in a refrigerator at 1.1°C. The chilled chitterlings were chopped rapidly in an electric meat chopper which had a ½-in. blade and the capacity to chop 10 lb of meat per minute. The chitterlings were passed through the electric meat chopper three times and mixed thoroughly after each grinding. Individual samples were mixed in an extra powerful electric mixer with a 5-qt stainless steel bowl (Kitchen Aid Model K5-A); the composite was mixed in a 60-qt electric mixer (Hobart mixer, Model 21 600

T-Timed Mixer). For chemical analyses, 100g composite raw and cooked chitterling tissues were sealed in polyethylene freezer bags and stored at −23.3°C in plastic freezer boxes.

Chemical analyses

All chemical analyses were accomplished by means of four replicate determinations on raw and cooked composite samples.

Official methods of the AOAC (1975) for meat and meat products were used to determine moisture (24.003), protein (24.024), fat (petroleum ether extractables (24.005), and ash (31.013). Percentage protein was calculated from the total Kjeldahl nitrogen using the factor for the protein(s) analyzed, i.e., N × 6.25 for meat protein. Protein and fat factors from *USDA Agriculture Handbook No. 74* (Merrill and Watt, 1955) were used to calculate the caloric content.

The methods described in "Meats and Fish," *Food Analysis by Atomic Absorption* (Rowe, 1973), were used to determine the minerals. Prior to the mineral determination the wet-ashing technique (2–5g) samples were digested with 30 ml of 3:2:1 nitric-percholoric-sulphuric acids in a long-necked Kjeldahl digestion flask. The Varian Techtron Model 1200 was used to determine calcium, magnesium, iron, phosphorus, sodium and potassium.

Phosphorus was determined by estimation of molybdenum content of certain molybdate complexes. The method depends upon the separation of two heteropoly acids by means of extraction with dimethylether. The molybdophosphoric acid was retained in the organic phase and back-extracted with a basic aqueous buffer solution. The heteropoly acid complex was decomposed to leave the molybdenum in the aqueous phase. Standard phosphorus solutions were treated in a like manner, and the molybdenum absorbance was calculated against known amounts of phosphorus. The phosphorus content of the original sample was then calculated by comparison with the phosphorus standards. Minerals were calculated in milligrams per 100-g sample.

Official methods of the AOAC (1975) were used for vitamin determinations. Chemical methods were used to determine vitamin A (43.001), thiamine (43.024), riboflavin (43.039), niacin (43.044), and vitamin C (ascorbic acid) (43.051).

The amino acid profile for the 18 most frequently occurring amino acids were determined by the Wisconsin Alumni Research Foundation (WARF) Laboratory, Madison, WI. These were run on the amino acid analyzer by the methods of Moore et al. (1958). Alkaline hydrolysis was used for tryptophan determination by the method of Henderson and Snell (1948) versus acid hydrolysis for the other amino acids. Each amino acid in Table 3 was calculated in grams of amino acid per 100 grams of amino acids.

The combined AOAC (1970) (28.052) and Food and Drug Administration methodology, Sheppard et al. (1974) were used to determine saturated triglycerides and cholesterol.

The saturated triglycerides, trilaurin, trimyristin, tripalmitin and tristearin (C_{12}–C_{18}) were determined by gas-liquid chromatography. The saturated fats were determined by fat extraction, saponification, and conversion to fatty acid methyl esters by a boron trifluoride-methanol reagent. The dried fatty acid methyl esters were taken up in N-hexane (pure grade). This solution also contains the sterols present in the original food extract. The sterols are in the "free state." A weight response plot for each component of interest was obtained using an equal weight mixture of the saturated fatty acid methyl esters (C_{12}–C_{18}).

The polyunsaturated triglyceride (trilinolein) was determined by an enzymatic method, Sheppard et al. (1974). Fatty acids were saponified to potassium salts. The salts of the cis, cis-methylene interrupted polyunsaturated fatty acids were then conjugated and oxidized to hydroperoxides by atmospheric oxygen in the presence of lipoxidase. The weight in grams of total cis, cis-methylene interrupted polyunsaturated acids per 100g of sample was calculated from the absorbance of the conjugated diene hydroperoxide.

Protein efficiency ratios

Estimated protein efficiency ratios (Est. PER) were calculated from grams of amino acid per 100g of amino acids. The estimated protein efficiency ratio (Est. PER) was calculated for each replicate of composite raw and cooked tissue by personnel from the USDA Eastern Regional Research Center, ARS by means of equations for predicting PER from amino acid analysis (Alsmeyer et al., 1974).

Statistical analyses

Statistical analyses of the data were made by the paired t test for means. The significance of the differences of the means of the raw and cooked composition values were calculated from the formula of Dixon and Massey (1957). According to t values from Snedecor (1946) for significance at the 5% level, t must be > 2.447. For significance at the 1% level, t must be > than 3.707.

Table 1—Yield, proximate composition and caloric content of thawed, trimmed, raw and cooked chitterling tissue

	Raw		After cooking		Losses due to cooking
Component	%[a]	g/kg[b]	%[a]	g/kg[b]	g/kg
Thaw-juice	14.5	145	–	–	–
Fat trim	5.2	52	–	–	–
Yield[c]	80.3	803	37.8[d]	378	425
Moisture	67.3	540	62.4	236	304
Dry matter	32.7	263	37.6	142	121
Protein (N X 6.25)	7.2	58	9.2	35	23
Fat	25.1	202	29.4	111	91
Ash	0.2	1.6	0.3	1.1	0.5
Calories/100g	257		304		

[a] As is basis
[b] Of starting material
[c] Based on original starting material
[d] Represents a cooking loss of 42.5% and a total wastage of 62.2% of the product as purchased

RESULTS & DISCUSSION

YIELD DATA are presented in Table 1. The percent by weight of thaw juice of Brand A indicated a range of 13.4–40.3, Brand B, 5.3–23.7 and Brand C, 3.7–6.

Proximate composition and caloric content data are presented in Table 1. Based on four replicates, the paired t test from variance of means of the raw and cooked tissue indicated a significant difference of moisture and protein at the 1% level. The fat content was not significantly different at the 1% level. The ash content showed no significant difference at the 1% level. *The AMIF (1964) Bulletin* reported no apparent difference in ash content of raw and cooked pork brain, heart, kidney, lung, pancreas, spleen, and tongue. From the yield data of raw and after cooking in Table 1, cooking produced significant losses of protein and very drastic losses of fat. There was a greater variation of moisture and fat in the raw samples.

The caloric content was significantly different at the 1% level. The high caloric content of raw and cooked tissue was due to the high fat content. Fat accounted for more than three-fourths of the calories.

Mineral content data are presented in Table 2. The t test indicated a significant difference of raw and cooked means at the 1% level for calcium, magnesium, iron, phosphorus and sodium. Potassium revealed no significant difference at the 1% level. Potassium indicated a decrease of 17%. Since 4.7 mg of potassium was lost to the cooking drip, all of the potassium was accounted for. In a private communication from WARF Institute (Aulik, 1976), it was stated that a loss of potassium but not other minerals is occasionally observed after cooking. It was stated that this phenomenon has been observed for 12 vegetables and one meat product. On a basis of 100g of raw and cooked chitterling tissue in this study as compared to a composite of trimmed lean cuts: ham, loin, shoulder, and spareribs as reported in *Agriculture Handbook, Number 8* (1963), there was higher calcium; lower magnesium, phosphorus, sodium, and potassium; and a similar iron content in the chitterling tissue.

Vitamin content data are also presented in Table 2. The t test indicated no significant difference of raw and cooked means at the 1% level for riboflavin and niacin. An overall

evaluation of these data indicates that chitterling tissue has a relatively low vitamin B nutritional value as compared to conventional pork cuts.

Data are presented in Table 3 for the amino acid profile. The t test indicates a significant difference of the raw and cooked means at the 1% level for trytophan, threonine, isoleucine, leucine, phenylalanine, tyrosine, alanine, glutamic acid, glycine and proline. Methionine, valine, arginine, histidine, aspartic acid, lysine and serine indicated no significant difference at the 1% level. The cystine content was not calculable.

Table 2—Mineral and vitamin content of a composite sample of thawed, trimmed, raw and cooked chitterling tissue

Component	Raw Mean value mg/100g	Cooked Mean value mg/100g
Calcium	19.9 ± 0.05	27.4 ± 2.09
Magnesium	5.5 ± 0.14	10.3 ± 0.72
Iron	1.6 ± 0.64	3.7 ± 0.39
Phosphorus	28.5 ± 1.74	47.2 ± 2.97
Sodium	19.9 ± 0.99	38.6 ± 1.60
Potassium[a]	9.4 ± 0.74	7.8 ± 0.47
Vitamin A[b]	0.00[c]	0.00[c]
Thiamine	0.01 ± 0.01	(Trace)[d]
Riboflavin	0.07 ± 0.00	0.08 ± 0.01
Niacin	0.08 ± 0.00	0.10 ± 0.04
Ascorbic acid	0.00	0.00

[a] The cooking loss accounted for 4.7 mg/100 g of potassium
[b] Indicates I.U/100g
[c] (0.00) Indicates none
[d] (Trace) Indicates less than 0.01 mg/100g

Table 3—Amino acid composition and estimated protein efficiency ratio of a composite sample of thawed, trimmed raw and cooked chitterling tissue (g amino acid/100g amino acid)

Amino acid	Raw Mean values	Cooked Mean values
Tryptophan	0.90 ± 0.06	0.6 ± 0.03
Threonine	4.2 ± 0.03	4.4 ± 0.03
Isoleucine	3.9 ± 0.01	4.1 ± 0.04
Leucine	7.6 ± 0.01	7.9 ± 0.04
Lysine	6.4 ± 0.03	6.4 ± 0.03
Methionine	1.7 ± 0.09	1.9 ± 0.02
Cystine	—[a]	—[a]
Phenylalanine	3.7 ± 0.02	4.0 ± 0.02
Tyrosine	3.2 ± 0.02	3.7 ± 0.03
Valine	4.7 ± 0.06	4.9 ± 0.06
Histidine	2.0 ± 0.03	2.1 ± 0.02
Alanine	7.4 ± 0.03	7.1 ± 0.02
Aspartic acid	9.4 ± 0.05	9.4 ± 0.02
Glutamic acid	13.4 ± 0.15	12.9 ± 0.20
Glycine	11.8 ± 0.08	10.7 ± 0.03
Proline	7.5 ± 0.01	7.0 ± 0.03
Serine	4.7 ± 0.08	4.8 ± 0.03
Arginine	8.0 ± 0.05	8.2 ± 0.05
Estimated PER		
Equation 1	2.23 ± 0.01	2.39 ± 0.02
Equation 2	2.45 ± 0.01	2.53 ± 0.03
Equation 3	1.93 ± 0.00	1.88 ± 0.06

[a] (—) Indicates not calculable

More than 50% of the amino acids studied showed a significant difference in the cooked as compared to the raw tissue.

In the cooked tissue more than 50% of the amino acids studied revealed a significant difference at the 1% level. More than 30% indicated no significant difference at the 1% level.

Raw and cooked chitterling tissue indicated a very high content of the nonessential amino acids: arginine, aspartic acid, glutamic acid and glycine. The glycine content was 11.8% and 10.7%, respectively, for raw and cooked tissue, which apparently indicates a high amount of collagen. With collagen present, there should be hydroxyproline and hydroxylysine.

It appears that the sulfur amino acids and trytophan are the first two limiting essential amino acids. On an overall basis the essential amino acid content of chitterling tissue was similar to amino acid content of beef intestines as compared to a study by Olsen (1970).

Since the equations of Alsmeyer et al. (1974) were based on an amino acid composition including hydroxyproline and hydroxylysine, the percentages of total amino acids and of histidine, methionine, leucine, proline and tyrosine were recalculated taking into account of hydroxyproline estimated from the proline content in collagenous tissue (6.09% for the raw and 5.6% for the cooked chitterlings) and 1% hydroxylysine in each replicate. Results of estimated PER values as calculated from amino acid analyses are presented in Table 3. The mean estimated PER's for raw tissue as calculated from Eq 1, 2 and 3 were 2.23, 2.45 and 1.93, respectively, as compared to cooked samples showing mean values of 2.39, 2.53 and 1.88. The results from Eq 1 and 2 are high. The estimated PER's calculated from Eq 3 are lower. Alsmeyer et al. (1974) reported that Eq 3 is a reliable estimator of PER when it is used with products that contain primarily meat, poultry, grain, or yeast origin and that Eq 1 and 2 were ineffective in predicting the estimated PER of foods containing little or no meat or poultry.

The PER observed range for most meats is from 2.3–2.9. On an overall basis, estimated PER's for chitterling tissue, as calculated from Eq 3, was similar to estimated PER's as calculated for certain soy products. Happich et al. (1975) reported calculated PER's for 70% lean beef and 30% soy protein concentrate, 2.4; 60% lean beef and 40% soy protein concentrate, 2.2%; meat patties with textured vegetable protein, 1.9; textured vegetable protein, 2.3; and 25% lean beef, 25% collagen and 50% whey protein concentrate, 1.8.

Results of saturated and polyunsaturated fats and cholesterol are presented in Table 4. The t test indicated a significant difference of raw and cooked means for C12:0, C14:0, and C16:0 at the 1% level and that C18:0 effected no significant

Table 4—Fat and cholesterol composition of a composite sample of thawed, trimmed raw and cooked chitterling tissue

Saturated and polyunsaturated fats and cholesterol		
Fat[a]	Raw Mean values g/100g	Cooked Mean values g/100g
12:0[a]	0.01 ± 0.01	0.05 ± 0.00
14:0	0.29 ± 0.01	0.36 ± 0.01
16:0	5.13 ± 0.22	6.32 ± 0.21
18:0	3.76 ± 0.19	4.27 ± 0.31
18:2[b]	3.06 ± 0.50	2.90 ± 0.27
Cholesterol[c]	160 ± 2.26	143 ± 3.02

[a] 12:0 = trilaurin; 14:0 = trimyristin; 16:0 = tripalmitin; 18:0 = tristearin.
[b] Trilinolein
[c] mg/100g

difference at the 1% level. On a gram of fat per 100g basis C16:0 accounted for more than 50% of the total saturated fats studied for the raw and cooked tissue. More than 90% of the total saturated fats was accounted for in C16:0 and C18:0 combined. Anderson (1976) reported saturated fat data for C14:0, C16:0, and C18:0 for raw pig tails similar to this raw chitterling tissue study.

The t test detailed no significant difference in polyunsaturated fats (C18:2) of raw and cooked means at the 1% level. *Agriculture Handbook No. 8* (1963) reported C18:2 data on cured pork (with and without bone) in agreement with our findings on chitterling tissue.

The t test confirmed a significant difference in cholesterol content of raw and cooked means at the 1% level. The cooked samples showed an 11% loss of cholesterol. *The AMIF Bulletin* (1964) reported that raw chitterling tissues contains 158 mg/100g of cholesterol. Lobanov et al. (1958) reported that cooking (i.e., boiling) lowers the cholesterol content. The authors conclude that cooking meat (beef and chicken) and fillet of ocean perch are prone to lose approximately 20% of their cholesterol. In these studies as well as ours, cholesterol was probably lost during the cooking process.

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LAURA L. ZAIKA, TATIANA E. ZELL, S. A. PALUMBO AND J. L. SMITH
USDA Eastern Regional Research Center, ARS, Philadelphia, PA 19118

EFFECT OF SPICES AND SALT ON FERMENTATION OF LEBANON BOLOGNA-TYPE SAUSAGE

ABSTRACT

Spices and different concentrations of NaCl added to sausage mixtures are shown to affect the course of fermentation during manufacture of Lebanon bologna. A mixture of nine spices used in Lebanon bologna formulation enhanced fermentation of these sausages. Sterile or nonsterile spices enhanced fermentation to the same extent. Similar stimulatory effects of spice were observed when sausages were prepared by natural flora fermentation or by addition of starter culture (a mixture of *Pediococcus cerevisiae* and *Lactobacillus plantarum*). Increasing concentrations of NaCl from 1 to 7% resulted in an inhibition of fermentation with an attendant decrease in lactic acid production. Use of less than 2% NaCl resulted in poor texture in the sausage. Sausages containing 7% NaCl showed very little fermentation in the absence of spices and showed enhanced fermentation when spices were added. From the standpoint of fermentation, texture, color and palatability, sausages containing 2 and 3% NaCl were the best. Generally, formation of cured meat pigment, using either nitrite or nitrate, and texture of the sausages were related to the degree of fermentation attained during processing.

INTRODUCTION

ALTHOUGH FERMENTED SAUSAGES, such as Lebanon bologna, summer sausage, and pepperoni, represent a significant portion of the variety of meat products available to the consumer, the significance of the various factors important to their production has not been completely elucidated. In order to develop a better understanding of these factors, we studied the effects of the nonmeat ingredients on the fermentation process and final product quality.

Spices and salt play important roles in sausage production besides their obvious function as flavor components. Since ancient times, both have been regarded as food preservatives. Antioxidant properties of spices have been reported, and a number of spices have been found to be effective in retarding rancidity during frozen storage of ground pork and beef (Dubois and Tressler, 1943), ground pork (Chipault et al., 1956), and pork sausage (Atkinson et al., 1947). Antimicrobial activity of spices has been demonstrated (Fabian et al., 1939; Corran and Edgar, 1933; Webb and Tanner, 1945; Dold and Knapp, 1948; Bullerman, 1974; Beuchat, 1976). The responses of different microorganisms to a given spice varied considerably, with cloves, cinnamon, and mustard having the most inhibitory effect on a variety of microorganisms.

Salt is the most common and the most important nonmeat ingredient of sausage. Its functions include flavoring, preservation, and production of the proper texture by solubilization of meat proteins. The tolerance of different species of microorganisms toward salt varies over a wide range of concentrations. Some microorganisms are inhibited by salt concentrations of less than 1%, while others can grow in saturated brines. Lactic acid producing bacteria, important in fermented foods, are reported to be fairly tolerant of salt and some are even stimulated by low concentrations (Irvine and Price, 1961).

Lebanon bologna, a fermented heavily smoked and spiced all-beef sausage originally produced in the Lebanon Valley area of Pennsylvania, was chosen as a model system for the study of fermentation processes in meat. In the traditional method of Lebanon bologna manufacture using nitrate as the curing agent, fermentation is produced by natural microflora present in ground meat. Some manufacturers are now using lactic acid starter cultures to achieve fermentation with nitrate and/or nitrite for curing.

The preparation of Lebanon bologna (Palumbo et al., 1973), the microbiology of the process (Smith and Palumbo, 1973), and the role of nitrite and nitrate in fermentation and cured meat color formation in Lebanon bologna (Zaika et al., 1976) have been reported. We now report the effect of salt and spices on the course of fermentation of Lebanon bologna prepared with a commercial starter culture containing *Lactobacillus plantarum* and *Pedicoccus cerevisiae*.

EXPERIMENTAL

Preparation of Lebanon Bologna

The sausages were prepared essentially as described by Palumbo et al. (1973).

Spices. The spice mixture used in the sausage formulation was prepared from commercially available spices (referred to as regular spices) according to the following concentrations:

black pepper	25.0g
nutmeg	12.5g
allspice	12.5g
red pepper	6.2g
cloves	6.2g
cinnamon	6.2g
ginger	6.2g
mustard	6.2g
mace	0.2g

A similar mixture was prepared using purified spices (Griffith Laboratories, Inc., Union, NJ). Total aerobic plate counts were determined on APT agar (Difco) incubated 3 days at 25°C. The count was 10^4/g for the regular spice mixture and less than 100/g for the purified spice mixture.

Starter culture method. Beef was ground through a 3/32 in. plate and divided into 2-kg batches. The sausages were prepared according to the formulation given in Table 1. All sausages were prepared with the addition of 2% glucose and 2% sucrose. Starter culture (Lactacel MC, Merck and Co., Inc., Rahway, NJ) containing both *Lactobacillus plantarum* and *Pediococcus cervisiae* was added at a level of 0.1%.

Natural microflora method. Beef ground through a 3/4-in. plate was mixed with 3% NaCl, aged for 10 days at 5°C, and then ground through a 3/32-in. plate. The aged meat was divided into 2-kg batches and mixed with 2% glucose, 2% sucrose, 200 ppm $NaNO_2$, and 0.8% spice mixture (if used).

Processing of sausages. In order to facilitate analysis, the sausage mixtures were stuffed into 55 mm moisture impermeable fibrous casings (Union Carbide Co.) and the sausages were incubated for 4 days at 35°C.

Analytical methods

Determination of pH, titratable acidity (expressed as percent lactic acid), cured meat pigment, and nitrite concentration during 4 days of fermentation of the sausages was carried out as described previously (Zaika et al., 1976).

Evaluation of the flavor, texture and firmness of the sausages was carried our informally by experienced laboratory personnel by comparison with sausages prepared by the formulation developed by Palumbo et al. (1973) in our laboratory.

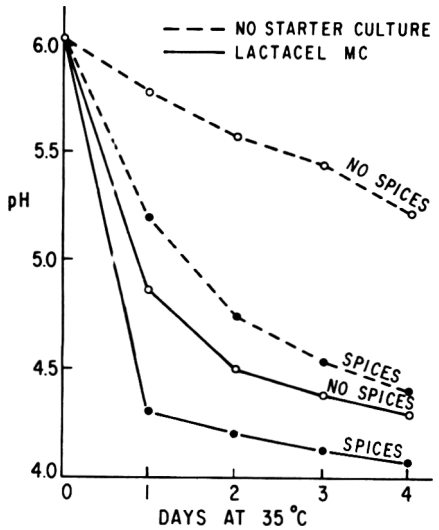

Fig. 1—Effect of spices on changes in pH during fermentation of Lebanon bologna prepared with and without starter culture (sausage Series A).

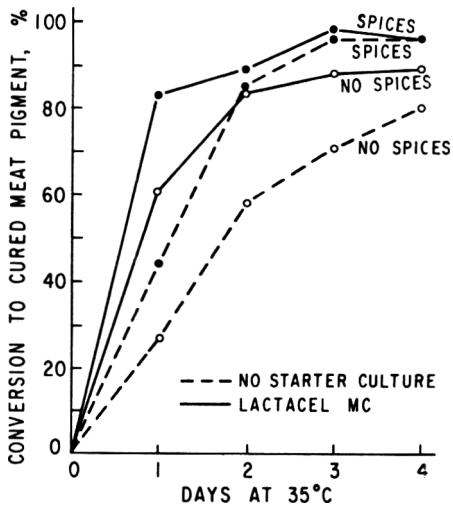

Fig. 2—Effect of spices on formation of cured meat pigment during fermentation of Lebanon bologna prepared with and without starter culture (sausage Series A).

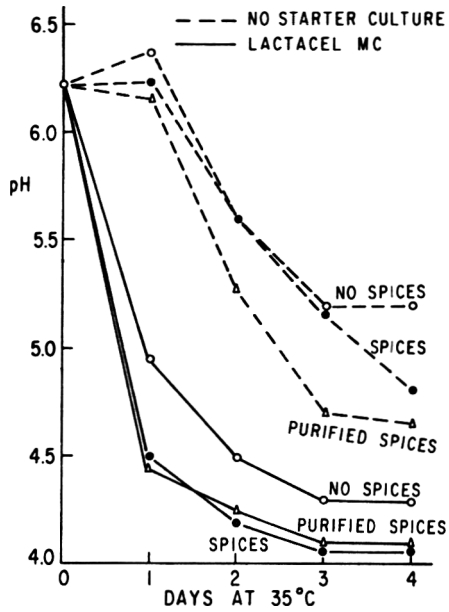

Fig 3—Effect of regular and purified spices on changes in pH during fermentation of Lebanon bologna prepared with and without starter culture (sausage Series B).

RESULTS & DISCUSSION

THE CHARACTERISTIC FLAVOR of a given type of sausage depends to a large extent on the spices used in its formulation. Our spice formulation consists of nine spices: black pepper, nutmeg, allspice, red pepper, cloves, cinnamon, ginger, mustard and mace. In our previous research on Lebanon bologna, we noticed that when spices were omitted from the sausage formulation, less fermentation was obtained, even with the use of starter culture, than in sausages containing spices. To investigate the effect of spices on fermentation, sausages (Series A, Table 1) were prepared from fresh meat with or without the addition of the spice mixture and starter culture, Lactacel MC. The results are shown in Figures 1 and 2. In sausages from which both the spice mixture and the starter culture were omitted, there was little fermentation (Fig. 1). The pH decreased from 6.0 to only 5.2 after 4 days at 35°C. With spices alone, the decrease in pH was almost as large as with starter culture alone. Addition of both spices and starter culture to the sausage mixture resulted in very rapid fermentation and the lowest final pH of 4.1.

In these sausages, as well as in those from other experiments, the development of cured meat pigment paralleled the production of acid. The most rapid formation of cured meat pigment was in sausages containing both spices and starter culture (Fig. 2). Series A sausages were prepared with 1000 ppm $NaNO_3$ as the curing agent; however, similar results were obtained with sausages prepared with 78 ppm $NaNO_2$ instead of $NaNO_3$. We have previously reported (Zaika et al., 1976) that satisfactory Lebanon bologna with good cured meat color can be prepared using nitrite alone.

Since spices are known to be heavily contaminated with micro-organisms, the possibility that these may contribute to fermentation of Lebanon bologna was investigated. Sausages (Series B, Table 1) were prepared without spices, with regular spices and with purified spices either with or without the addition of starter culture. Microbiological analysis of the purified spice mixture indicated that the total bacterial count was less than 100/g compared to a count of approximately 10^4/g for the regular spice mixture. Changes in pH during the fermentation of these sausages are shown in Figure 3. These results suggest that the enhanced fermentation in the spice-containing sausages, both in the presence and in the absence of starter culture, was not due to microbial contaminants in spices, since the regular and the purified spices behaved similarly. In an experiment using starter culture, regular spices sterilized by autoclaving gave the same enhancement of fermentation as did unsterilized spices.

Table 1—Formulation of sausages prepared by the starter culture method

Sausage series	NaCl (%)	$NaNO_2$ (ppm)	$NaNO_3$ (ppm)	Spices (%)	Lactacel MC (%)
A	3	–	1000	0 or 0.8[a]	0 or 0.1
B	3	–	200	0 or 0.8[a,b]	0 or 0.1
C	3	78	–	0–1.6[b]	0.1
D	0-4	78	–	–	0.1
E	3-7	78	–	0 or 0.8[b]	0.1

[a] Regular commercial spices
[b] Purified spices

The effect of spices in Lebanon bologna prepared by the traditional method, which involves fermentation by microflora naturally present in ground meat, was also investigated. Sausages were prepared by the natural flora fermentation method with regular spices, purified spices, or without spices. As in the previous example with starter culture, the results of this experiment (Fig. 4) show that purified spices gave the same enhancement of fermentation as did the regular spices. Development of cured meat color during fermentation of sausages by natural microflora is shown in Figure 5, which is a composite of several experiments. In each case 200 ppm $NaNO_3$ was used as the curing agent. Conversion to cured meat pigment was better and proceeded at a faster rate in spice-con-

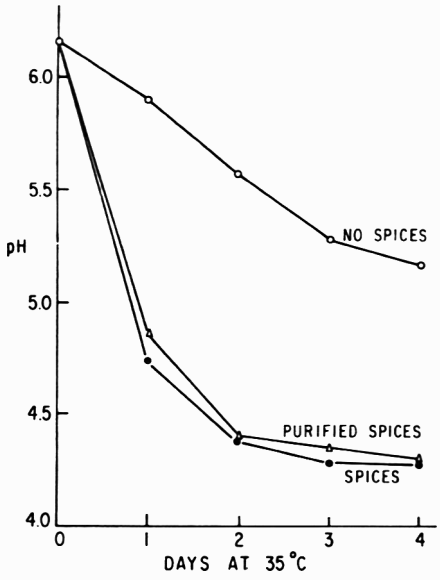

Fig 4—Effect of regular and purified spices on changes in pH during fermentation of Lebanon bologna by natural flora.

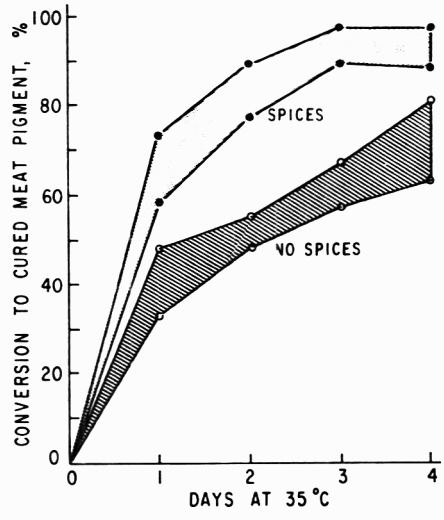

Fig. 5—Effect of spices on formation of cured meat pigment during natural flora fermentation of Lebanon bologna in presence of 200 ppm added $NaNO_3$.

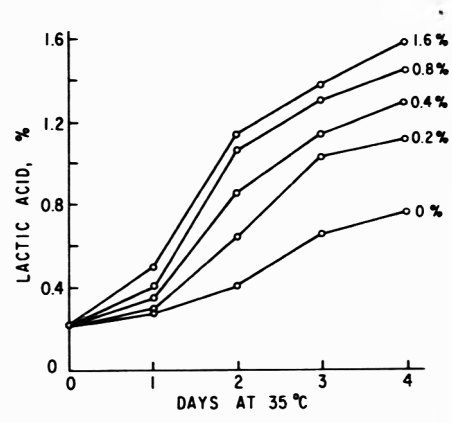

Fig. 6—Effect of spice concentration on production of lactic acid during fermentation of Lebanon bologna (sausage Series C).

taining sausages than in sausages prepared without spices. Generally, an acceptable cured meat color was produced in sausages without spices after 4 days of fermentation.

Although we are unable to offer a satifactory explanation for the stimulatory effect that spices have on fermentation of Lebanon bologna, all our experiments suggest that some chemical constituent of one or more of the spices used in our formulation may be involved. Previously, Corran and Edgar (1933) suggested that black pepper contains a yeast stimulant. Wright et al. (1954) showed that a number of spices at low levels, namely caraway, cardamon, cinnamon, ginger, mace, nutmeg and thyme, exhibited marked promoting effects on gas production in yeast-sugar suspensions, as well as in more complex media.

Although the amount of spice mixture normally used in our sausage formulation is 0.8%, the effect of spice concentration of 0–1.6% on titratable acidity was examined (sausage Series C, Table 1). Titratable acidity, expressed as % lactic acid, increased with increasing spice concentration, the greatest increase in acidity was produced by the first increment of spices used (Fig. 6).

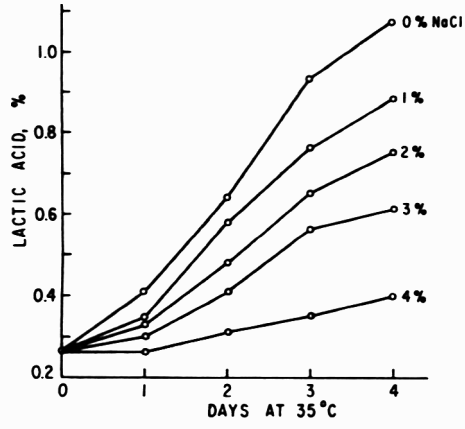

Fig. 7—Effect of NaCl concentration on production of lactic acid during fermentation of Lebanon bologna prepared with starter culture but without spices (sausage Series D).

Some aspects of the effect of salt on the preparation of Lebanon bologna by the natural microflora method have been reported previously (Palumbo et al., 1973; Smith and Palumbo, 1973). We conducted experiments to study the effect of salt on fermentation using the starter culture organisms *Lactobacillus plantarum* and *Pediococcus cerevisiae*. *P. cerevisiae* apparently grows well in solutions containing up to 5.5% salt and poorly in concentrations of salt up to 10% (Frazier, 1958). *L. plantarum* was reported to grow in soy sauce containing 10% NaCl (Yoshii and Kato, 1966). Sausages (Series D, Table 1) were prepared with 0, 1, 2, 3 and 4% NaCl, using Lactacel MC starter culture but no spices. Figure 7 shows the changes in titratable acidity during 4 days of fermentation of these sausages. Increased amounts of NaCl inhibited fermentation and decreased lactic acid production. Using 78 ppm $NaNO_2$, conversion to cured meat pigment was 85% or better in sausages containing up to 3% NaCl; in sausages containing 4% NaCl, it was only 70%, accompanied by a slower disappearance of $NaNO_2$.

A certain amount of NaCl is necessary for the production of sausage having the proper texture. Sausages containing 2–4% NaCl and starter culture had a good firm texture. Use of less than 2% NaCl resulted in poor texture. From the standpoint of texture, flavor and fermentation, sausages containing 2 and 3% NaCl were the best.

To investigate the possibility that higher levels of NaCl may completely inhibit the activity of the starter culture, sausages (Series E, Table 1) were prepared containing 3, 5 and 7% NaCl. A mixture of the purified spices was added to some of the sausages to see whether or not the stimulatory effect of spices would counteract the inhibitory effect of higher levels of salt. The results of this experiment are summarized in Table 2.

There was very little fermentation in samples containing 5 and 7% NaCl in the absence of spices. With the added spices there was considerable increase in lactic acid production in sausages with 3 and 5% NaCl and a small increase in sausages with 7% NaCl.

In the Series E experiment, 78 ppm $NaNO_2$ was used as the curing agent. Conversion to cured meat pigment was good (>84%) in sausages containing 3% NaCl both in the presence and in the absence of spices. At the 5% NaCl level, either with or without spices, and at the 7% NaCl level with spices the color conversion was ∿70%. However, in sausages prepared

Table 2—Effect of NaCl and spices on the properties of Lebanon bologna (sausage Series E) during fermentation at 35°C.

		No spices				Spices (0.8%, purified)			
NaCl	Days	pH	Lactic Acid, %	$NaNO_2$ ppm	Color % conversion	pH	Lactic acid, %	$NaNO_2$ ppm	Color % conversion
3%	0	—	—	—	—	6.06	0.18	49	—
	1	5.40	0.25	7	46	4.75	0.42	6	74
	2	4.80	0.43	7	70	4.15	1.03	2	81
	3	4.55	0.61	4	79	4.10	1.24	0	86
	4	4.44	0.75	4	84	4.06	1.39	2	88
5%	0	—	—	—	—	6.05	0.18	49	—
	1	5.83	0.22	19	36	5.56	0.26	20	41
	2	5.30	0.29	11	54	4.63	0.50	5	73
	3	5.25	0.32	9	62	4.36	0.75	3	74
	4	4.92	0.42	8	72	4.24	0.98	2	73
7%	0	—	—	—	—	6.05	0.18	51	—
	1	6.15	0.17	20	32	6.14	0.17	23	33
	2	5.80	0.23	15	40	5.73	0.23	16	38
	3	5.55	0.27	10	47	4.89	0.41	7	66
	4	5.40	0.30	10	56	4.64	0.54	6	69

with 7% NaCl without spices, color conversion was only 56%. From the standpoint of acid production, texture and color, sausages containing 5% NaCl were satisfactory. The use of 7% NaCl resulted in sausages of borderline acidity with pH values of 4.6–4.8. In our opinion, a fermented sausage should have a pH value of 4.7 or less to assure microbiological safety. Considerations of palatability, as well as potential adverse effects on the health of individuals with high blood pressure, make the use of 5 or 7% NaCl impractical for sausage preparation.

SUMMARY

FERMENTATION of Lebanon bologna-type sausages containing from 1 to 7% NaCl was studied. Fermentation was inhibited by increasing amounts of NaCl. A mixture of nine spices exerted a stimulatroy effect on fermentation both by Lactacel MC starter culture and by natural microflora. Increased fermentation was not due to microbial contamination of spices.

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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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R. J. KRAUSE, R. F. PLIMPTON, H. W. OCKERMAN and V. R. CAHILL
Dept. of Animal Science, The Ohio State University, Columbus, OH 43210
and Ohio Agricultural Research & Development Center, Wooster, OH 44691

INFLUENCE OF TUMBLING AND SODIUM TRIPOLYPHOSPHATE ON SALT AND NITRITE DISTRIBUTION IN PORCINE MUSCLE

ABSTRACT

The effect of tumbling and sodium tripolyphosphate on the migration of salt and nitrite in pork muscle tissue was studied using the semimembranosus muscles from 20 matched hams. Uniform sections of these muscles were injected with a column of cure placed in the geometric center of the muscle section perpendicular to the muscle fibers. Portions of these muscle samples were subsequently analyzed after tumbling or holding for extent of cooked cured color development. Uncooked portions were analyzed for salt and nitrite concentration at specific distances from the injection site. Both sodium tripolyphosphate and tumbling independently significantly increased the migration of salt and nitrite and resulted in a significant increase in color development. Although percent cured color area was significantly increased, both tumbling and sodium tripolyphosphate increased the residual nitrite content. Sodium tripolyphosphate added to the effect of tumbling on the percent of cured area.

INTRODUCTION

THE MEAT INDUSTRY has experienced numerous innovations in the curing of pork products. One such innovation is the tumbling or massaging of meat tissue by mechanical agitation. At this point it would be relevant to establish the difference between tumbling and massaging. Weiss (1973) has reported that the term tumbling has been used in the domestic cured meat industry to include both the tumbling and massaging action.

Tumbling actually involves the influence of impact energy on the muscle tissue. This occurs when the meat falls from the upper part of a rotating drum or it can be caused by striking the meat with paddles or baffles. This impact energy theoretically leads to the transfer of kinetic energy to the muscle tissue mass and results in a temperature rise in the processing material.

Massaging is a less physically rigorous process and involves frictional energy resulting from the rubbing of one meat surface on another or on a smooth surface of a container.

Viskase (1974) has stated that tumbling has several functions: (1) to aid in the distribution of the curing ingredients and (2) to increase the extraction of the salt soluble proteins due to the addition of salt and the tumbling action as previously defined.

The function of phosphate in tumbled products is of major interest to the meat industry. Polyphosphates have been shown to increase the pH of meat and to improve the water-holding capacity due to their sequestering ability of the alkaline-earth and heavy metal ions naturally present in meat (Ellinger, 1972; Hamm and Grau, 1955). This latter effect results subsequently in less shrinkage during heat processing particularly in the presence of low concentration (1, 2.4 and 2.15%) of sodium chloride (Wierbicki et al., 1976; Krause et al., 1976).

In the presence of sodium chloride, polyphosphates increased the binding properties of cured meat by increasing the solubility of myosin and actomyosin (Ellinger, 1972; Yasui et al., 1964). This binding effect is further enhanced by mechanical actions such as tumbling or massaging (Rahelic et al., 1974; Siegel et al., 1976; Krause et al., 1976).

Polyphosphates have been credited with preventing discoloration of cured meats (Watts, 1954; 1957; Savic et al., 1965). Rahelic et al. (1966) reported that polyphosphates increased cured pigment quantity. Allen (1938) suggested that some phosphates have the ability to maintain the flow properties of the spices and the curing salt mixtures in meat.

These reports suggest that the combined action of tumbling and polyphosphates might permit the development of stable cured meat color using reduced levels of sodium nitrite.

This project was designed to determine the effect of tumbling and sodium tripolyphosphate on the migration of salt and nitrite in pork muscle tissue.

MATERIALS & METHODS

THE SEMIMEMBRANOSUS MUSCLES from 20 paired hams were assigned to four treatment groups (see Fig. 1). Each muscle was trimmed to a section, 15 cm long, 10 cm wide and 7.5 cm deep. These sections were pumped 3% by weight with a single injection curing needle placing a column of cure in the geometric center and parallel to the long axis of the muscle section which was perpendicular to the muscle fibers (Fig. 2). Sections from ten hams were pumped with a brine containing 76.8% water, 16.5% salt, 3.3% sugar, 3.3% sodium tripolyphosphate and 0.092% nitrite. Sections from ten hams were pumped with a brine containing 80.1% water, 16.5% salt, 3.3% sugar and 0.092% nitrite. Following this injection, ten hams (5 with NaTPP

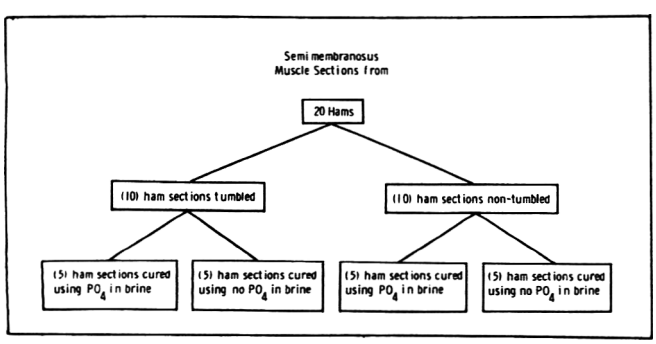

Fig. 1—Experimental design

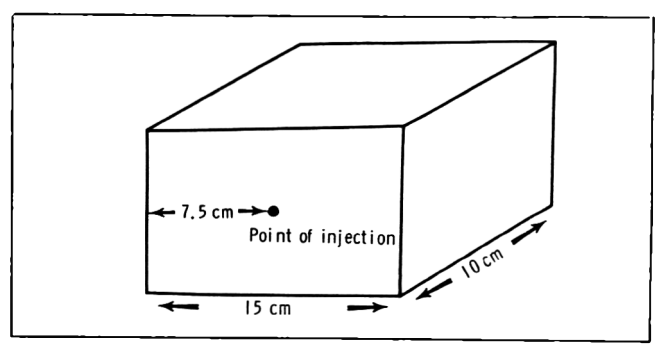

Fig. 2—Schematic representation of muscle prior to injection

and 5 without NaTPP) were tumbled 10 min out of each hour for 18 hr at a room temperature of 20°C. The remaining ham sections were held at 20°C for 18 hr. This temperature was selected in order to duplicate tumbling practices found in many American and European Meat Plants. After the 18-hr period, a 1.4 cm slice was taken from each muscle section perpendicular to the cure column (Fig. 3) and baked at 121°C for 15 min. Tracings were then made of the cured surface area and total muscle surface area and these areas were calculated using the compensating polar planimeter. At that time samples (6.35 mm slices) were also taken perpendicular to the previous slice (Fig. 3), from the remaining uncooked muscle section in eight areas:

1. site of injection
2. site of injection
3. 1.27 cm to the right of the injection site
4. 1.27 cm to the left of the injection site
5. 3.17 cm to the right of the injection site
6. 3.17 cm to the left of the injection site
7. 5.46 cm to the right of the injection site
8. 5.46 cm to the left of the injection site

These samples were analyzed for salt and nitrite content using procedures outlined by AOAC (1965) as modified by Ockerman (1974).

Statistical analysis of the salt and nitrite data was performed using the Statistical Analysis System (SASS) (Barr and Goodnight, 1972). The data on cured area were subjected to simple correlation analysis (Snedecor and Cochran, 1971).

RESULTS & DISCUSSION

THE RESULTS reported in Table 1 demonstrate that tumbling increased the migration of salt. Significantly ($P < 0.05$) greater concentrations of both salt and nitrite (Tables 1 and 2) were found at the injection site and at 1.27 and 3.17 cm beyond the injection site. There was no significant difference at the 5.46 cm site for nitrite content but there was a significant difference for salt at this point. This increased cure migration may be influenced by the impact energy on the muscle tissue during tumbling. In a normal curing operation the brine movement would be due to osmotic pressure alone. When tumbling is employed, the distribution of the curing ingredients is not only influenced by osmotic pressure but also by the movement of the muscle tissue due to tumbling and by the disruption of the muscle sarcolemma as reported by Theno et al., (1976). This will promote the migration of the curing ingredients both between muscle bundles and fibers and into those fibers with fragmented sarcolemma, resulting in a quicker and more uniform distribution of the curing ingredients.

Tumbling also had a significant effect on residual nitrite concentration (Table 2). Greater nitrite concentrations were noted at the site of injection and at both 1.27 and 3.17 cm distant from that site. With the effects of phosphate, distance, and ham section variation statistically removed, tumbled ham sections averaged 39.7 ppm residual nitrite, while nontumbled ham sections averaged 26.05 ppm ($P < 0.01$).

The influence which tumbling had on improving the distribution of the curing ingredients resulted in the significant ($P < 0.01$) increase in percent cured cooked color area shown in Tables 3 and 4. Examination of these data indicates that tumbling had a far greater effect on percent cooked cured color area than did sodium tripolyphosphate alone. It is interesting to note that although the cured cooked color area was more extensive in the tumbled muscle sections, there was also a higher level of residual nitrite (Table 2) at three of the four sampled sites in these muscle sections.

With tumbling causing a disruption of the sarcolemma as reported by Theno et al. (1976), myoglobin from within the muscle fiber is more quickly available to the nitrite in the spaces between the cells and an acceptable color is more rapidly developed as the nitrite is reduced to nitric oxide. The efficiency of the system is increased.

This would suggest that tumbling could permit a reduction

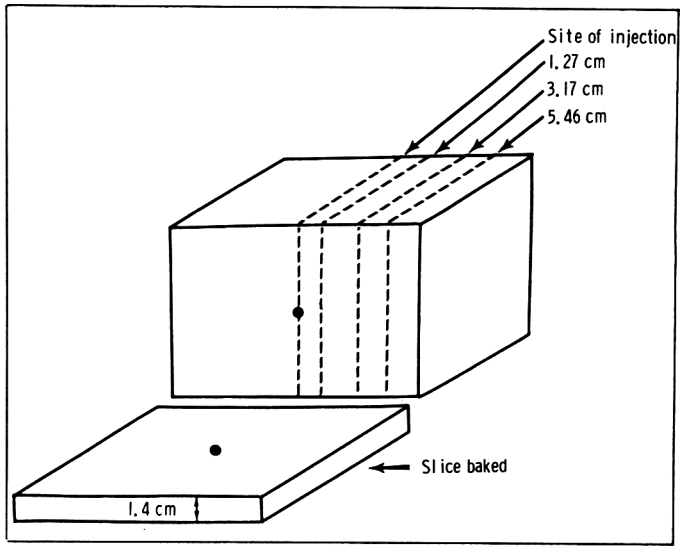

Fig. 3—Schematic representation of sample areas

Table 1—Salt concentration at distances from the injection site as influenced by tumbling

Distance from injection site (cm)	Salt conc (%)	
	Tumbled	Nontumbled
0	3.92	4.18
1.27	2.56	1.88
3.17	1.38	0.80
5.46	0.71	0.05
$\bar{X}$	2.14*	1.73

* Significant $P < 0.05$ (Effects of PO_4, distance and ham variation statistically removed)

Table 2—Nitrite concentrations at distances from the injection site as influenced by tumbling

Distance from injection site (cm)	Nitrite, ppm	
	Tumbled	Nontumbled
0	60.3	47.2
1.27	60.4	30.9
3.17	20.9	15.3
5.46	8.6	10.8
$\bar{X}$	39.7**	26.05

** Significant $P < 0.01$ (Effects of PO_4, distance and ham variations statistically removed)

Table 3—Cooked, cured area of ham sections as influenced by tumbling without sodium tripolyphosphate

	Nontumbled	Tumbled
Total muscle section cooked area (sq cm)	69.72	71.85
Cured cooked color area (sq cm)	11.00	25.72
Cured cooked color area (%)	1.68	36.00**

** Significant ($P < 0.01$)

in the amount of nitrite put into the curing solution without adversely affecting cured cooked color development or could significantly reduce the time required for color development.

The effect which NaTPP had on the migration of the curing ingredients is presented in Table 5. With the effects of tumbling, distance and ham statistically removed, the presence of sodium tripolyphosphate resulted in a significant ($P < 0.05$) increase in percent salt ($\bar{X}$ = 2.20 vs $\bar{X}$ = 1.67) and a highly significant ($P < 0.01$) increase in residual nitrite ($\bar{X}$ = 48.2 ppm vs $\bar{X}$ = 15.4 ppm).

By comparing the mean percent cured areas in Tables 3 and 4, it is apparent that NaTPP improved the area of color development in both tumbled and nontumbled porcine muscle in addition to increasing the residual nitrite. The polyphosphate effect on area of color development was more dramatic for nontumbled hams since tumbling independently has a significant effect on cure migration and color development. These data support the reports which indicated that polyphosphates increased the cooked cured pigment quantity (Rahelic et al., 1966).

The means by which NaTPP increased cure migration is not completely elucidated. If phosphate has the ability to break the actomyosin complex of the myofibril it should provide more opportunity for the free migration of salt and nitrite. It is also suggested that the increased migration of salt and nitrite due to NaTPP may be aided by the increased migration of actin and myosin. Siegel et al. (1976), Vartorella, (1975) and Krause et al. (1976) have reported that NaTPP increased the level of muscle exudate independent of the effect of salt or mechanical action. The effect of phosphates on muscle water retention is well documented in the literature.

Table 4—Cooked, cured color area of ham sections as influenced by tumbling with sodium tripolyphosphate

	Nontumbled	Tumbled
Total muscle section cooked area (sq cm)	64.25	62.60
Cured cooked color area (sq cm)	15.68	29.03
Cured cooked color area (%)	25.20	47.00**

** Significant ($P < 0.01$)

Table 5—Influence of sodium tripolyphosphate (NaTPP) on the migration of salt and nitrite in cured pork muscle tissue

Sample distance from site of injection (cm)	Salt (%) NaTPP		Nitrite residue (ppm) NaTPP	
	3.3%	0%	3.3%	0%
0	4.28	3.82	71.5	36.0
1.27	2.66	1.78	74.4	16.9
3.17	1.28	0.90	31.5	4.7
5.46	0.58	0.18	15.6	4.0
$\bar{X}$	2.20*	1.67	48.2**	15.4

* Significant $P < 0.05$ (Effect of tumbling, distance and ham statistically removed)

** Significant $P < 0.01$ (Effect of tumbling, distance and ham statistically removed)

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T. C. TSAI,[1] G. H. WELLINGTON and W. G. POND
Dept. of Food Science and Dept. of Animal Science
Cornell University, N.Y. State College of Agriculture & Life Sciences
Ithaca, NY 14850

IMPROVEMENT IN THE OXIDATIVE STABILITY OF PORK BY DIETARY SUPPLEMENTATION OF SWINE RATIONS

ABSTRACT

The extent of improvement in the oxidative stability of pork through dietary supplementation was determined by feeding pigs rations containing α-tocopherol acetate and ascorbic acid. Approximately 7.7, 5 and 4.3 μg of α-tocopherol per gram of tissue were deposited in the renal fat, the subcutaneous fat and the triceps muscle respectively by 200 ppm α-tocopherol acetate supplementation of the ration fed between beginning weights of 9–20 kg and a slaughter weight of 91 kg. The oxidative stability of the pork tissues was unaffected by ascorbic acid supplementation. However the stability of the adipose tissues was significantly increased ($P < 0.01$) by α-tocopherol acetate supplementation as indicated by the oxygen bomb stability test. There was a linear relationship between the stability of adipose tissues and their α-tocopherol content. Supplementation of 100 ppm of α-tocopherol acetate in the ration appeared to be the optimum level for improvement of the oxidative stability of the muscle.

INTRODUCTION

UNDESIRABLE ODORS AND FLAVORS result from the oxidation of meat lipids. This is frequently accompanied by the discoloration of pigments. In fact oxidation has been one of the major problems in maintaining high market quality. This is particularly the case with pork because of its larger proportion of polyunsaturated fatty acids.

Lipid oxidation can be inhibited by antioxidants and the tocopherols are classified among the natural antioxidants (Bauernfeind and Cort, 1947). The antioxidant effect on rat body tissues following dietary supplementation with tocopherol was shown in 1943 by Barnes et al. The dietary application of tocopherol to stabilize pork fat has been investigated by Watts et al. (1946) and Hvidsten and Astrup (1963). It was found that a slight improvement in keeping quality could be achieved by this method although the improvement was felt to be too small for practical significance. More recently the effectiveness of dietary supplementation for the improvement of meat quality in turkeys fed on tuna oil was extensively studied by several additional workers (Crawford et al., 1975, 1974; Webb et al., 1972a, 1973; Webb et al., 1972b).

Ascorbic acid, which functions by oxygen scavenging, has frequently been used as an antioxidant. These applications of ascorbic acid have been in closed systems to remove the oxygen in a package head space or for the removal of oxygen from solutions (Bauernfeind and Pinkert, 1970). In the dietary supplementation of ascorbic acid together with α-tocopherol acetate a synergistic effect has been reported that enhanced the deposition of α-tocopherol in the adipose tissues of chicks fed cod liver oil (Prange, 1949). However, there are no similar reports of this synergistic effect with pigs.

The objectives of this study were first to find the individual effect of ascorbic acid and α-tocopherol acetate as dietary supplements in swine rations upon the oxidative stability of pork and second to determine whether there might be a synergistic effect influencing the deposition of α-tocopherol by the simultaneous feeding of ascorbic acid.

MATERIALS & METHODS

Animals and diets

Four levels of α-tocopherol acetate and two levels of ascorbic acid, factorially arranged as eight treatments, as shown in Table 1, were added to a basic swine ration and fed ad libitum to random groups of four pigs each.

The basic diet was a commercial type maize-soybean meal based diet supplemented with vitamins and minerals to meet nutritional requirements. Ascorbic acid and α-tocopherol acetate dissolved in corn oil were added during the mixing of the basic ration. Batches of feed were prepared in quantities to last 2–4 wk with ad libitum consumption. The mixed feed was stored in polyethylene bags.

The pigs were kept on concrete floors and each pen was equipped with self-feeders and waterers. During the early portion of the feeding period the basic ration contained 18% protein. After 1 month the pigs were switched to a 16% protein ration. The latter ration was fed until the animals were slaughtered at 91 kg.

Tissue sampling

Slaughtering was done according to standard procedures. After evisceration, renal or leaf fat samples were removed. The carcasses were then placed in a chilling room (0°C) where they were cooled over 30 hr to an internal temperature of 0°C. Following chilling, 15 × 15 cm sections of the subcutaneous fat that covered the Bostom butt and the triceps muscle (caput longum, caput mediale and caput lateral) were removed. Skin and any muscle tissue or blood vessels accompanying the subcutaneous fat sample were discarded. Covering fat and intermuscular fat were trimmed from the triceps muscle as completely as possible. The trimmed tissue samples were vacuum packaged in a plasticized vinylene chloride-vinyl chloride polymer pouch by means of a Cryovac vacuum machine (Cryovac Division, W.R. Grace & Co.) and stored at −23°C for 1–3 days prior to grinding.

Grinding, mixing and preparation

Before grinding, the frozen samples were allowed to warm and soften at 0°C. Next the tissue samples were cut into approximately 19 mm cubes and held below freezing. They were mixed with pulverized dry ice and ground through a 9.5 mm grinder plate which provided a spaghetti-like product. The samples were ground a second time and held at −23°C overnight to eliminate any dry ice residue. The samples were

Table 1—Dietary supplementation to the basic diet

Treatment no.	Ascorbic acid (ppm)	α-tocopherol acetate (ppm)
1	0	0
2	0	50
3	0	100
4	0	200
5	2000	0
6	2000	50
7	2000	100
8	2000	200

[1] Present address: Food Science & Nutrition Dept., Providence College, Taichung, Taiwan

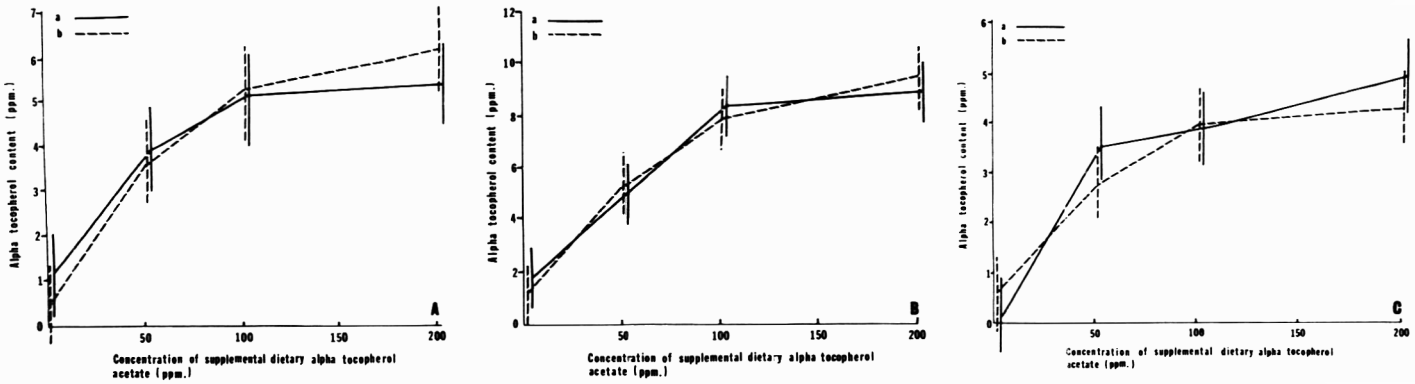

Fig. 1 — Effect of supplimental dietary ascorbic acid and α-tocopherol acetate on the α-tocopherol content of selected tissues. Vertical lines denote 95% C.I. of the treatment means: (a) with 2,000 ppm ascorbic acid supplement; (b) without ascorbic acid supplement. A—Subcutanious fat; B—Leaf fat; C—Triceps muscle fat.

then vacuum packaged in a polyester Saran-type pouch using a Multivac vacuum packaging machine and stored at −23°C.

For thiobarbituric acid and peroxide value measurements, approximately 250g of the frozen ground meat were evenly spread into a thin layer (approximately 5 mm) on a meat packaging paper tray and then covered with polyvinyl chloride film. The packaged samples were stored at 0°C. After 9 days sub-samples were taken from each package for analysis.

Laboratory procedures

Lipid extraction for iodine number measurement and fatty acid composition analyses were conducted according to Ostrander and Dugan (1962). Iodine number was determined according to the Official Hanus method (AOAC, 1965). Esters of fatty acids were prepared according to Van Wijngaarden (1967) and fatty acid composition analysis was conducted on a Perkin-Elmer Model 881 gas liquid chromatograph with a column of 15% diethylene glycol succinate coated on Chromasorb P of 100–120 mesh.

For the determination of α-tocopherol, the procedure included saponification, extraction, separation and quantitation. The saponification and extraction were essentially conducted according to Bro-Rasmussen and Hjarde (1957). The extract was evaporated to dryness in a water bath under prepurified nitrogen and the residue was dissolved in 2 ml analytical benzene for separation. The separation of α-tocopherol from cholesterol was conducted on a florisil column. The procedure used was essentially that of Pudelkiewicz et al. (1960), except that activated Florex (Sigma Chemical Company) was layered with a pipette to attain a column 12.5 cm long and 20 ml of eluant were collected. The eluant was then evaporated to dryness under nitrogen and the residue was redissolved in 1 ml of methanol for quantitation of α-tocopherol by gas-liquid chromatograph with a column of 3% QF-1 on Gas Chromo. Q of 60–80 mesh (Applied Science Laboratories).

The oxidative stability of fresh samples was evaluated by three methods. The oxygen bomb was used to predict the stability of fat tissues and a standard procedure, No. 13A of Eastman Food Laboratory, Kingsport, TN (1973) was followed. In this method 20-g samples were used and the temperature of the oven and the oxygen pressure were set at 100°C and 110 psi respectively. Oxidative stability of the triceps muscle was measured by the peroxide value (AOCS, 1975) and the TBA value (Witte et al., 1970).

Table 2—Summary of the effect of supplemental dietary ascorbic acid and α-tocopherol acetate on the fatty acid composition and its saturation[a]

	Subcutaneous fat		Leaf fat		Triceps fat	
Fatty acid	E	C	E	C	E	C
C 14:0	±	±	±	±	±	±
C 15:0	/	/	/	/	±	±
C 16:0	±	±	±	$P < 0.05$	±	±
C 16:1	±	±	±	±	±	±
C 18:0	±	±	±	$P < 0.01$	±	±
C 18:1	±	±	±	±	±	±
C 18:2	±	±	±	±	±	±
C 18:3	±	±	±	±	±	$P < 0.05$
C 20:4	/	/	/	/	±	±
Satur.	±	±	±	±	±	±
Mono "="	±	±	±	±	±	±
Poly "="	±	±	±	±	±	±
Iodine	±	±	±	±	/	/

[a] ± No effect; P—Significant level; 1—Not measured; E—α-tocopherol acetate supplement; C—Ascorbic acid supplement

RESULTS & DISCUSSION

Fatty acid composition

The influence of dietary supplementation of ascorbic acid and α-tocopherol acetate on the fatty acid composition are summarized in Table 2. There were no effects of supplementation of α-tocopherol acetate on the fatty acid distribution nor on the amount of unsaturation. The observations thus are in agreement with those of Hvidsten and Astrup (1963) that the degree of unsaturation of the body fat is unaffected by the supplementation of α-tocopherol acetate. In addition, Bunnell et al. (1956) concluded that there is little evidence to show an effect of vitamin E on the overall fatty acid composition of the tissues.

Dietary supplementation with ascorbic acid seemed to show some effect on the fatty acid distribution in tissues studies. Ascorbic acid tended to increase the C 16:0, C 16:1 and to decrease the C 18:0 and C 18:2 in the subcutanious fat. However, none of these trends was sufficient to be statistically significant. With the leaf fat, a significant increase ($P < 0.05$) in C 16:0 compensated for the significant decrease in C 18:0 and no change in the saturation or iodine value could be attributed to the ascorbic acid supplementation. A significant increase ($P < 0.05$) in C 18:3 was found in the triceps intramuscular fat. However, this increase in degree of unsaturation was too small (0.07%) to be statistically significant.

α-tocopherol content

It is well known that α-tocopherol is the predominant tocopherol in animal tissues. Thus in this study only the α-tocopherol was analyzed. Figure 1 shows the influence observed of dietary supplementation of ascorbic acid and α-tocopherol acetate upon the α-tocopherol contents of selected tissues.

There was no significant effect from ascorbic acid supplementation upon the tissue α-tocopherol content. The synergistic effect of ascorbic acid supplementation on α-tocopherol content of chicken tissues as reported by Prnage (1949) was not observed in the tissues of swine. It is unknown whether this difference in effect from ascorbic acid supplementation was due to differences in species, in experimental conditions, or possibly due to both.

The supplementation of α-tocopherol acetate significantly ($P < 0.005$) increased the content of α-tocopherol of all tissues selected for analysis. It can be observed from the slopes of the curves that the deposition of α-tocopherol by supplementation of α-tocopherol acetate is relatively inefficient and the efficiency of deposition was decreased when the concentration of α-tocopherol acetate supplement was increased. Of the three tissues studied, the increase in α-tocopherol content was most marked in leaf fat and least in the triceps muscle. Bratzler (1950) reported that, among tissues he studied, liver, leaf and ruffle fat showed the largest increases in α-tocopherol.

The small amount of α-tocopherol in tissues from animals that received no supplementation of α-tocopherol acetate suggests that the basic rations contained low levels of α-tocopherol. By dietary supplementation of 200 ppm α-tocopherol acetate, about 5.0, 7.7 and 4.3 μg of α-tocopherol/g tissue were deposited in subcutaneous fat, leaf or renal fat and triceps muscle respectively. The lipid content of adipose tissues was about 20 times as high as that of triceps muscle. In terms of the α-tocopherol content per unit of fat, the concentration in the triceps tissue was found to be greater (107 μg) than in adipose tissue (8.5 μg).

Oxidative stability

Adipose tissues. The effect of dietary supplementation with ascorbic acid and α-tocopherol acetate on the stability of subcutanious fat and leaf fat as measured by the oxygen bomb at 100°C and 110 psi of O_2 pressure is shown in Figure 2. The oxidation induction period was defined as the elapsed time between the placing of the bomb in the controlled temperature oven and the declared endpoint of a 2 psi drop in pressure. From these data, ascorbic acid supplementation seemed to be ineffective for improvement of oxidative stability. This was expected because the addition of ascorbic acid failed to change either the degree of saturation of lipid or the α-tocopherol content of tissues. Nevertheless, as shown in Figure 2b, the addition of ascorbic acid did show a tendency to improve the stability of leaf fat at low levels of α-tocopherol acetate supplementation. In contrast to ascorbic acid, α-tocopherol supplementation was effective in increasing the tocopherol content of tissues, and thus significantly increased ($P < 0.01$) the stability of fresh adipose tissues.

The efficiency for the improvement in oxidative stability by α-tocopherol supplementation tends to decrease when the concentration of the supplement is increased as shown by the stability curves. In the case of subcutaneous fat, an increase of about 30 min on the OBM stability test could be achieved with the first 50 ppm of α-tocopherol acetate supplementation, while only about 18 min could be increased by an additional 50 ppm of supplementation. At the same level of α-tocopherol acetate supplementation the stability of leaf fat was usually higher than that of subcutaneous fat. This was predictable because the leaf fat had a higher α-tocopherol content and a higher degree of saturation of lipids.

The linear relationships between the OBM stability test for tissues and their α-tocopherol content are given in Figure 3. A high correlation was observed between oxidative stability and α-tocopherol content.

Triceps muscle. The oxidative stability of triceps muscle could not be predicted by the OBM method due to its low fat content. Thus its oxidation could not be shown by an obvious drop in pressure. Peroxide values and TBA values of the triceps muscle are shown in Table 3. There were relatively large deviations in these observations. No statistically significant effects were found on the stability of triceps muscle as related to treatments. Obviously, the effect on the stability from adding α-tocopherol acetate was higher than from the addition of

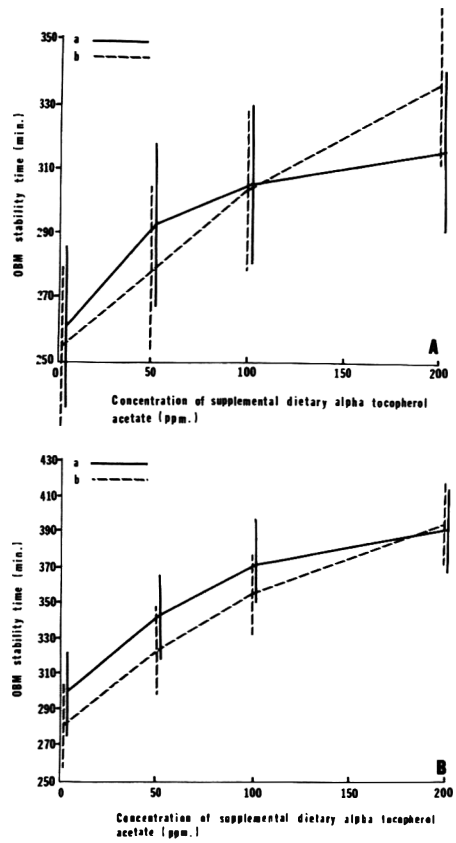

Fig. 2—Effect of supplemental dietary ascorbic acid and α-tocopherol acetate on the stability of fat tissues. Vertical lines denote 95% C.I. of the treatment means. (a) with 2,000 ppm ascorbic acid supplement; (b) without ascorbic acid supplement. A—Subcutaneous fat; B—Leaf fat.

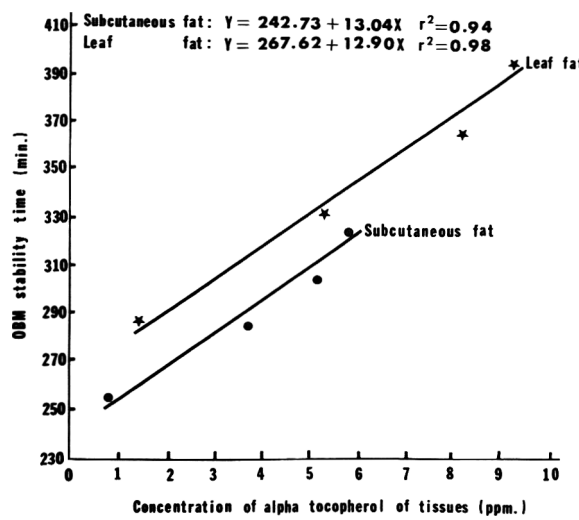

Fig. 3—linear relationships between the OBM (oxygen bomb method) stability times of tissues and their concentration of α-tocopherol content.

Table 3—Effect of supplemental dietary ascorbic acid and α-tocopherol acetate on the stability of triceps muscle

Treatment no.	Stability	
	Peroxide value	TBA value
1	32.68 ± 21.60[a]	2.42 ± 1.26
2	16.91 ± 16.62	1.20 ± 1.56
3	10.66 ± 7.33	0.46 ± 0.43
4	15.30 ± 8.45	1.34 ± 0.93
5	24.94 ± 26.40	1.69 ± 1.64
6	13.65 ± 1.58	0.8[illegible] ± 0.22
7	11.58 ± 7.33	0.56 ± 0.48
8	16.17 ± 9.34	1.06 ± 1.03

[a] Treatment mean ± standard deviation

ascorbic acid. Thus to statistically evaluate the trend, the results were considered as a single factor experiment with different levels of α-tocopherol acetate supplementation and with the effect of ascorbic acid ignored. The data suggest that there is an optimum level of the α-tocopherol acetate supplementation for increased stability. Supplementation at 100 ppm of α-tocopherol acetate appears to be near this optimum.

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because the collagen fibers, in this case, are nearly perpendicular to the main muscle fiber axis (Rowe 1974, Swatland, 1975) so that prevention (or reduction) of shortening along that axis will have little or no restraining effect on the collagen fibers. It could be helpful to the elucidation of the structural significance of other treatments on meat to have methods available for selectively altering either the connective tissue or myofibrillar contributions to the structural strength of meat samples.

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T. L. MAYFIELD,[1] K. K. HALE JR.,[1] V. N. M. RAO and I. A. ANGULO-CHACON[2]
Departments of Poultry Science and Food Science
University of Georgia, Athens, GA 30602

EFFECTS OF LEVELS OF FAT AND PROTEIN ON THE STABILITY AND VISCOSITY OF EMULSIONS PREPARED FROM MECHANICALLY DEBONED POULTRY MEAT

ABSTRACT

A temperature controlled, capillary extrusion viscometer was used to compare meat batters prepared from mechanically deboned poultry meat (MDPM). Protein levels of 12% produced more viscous batters and less release of gel water and fat during emulsion stability tests than 11% protein. Product formulated with 21% fat gave less stable batters than meat with 16% fat; however, the viscosity of the meat batter increased with increased level of fat. Although meat batters prepared from two different sources of MDPM exhibited similar emulsion stability and extrusion capillary viscometry characteristics, a mixture of the meat from the two sources was lower in all components released during the emulsion stability test.

INTRODUCTION

MECHANICAL DEBONING of raw poultry meat has been one of the most important advances in poultry processing. Backs and necks of broilers, turkey frames, and whole spent layers are now important sources of edible poultry meat. Because of small particle size this product is most useful in comminuted products such as frankfurters and sausage. The production of stable raw meat batters in which the water and fat do not separate during processing and cooking is economically important.

The effect of fat and protein levels of mechanically deboned poultry meat (MDPM) on emulsified products is of significant importance from the standpoint of obtaining quality products. The effect of fat levels in emulsion-type products includes work with beef bologna (Swift et al., 1954); hand-deboned chicken meat frankfurters (Baker et al., 1969); beef frankfurters (Townsend et al., 1971); beef, pork and mutton frankfurters (Carpenter et al., 1966); and mechanically deboned turkey meat frankfurters (Baker and Darfler, 1975). Research on the effect of level of protein on these products includes studies with beef frankfurters (Simon et al., 1965), turkey franks made from MDPM (Baker and Darfler, 1975), and hand-deboned chicken meat frankfurter (Baker et al., 1969).

Viscosity measurements of raw meat batters have also been used to help determine the functional property characteristics of meat mixtures. Many methods of measurement have been employed including work with the Brookfield viscometer (Townsend et al., 1971; Acton and Saffle, 1970). Measurement of viscosity by extrusion through an aperture with the Kramer shear press was performed by Baker et al., (1974) and Schnell et al. (1973). However, extrusion capillary viscometers have been found to be more suitable for determining viscosity of thick materials such as meat batters (Badakhsh, 1976).

Viscosity of meat emulsions (beef) was determined using a capillary viscometer attached to an Instron. The temperature of the viscometer apparatus was kept at approximately 2°C by immersion in ice, but fluctuations in temperature often occurred due to the time required to extrude the material.

In this study the effects of selected composition levels (fat and protein) of MDPM, different meat sources, and various chopping times on the emulsion stability and viscosity of MDPM batters were studied. A newly designed cylinder (jacketed) which could be temperature controlled to within ± 0.5°C was used in determining viscosity.

MATERIALS & METHODS

Sources of meat

Mechanically deboned poultry meat (MDPM) processed the previous week was obtained frozen from two local producers (A and B) and a mixed source was produced by combining equal amounts of A and B. Both producers A and B used primarily a combination of skinless necks and frames from broiler chickens for mechanical deboning. These sources of meat were stored at −25°C and used within 2 wk. Protein, fat and moisture of the MDPM was determined by the methods of AOAC (1970). The fat source utilized in this study was abdominal fat of broilers.

Methods

The experimental design included a factorial arrangement of treatments with two fat levels (16 and 21%), two protein levels (11 and 12%), three sources of MDPM (A, B, and Mix), and three chop times (5, 10 and 15 min). Fat and protein levels were adjusted by combining broiler abdominal fat, ice and MDPM according to the information presented in Table 1.

The MDPM, fat and ice combinations of Table 1 were allowed to reach an average temperature of −12°C before combining with 58g of salt, 12.8g of a commercial spice mixture, 0.58g of sodium nitrite and 0.58g of ascorbic acid. The batters were prepared by chopping the mixture in a 45 cm diameter Hobart cutter. Raw emulsion samples were collected after 5, 10 and 15 min of chop time (−6°, −1° and 6°C batter temperatures, respectively) for the emulsion stability tests and viscosity measurements. The samples were stored in sealed containers at 4°C for about 4 hr until evaluated.

Objective tests

Emulsion stability. The method of Townsend et al. (1968) was used

Table 1—Amounts of MDPM, fat and water combined to obtain desired fat and protein composition levels.

Fat level (%)	Protein level (%)	Source A MDPM	A Fat (g)	A H_2O	Mix MDPM	Mix Fat (g)	Mix H_2O	B MDPM	B Fat (g)	B H_2O
16	11	1719	43	236	1572	115	313	1457	172	371
16	12	1875	14	110	1714	92	194	1589	154	257
21	11	1719	159	122	1572	230	198	1457	287	256
21	12	1875	125	–	1714	207	79	1589	269	142

[1] Present address: Poultry Science Dept., Clemson University, Clemson, SC 29361
[2] Present address: Instituto de Investigaciones Veterinarias, Programa Nacional de Produccion Avicola, Apartado 70, Maracay, Venezuela

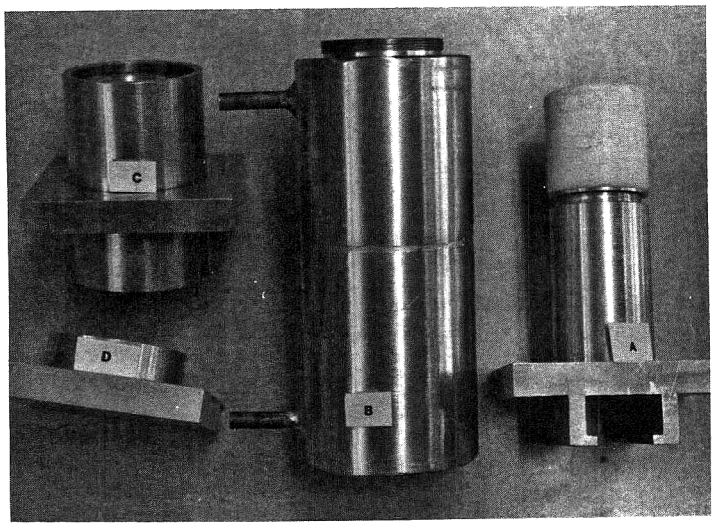

Fig. 1—Extrusion apparatus.

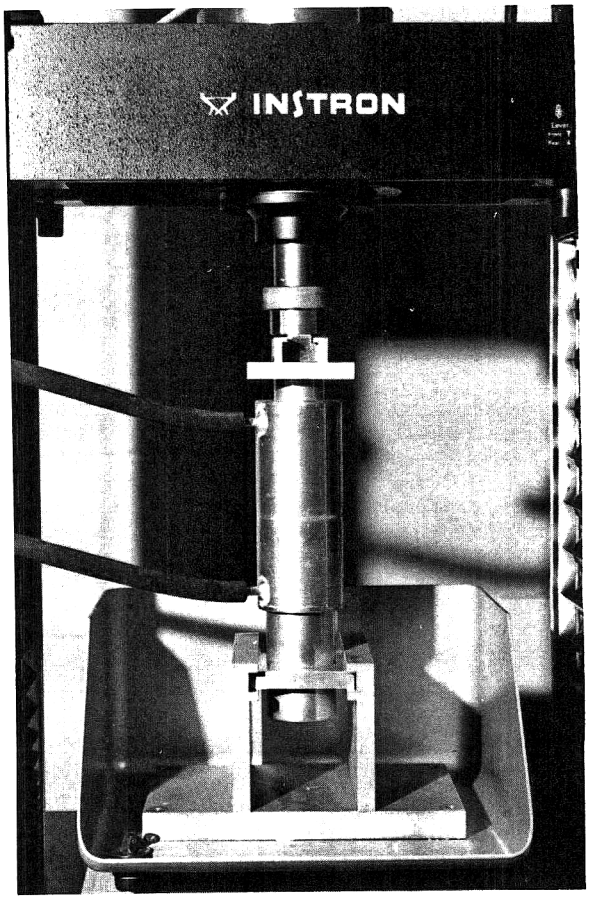

Fig. 2—Extrusion apparatus fitted to Instron.

to evaluate the mixture from each treatment. The amount of gel-H_2O, fat and total volume released during cooking was used as an indicator of emulsion stability.

Viscosity of raw emulsion. Viscosity of the raw meat batter was measured by extrusion through a capillary. The extrusion instrument was designed and constructed at the Machine Shop of the University of Georgia and all instrument parts were made from high grade aluminum alloy. The viscosity device included the following: (A) piston (Teflon); (B) cylinder (jacketed for temperature control); (C) capillary attachment; (D) orifice attachment (Fig. 1). The piston-cylinder assembly was fitted to a Model 1122 Instron (Fig. 2).

A temperature-controlled circulating water bath was connected to the jacketed cylinder portion of the apparatus in order to maintain a constant temperature of 5 ± 0.5°C for the viscosity device and meat batter throughout the experiment. Meat mixtures were removed from refrigerated storage at 4°C and stuffed into the cylinder as quickly as possible using care to eliminate air-pockets.

The principle objective in this part of the experiment was to determine the force required to extrude the material through the capillary. This force was determined by extruding the batter once through the orifice attachment and once with the capillary attachment for the same crosshead speed. The force attained for the case with the orifice attachment was subtracted from the force with the capillary attachment and the result was recorded as the force necessary to extrude the material through the capillary. This procedure accounts for corrections such as the kinetic energy loss, and effect and the entrance pressure loss (Van Wazer et al., 1963).

The measurements were replicated two times at each speed for the orifice and the capillary attachments. The experiment was performed using crosshead speeds of 0.1, 0.5, 1.0, 5.0 and 10.0 mm per min.

Adequate material for density and weight measurements for every replication at each speed was collected into a preweighed aluminum pan. The time of extrusion and average peak force were measured directly from the Instron output. Collection of material (and subsequent weight) was carefully coordinated with time of extrusion and stabilized force readings for each crosshead speed.

The weight of the extruded batter was recorded and the volume was calculated from the density. Density was measured by immersing a quantity of emulsion in a weighed graduated cylinder containing water. The difference in weight was divided by the volume of water displaced in the cylinder and the result was taken as the density in each replication. The above procedure was repeated three times for each treatment and the average in each case was recorded as the density of the batter.

However, initial data analysis indicated that the above procedure can be modified. One can directly calculate the volumetric flow rate (Q) by knowing the crosshead speed and the area of the cylinder as long as there are no air pockets in the extrusion process. Volumetric flow rate is then calculated as follows:

$$Q = \text{crosshead speed} \times \text{area of cylinder}$$

Calculation of Q by this method eliminates (1) the numerous weighings of each replication at each crosshead speed, (2) the calculation of volume from the density and (3) the determination of time of extrusion for each replicate. Therefore, the overall procedure is made faster, simpler, and there exists less possibility for error.

Analysis of data

Data obtained from the capillary extrusion viscometer assembly attached to the Instron, comprises volumes of material extruded through the capillary during specific lengths of time and the corresponding forces measured by the Instron force transducer. The shear stress, τ, was evaluated as:

$$\tau = \frac{\Delta P R}{2L}$$

where ΔP is the corrected pressure drop across the capillary

$$\Delta P = \frac{F_c - F_o}{A}$$

where F_c is the force required to extrude the material through the capillary; F_o is the force required to extrude the material through the orifice; A = area of cylinder (1140 mm^2); R is the radius of the capillary (1.6 mm); and L is the length of the capillary (50.8 mm).

The shear rate, $\dot{\gamma}$, was evaluated following the general approach of Van Wazer et al. (1963) using the Rabinowitsch's equation. Assuming a generalized single valued functional form of shear stress and shear rate, such that

$$\dot{\gamma} = (dV/dr) = f(\tau)$$

The volumetric flow rate, Q is

$$Q = \int_0^R 2\pi r\ V(r)\ dr$$

where V(r) is the velocity as a function of the radial coordinate r. Integrating by parts and using the boundary conditions at r = o and r = R yields

$$\frac{4Q}{\pi R^3} = \frac{4}{\tau^3}\int_0^R f(\tau)\ \tau^2\ d\tau$$

Differentiating with respect to r, and rearranging

$$\dot{\gamma} = (dV/dr) = \frac{Q}{\pi R^3}\quad (3 + b)$$

where $b = [d \log (4\ Q/\pi R^3)]/d \log (\Delta P\ R/2L)$. The term b is referred to as the correction factor. In the case of Newtonian fluid the value of b would be unity. The correction factor b was obtained as the slope of the linear regression of log Q on log (ΔP/L). After obtaining the shear stresses and corresponding shear rates a generalized power law model was assumed to fit the data. The model is

$$\tau = k\dot{\gamma}^n$$

where τ is the corrected shear stress including yield stress; $\dot{\gamma}$ is shear rate; k is the coefficient of shear rate; and n is the flow index.

The parameters of the flow model were evaluated using the steepest ascent method. The computer program and description of the procedure are given by Rao (1974). The apparent viscosity of the meat batters was evaluated at two shear rates (0.5 and 5.0 mm/min) as the ratio of shear stress to Newtonian shear rate. Apparent viscosity,

$$\eta\ app = \frac{(\Delta P \cdot R)/2L}{4Q/\pi R^3} = \frac{\tau}{4Q/\pi R^3}$$

During initial analysis of the data, crosshead speeds of 0.1, 1.0 and 10.0 mm/min gave relative values similar to 0.5 and 5 mm/min and were not used in further analysis. Data from emulsion stability tests, and the parameters of the flow model were subjected to a BMD 02V factorial analysis program (Dixon, 1970). Significant differences between means were determined by the method of Student-Newman-Keuls as outlined by Steele and Torrie (1960). The 0.05 level of probability was used for all statistical tests.

RESULTS & DISCUSSION

PROTEIN, FAT AND MOISTURE composition for the different meat sources are presented in Table 2. Data from Table 3 indicated that product made with higher fat (21%) or lower protein (11%) levels as well as 15 min chopping times released significantly greater amounts of gel-water, fat and total liquid. The mixture of meat sources A and B was found to be lower in all components released than either source A or B. However, it was noted that source A was higher in fat released than source B.

Data from the significant interaction of protein X source X chop time for gel-water and fat released (Fig. 3) indicated that the mixture formed the most stable product over the chopping ranges at both protein levels. This may be related to the difference in particle size of the two sources of meat. Source A was observed to have larger meat particles than source B and it seemed apparent that the mixture of these two sources produced a more stable product. In general, the 12% protein level produced a more stable product than the 11% level. However, it was noted that source A was most stable at high protein levels while source B was observed to be most stable at low protein levels.

The significant interaction of fat X source X chop time for gel-water and fat released (Fig. 4) demonstrated that little differences existed between meat sources using the 16% fat level indicating that it was more difficult to overchop at this lower fat level. However, with the higher fat level of 21%, chopping times of 15 min produced batter instability. The mixture of meat from sources A and B withstood chopping better at both fat levels. Although the two sources of MDPM used for this study were processed from similar parts (primarily skinless necks and frames), the differences in the deboning processes may have yielded different proteins or other components that complemented their emulsion stability characteristics.

The significant interaction of fat X protein X chop time for gel-water and fat released (Fig. 5) indicated that the higher protein level of 12% exhibited better stability than the 11% level. The low-protein-low-fat product seemed to have an optimum chopping time of around 10 min. The 16% fat level was not greatly affected by chopping time and the 21% level followed basically the same trend except that more "fatting out" occurred at 15 min of chopping than with 5 or 10 min chopping time. The high-fat/high-protein product was protected from breakdown for about 10 min but became unstable after 15 min of chopping.

Effects of fat and protein levels, sources of meat and chopping times on apparent viscosity, flow index and coefficient of shear rate are summarized in Table 4. Higher fat and higher protein levels produced more viscous emulsions. These results were similar to the turkey frame data of *Baker and* Darfler (1975), but appear to be in opposition to the findings of Townsend et al. (1971) who noted little difference in viscosity due to fat level. The measurements of Townsend et al. (1971) were taken at the end of chopping and at temperatures which intentionally varied considerably, whereas in this study measurements were obtained at 5°C about 4 hr after chopping.

Table 2—Moisture, fat and protein content of various sources of MDPM[a]

Source	Moisture	Fat	Protein
A	69.1	16.4	12.8
Mix[b]	70.3	14	14
B	71.5	11.7	15.1

[a] MDPM—Mechanically deboned poultry meat
[b] Values for mix are the average of A and B.

Table 3—Effect of fat, protein, source of meat and chop times on the stability of batters made from MDPM[a]

Variable	Level (%)	Components released per 100g emulsion[b] Gel-H_2O (ml)	Fat (ml)	Total (ml)
Fat	16	3.71b	0.06b	3.77b
	21	6.02a	0.69a	6.71a
Protein	11	5.94a	0.58a	6.52a
	12	3.79b	0.17b	3.96b
Source	A	4.85b	0.81a	5.68a
	Mix	3.75c	0.13b	3.87b
	B	5.99a	0.18b	6.17a
Chop time	5	4.37b	0.16b	4.53b
	10	3.65b	0.14b	3.79b
	15	6.57a	0.82a	7.39a

[a] MDPM—Mechanically Deboned Poultry Meat
[b] Different letters within the same column and variable indicate that values are significantly different ($P < 0.05$).

In addition, Townsend et al. (1971) used the Brookfield viscometer to measure viscosity.

There were proportional increases in apparent viscosity as chopping times increased (Table 4). The low value for viscosity at 5 min chopping time indicates that a stable batter had not yet been formed. No differences in viscosity for meat sources were found. Values for the flow index (n) were similar for all batters indicating the same type of flow characteristics. The coefficient of shear rate followed the same trend as apparent viscosity since the flow index was similar for all variables.

It was observed that the viscosity data for fat level responded somewhat differently from the gel-water data for fat level. The higher level of fat tended to decrease emulsion stability while increasing fat increased apparent viscosity. These differences in emulsion stability and viscosity may be attributed to the fact that (1) fat is more viscous than water at the testing temperature of 5°C thereby resulting in a more viscous product, and (2) higher levels of fat may be causing greater gel-water and fat release due to insufficient fat entrapment by the protein-water network (Brown, 1972).

Based upon these findings, it is evident that apparent viscosity of meat batters is affected by fat and protein content of the meat mixture and time chopped. Corresponding gel-water and viscosity values were plotted, but no obvious or readily discernible relationships were noted to exist. Attempts at regression and correlation analysis did not prove any strong relationships either. From the results of this study, it appears that capillary viscometry is a tool which can be used to investigate the functional and rheological properties of meat emulsions. However, in order to use viscosity as a functional property measurement, it is probably necessary to fix variables such as fat, protein, and chop time in order to investigate other variables individually.

Table 4—Effect of fat, protein, source of meat and chop times on the apparent viscosity, flow index and coefficient of shear rate of batters made from MDPM[a]

Variable	Level	Apparent viscosity (0.5 mm/min)	Apparent viscosity (5.0 mm/min)	Flow index (n)	Coefficient of shear rate (k)
Fat	16%	33.0b	5.2b	0.15a	83.7b
	21%	41.6a	6.4a	0.15a	103.7a
Protein	11%	34.2b	5.3b	0.15a	83.8b
	12%	40.5a	6.3a	0.14a	103.6a
Source	A	35.4a	5.7a	0.16a	89.4a
	Mix	39.2a	5.9a	0.15a	96.4a
	B	37.5a	5.8a	0.15a	95.2a
Chop time	5	31.4c	5.2c	0.17a	80.0b
	10	38.2b	5.9b	0.15a	95.9ab
	15	42.5a	6.3a	0.13a	105.2a

[a] Means within the same column and variable followed by different letters are significantly different from each other ($P < 0.05$).

Determination of the functional and rheological properties of meat batters by the use of capillary viscometers should find wider use in the future by researchers and industry especially when used in conjunction with other methods such as taste panel evaluations, stability tests, and texture measurements. This area, however, needs further investigation.

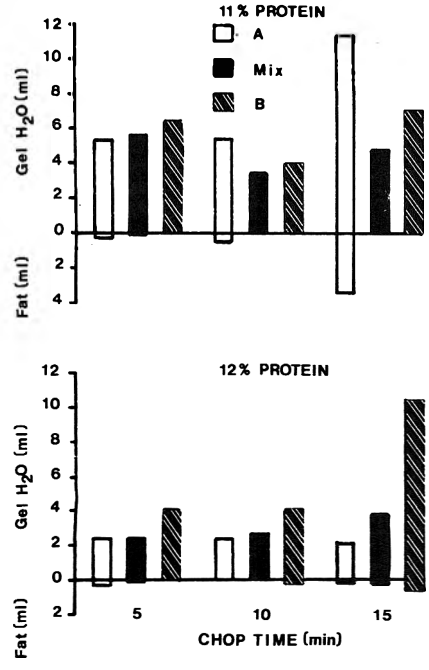

Fig. 3—Histogram of statistically significant protein X source X chop time interaction for gel-water and fat released from batters made from MDPM.

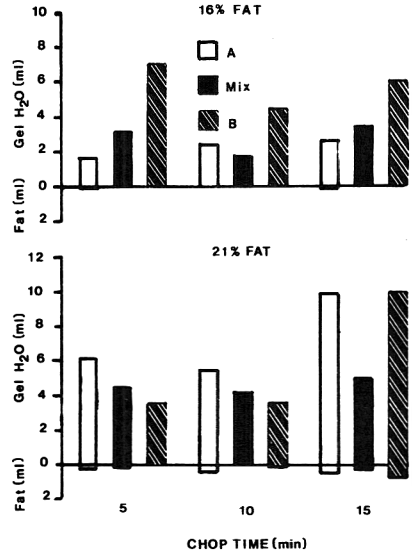

Fig. 4—Histogram of statistically significant fat X source X chop time interaction for gel-water and fat released from batters made from MDPM.

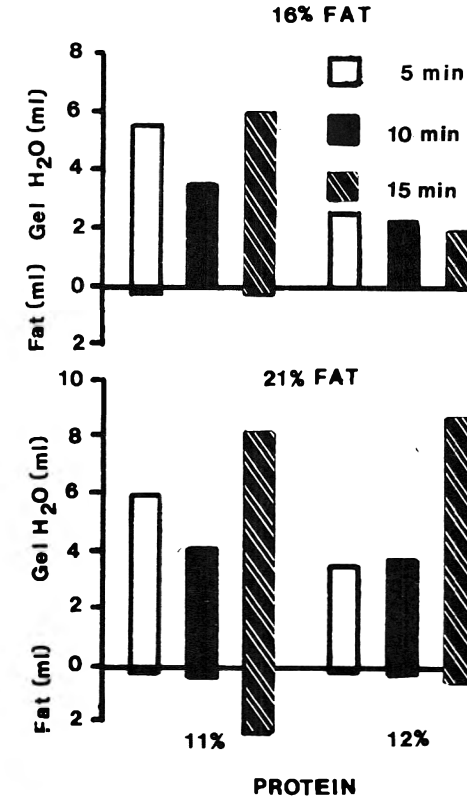

Fig. 5—Histogram of statistically significant f X protein X chop time interaction for gel-wa and fat released from batters made fr MDPM.

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ECHANICAL PROPERTIES OF COLLAGENOUS TISSUE . . .

From page 181

the various samples. Some variation in properties between collagenous tissues obtained from animals of similar age would be anticipated where the collagen in these tissues had differing degrees of crosslinking. This is of particular relevance in the meat context as it has been shown (Mohr and Bendall, 1969) that the intramuscular connective tissue is more heavily cross linked than the tendinous tissue from the same animal. However isolation of samples of this intramuscular tissue suitable for the investigations of the type described in this present paper would be extremely difficult.

It is often assumed that collagen in meat is converted to gelatin during cooking and thus its contribution to the mechanical strength of the cooked meat is likely to be low. Collagen fibers in situ will be, at least, partly restrained from thermal shrinkage by the interstitial myofibrillar structure. The properties of collagen fibers measured ex situ and without restraint are able to contract to $< 0.3\ L_o$ and would thus be expected to display different mechanical behavior to the collagen in intramuscular connective tissue. The relative restrained length (L_r/L_o) of the collagen fibers in situ will be determined, in part, by the physical properties of the myofibrillar structure. The stress-strain properties of the collagenous network in meat will thus not only be determined by the intrinsic properties of the collagen itself and by cooking conditions but also by the changes produced in the myofibrillar structure by cooking. Other work (Bouton et al., 1976b) has shown that myofibrillar contraction state has considerable influence on the dimensional changes which occur in meat during cooking so it would not appear surprising if myofibrillar contraction state had an effect, albeit indirect, on the connective tissue properties.

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HONG-MING SOO, EUGENIA A. DAVIS and EUGENE H. SANDER
Dept. of Food Science and Nutrition
University of Minnesota, St. Paul, MN 55108

APPLICATION OF SCANNING ELECTRON MICROSCROPY TO TEXTURE DEFINITION IN NATURAL AND FABRICATED SHRIMP

ABSTRACT

Scanning electron microscopy (SEM) was used to investigate the microstructure of two geographic sources of shrimp, binding matrix agents and their mixtures. Observed SEM microstructural differences were related to the Instron textural characteristics of cooked shrimp patties; microstructure differences were related to more intact and longer myofibrils in the natural shrimp. Slight differences among microstructures were observed in singular matrix agent cooked gels. Observed microstructures of shrimp-matrix agent mixtures showed differences which related to measured textural parameters and matrix agent choice.

INTRODUCTION

FABRICATION OF SHRIMP PATTIES involves combining comminuted shrimp with a suitable binding-matrix agent at a level required to produce and retain the desired shape using a mechanical, low temperature, low pressure extruder (Sander et al., 1976). The shrimp and the binding-matrix agent are the two major components in fabricated shrimp. Therefore, geographic source of shrimp, choice of binding-matrix agent, and levels of each used in shrimp-binding matrix compositions are important parameters which contribute to the texture of the final product (Soo and Sander, 1977b).

In order to evaluate how these parameters contribute to texture, it is essential to define the microstructure changes which actually determine the sensory and mechanical characteristics of foods. This type of study was discussed in excellent and comprehensive reviews by Stanley and Tung (1976) on various types of foods. However, no similar information for natural and fabricated shrimp appears to be available.

The purpose of this study was to investigate the merits of two scanning electron microscopy (SEM) techniques (Davis et al., 1976) as applied to the study of natural and fabricated shrimp microstructure. Specific objectives were (1) to use one of these SEM methods in the identification of microstructural differences that result in the observed Instron textural differences in natural and fabricated shrimp, and (2) to investigate the effects of shrimp from different geographic sources and binding matrix agents of different types on the textural properties of natural and fabricated shrimp.

EXPERIMENTAL

Preparation of natural and fabricated shrimp

Shrimp from two geographic sources (from waters surrounding India and from the Gulf of Mexico) in frozen blocks of whole and broken pieces (400–600 count/kg) were obtained from seafood processors. A frozen block (2.3 kg) tempered to −1°C was used to prepare the natural and fabricated shrimp sample. The cooked natural shrimp was prepared by cooking in boiling water for 1 min. Two binding-matrix agents were used: (1) Hercules 30, a commercial product containing starch and gums, and (2) isolated soy protein (ISP), (Supro 620T, Ralston Purina Co.).

The cooked fabricated shrimp patties (which contain 95% shrimp and 5% binding-matrix agent) were prepared by the method of Soo and Sander (1977a). When the contribution of geographic source of shrimp to the texture and microstructure was investigated, cooked shrimp patties were prepared using comminuted shrimp without matrix agent.

Instron measurement of TPA parameters

The objective (Instron) textural characteristics (hardness, cohesiveness and springiness values) of cooked shrimp patties were obtained by using previously described method (Soo and Sander, 1977a).

SEM preparation methods

Two methods of sample preparation as described in detail by Davis et al. (1976) were used in this investigation. One set of samples of shrimp and of shrimp with binding matrix samples was prepared using mechanical excision. The pieces were no larger than 3 mm on any side. Another similar set was prepared by liquid N_2 cryofracture (Cryofracture technique) which resulted in pieces no bigger than 3 mm on any one side. Both sets of samples were subsequently fixed in glutaraldehyde and osmium tetroxide, and were critical-point dried after successive dehydration with acetone. All samples were coated with carbon and platinum-palladium prior to viewing in a JEOL JSM-36 (JEOL Analytical Instruments, Inc., Cranford, NJ) operated at 15 kv, at various magnifications.

RESULTS & DISCUSSION

Comparison of the two SEM speciment preparation methods

The mechanical excision and cryofracture methods for SEM sample preparation were evaluated on raw and cooked natural shrimp and fabricated shrimp patties. Figure 1 is a series of micrographs comparing these methods. The cryofractured samples (Fig. 1A, 1C, 1E) had a more intact, less damaged appearance then those that were mechanically excised (Figures 1B, 1D, and 1F). The cryofracture method cleaved cleanly and smoothly through muscle fibers, while those that were mechanically excised appeared jagged, and fibers and myofibrils were randomly pulled apart and damaged. It is believed that in the cryofracture method the muscle was fixed frozen in place without damage prior to chemical fixation, so that longitudinal fiber breaks occurred along lines of least resistance (Schaller and Powrie, 1971). Thus there is exposure of more intact fiber structure. This was similar to the results found by Davis et al. (1976) on the cellular integrity of carrot xylem and phloem tissue. Ice crystal damage is believed to be minimal due to the rapid rate of freezing in liquid nitrogen and moderate thaw rate in the chemical fixatives. This was studied by Mohr and Stein for tomatoes (1969). Also, Baker (1962) reported that thawing of frozen mouse kidney tissue in chemical fixatives produced microstructures comparable to that of normally fixed tissue. It was, therefore, concluded that the cryofracture method was best for our shrimp samples, and all subsequent SEM micrograph discussion will related to samples prepared by this method, unless otherwise indicated.

Contribution of geographic source of shrimp to texture and microstructure of natural and fabricated shrimp

The contribution of geographic source of shrimp to texture of fabricated shrimp was discussed earlier by Soo and Sander (1977b). It was concluded that frozen blocks of shrimp from different geographical regions of the world affect the final Instron texture of cooked shrimp patties and sensory preferences of fabricated shrimp (Soo and Sander, 1977a). The microstructure, originating from chemical composition and

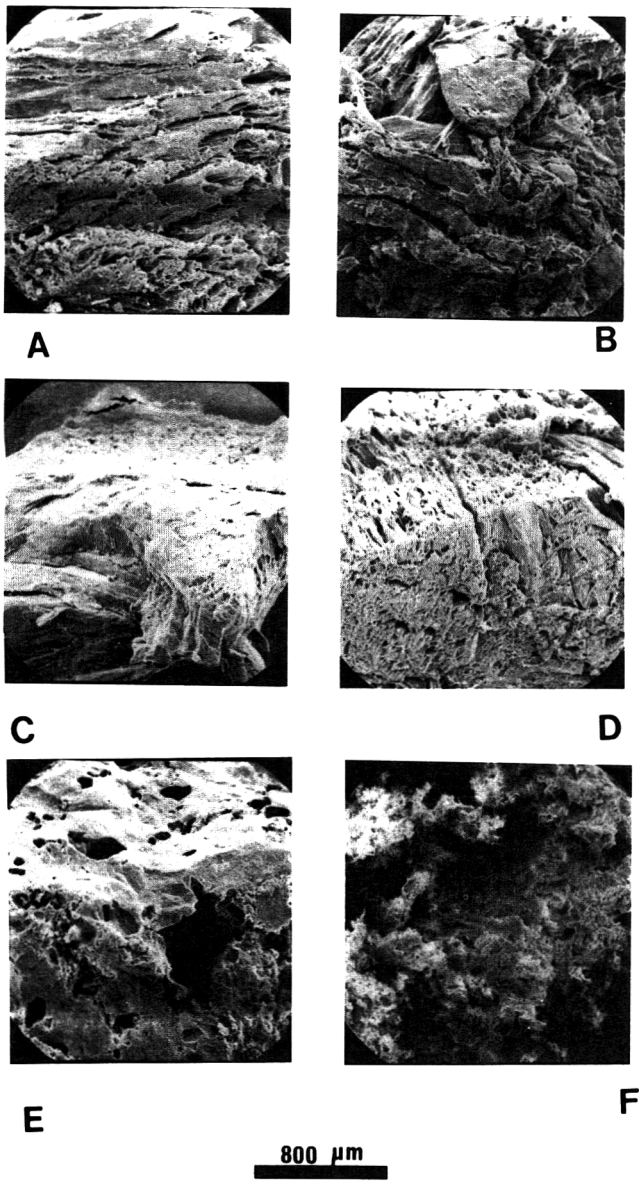

Fig. 1—Observed SEM microstructures of raw and cooked natural and fabricated shrimp prepared by cryofracture and mechanical excision methods: (A) cryofracture preparation method for raw natural shrimp; (B) mechanical excision for raw natural shrimp; (C) cryofracture for cooked natural shrimp; (D) mechanical excision for cooked natural shrimp; (E) cryofracture for cooked fabricated shrimp; and (F) mechanical excision for cooked fabricated shrimp.

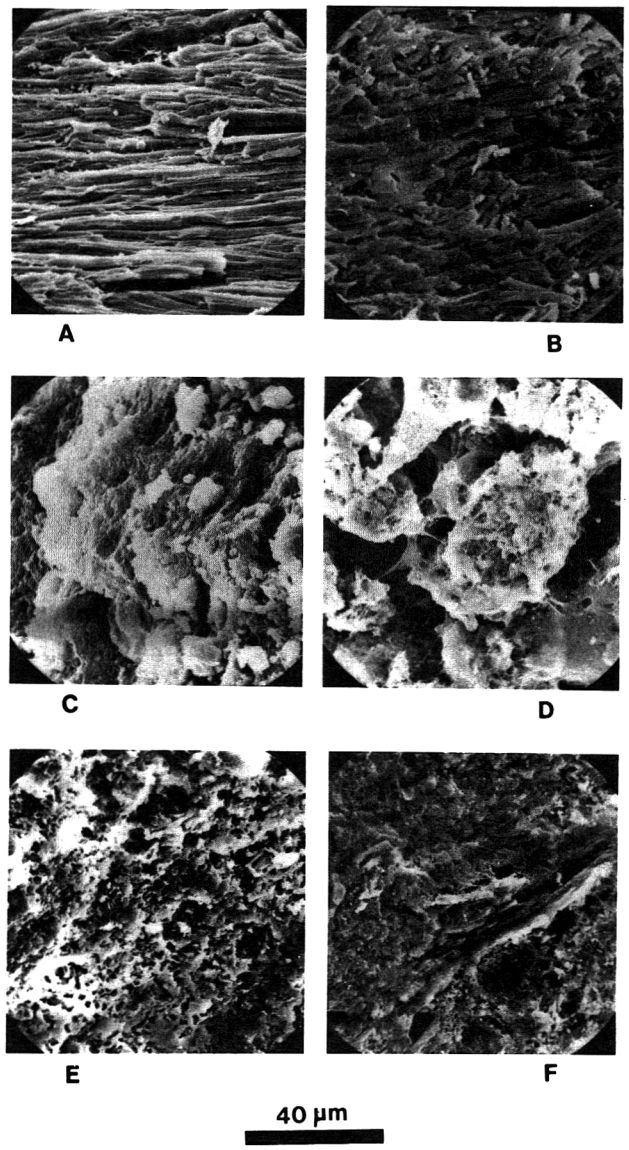

Fig. 2—Microstructures of raw natural shrimp from water around India and Gulf of Mexico, singular binding matrix agent cooked gels, and shrimp-binding matrix agent mixtures: (A) raw Indian shrimp; (B) raw Gulf shrimp; (C) ISP; (D) Hercules 30; (E) Indian shrimp-ISP mixture; and (D) Indian shrimp-Hercules 30 mixture.

physical forces, actually determines the texture characteristics of foods (Stanley and Tung, 1976). It was of interest to investigate the microstructure of natural shrimp and to see if there was a correlation between the observed texture changes in cooked patties which result from using different geographic source of shrimp and the corresponding alternation in microstructure.

Two geographic sources (from waters surrounding India and Gulf of Mexico) of frozen block shrimp with significant (at 1% level) textural differences were investigated. Texture Profile Analysis (TPA) values of cooked shrimp patties for Instron hardness, springiness, and cohesiveness (Soo and Sander, 1977a) prepared from these two geographic sources of shrimp are presented in Table 1. The shrimp patties, prepared using Indian shrimp, showed higher Instron TPA values than did patties prepared using Gulf shrimp. For example, the Instron hardness values of shrimp patties prepared from Indian and Gulf shrimp were 51.69 and 17.97 kg force, respectively.

The SEM micrographs of raw Indian and Gulf shrimp are presented in Figure 2. The Indian shrimp (Fig. 2A) appeared more intact with longer and less broken myofibrils than those of the Gulf shrimp (Fig. 2B). Similar microstructure differences were observed between the cooked Indian and Gulf shrimp (micrographs not shown). Stanley (1974) showed SEM micrographs of beef muscle myofibrils taken at various stages of aging, and demonstrated a correlation among certain textural properties of beef muscle and myofibrils. He reported that in the initial specimens (at 0 day postmortem) myofibrils are unbroken, and result in higher shear values and sensory texture response (Stanley and Tung, 1976). The higher TPA

values obtained for cooked Indian shrimp patties might thus be explained on the basis of observed microstructural differences of the natural shrimp. The use of raw shrimp which have more intact, longer and unbroken myofibrils could result in higher TPA values and better sensory preference for fabricated shrimp.

Effect of shrimp-binding matrix composition on texture and SEM microstructure of cooked patties

The effect of binding matrix agent on texture of fabricated shrimp and on extruder performance can be explained on the basis of matrix agent compositional differences (Soo and Sander, 1977b). The use of a protein source such as ISP for fabricated shrimp generated higher Instron TPA and sensory texture values, while the use of starch-gum base (Hercules 30) produced lower values (Soo and Sander, 1977a). The data in Table 1 show Instron values for hardness, cohesiveness and springiness of these two cooked shrimp-binding matrix mixture patties: ISP generated significantly (at 1% level) higher values than did Hercules 30.

SEM micrographs of individual matrix agent cooked gels and cooked shrimp-matrix agent mixtures are presented in Figure 2. Since the shrimp and matrix agent form a complex physical mixture, the microstructure of matrix agent gels alone was studied in an attempt to better evaluate the effects of component variations. Individual matrix agent gel (15% solution) was prepared in test tubes (Pyrex, 15 X 150 mm). The test tube plus content were placed in boiling water for 5 min. The cooked starch-gum base matrix agent (Hercules 30) appeared to have a gummy structure (Fig. 2D), whereas the ISP gel appeared to have a porous or spongy structure (Fig. 2C). Heat denaturation of protein (ISP) solution which results when intermolecular crosslinks are formed by sulfhydryl-disulfide interchange and/or intramolecular disulfide bonds (Wolf and Cowan, 1975) probably stabilizes the protein network and forms the SEM spongy appearance (Fig. 2C). Pregelatinized corn starch and corn flour are the key components of Hercules 30; the heat gelatinization of starch is characterized by granular swelling with water and results in a gummy appearance in the micrographs.

The microstructure of cooked shrimp-ISP mixture (Fig. 2E) appeared less compact and more spongy than the microstructure of shrimp-Hercules 30 mixture (Fig. 2F). These observed microstructural differences seemed to correlate well with the higher Instron textural values in all the parameters studied (Table 1). Spongy protein fibers which might assist in maintaining conformations of mixture structure seem responsible for the textural and SEM microstructural differences. However, the gummy starch molecules of Hercules 30, which increased gel strength and water-holding capacity in cooked shrimp patties (Soo and Sander 1977b) by heat gelatinization of starch, probably resulted in the more compacted microstructure and lower Instron TPA values.

We conclude that samples prepared by the cryofracture technique for SEM are less damaged than those prepared by the mechanical excision method. Also the use of natural shrimp with more intact, longer and less broken fibers and myofibrils will give significantly different textural analysis from shrimp that are more fragile and which therefore result in shorter and broken fibers and myofibrils. Observed SEM microstructural differences using different geographic sources of shrimp were consistent with the Instron textural characteristic differences of cooked shrimp patties. The effect of binding matrix agent composition on texture and SEM microstructure seemed to be related to (1) the apparently spongy SEM microstructure of cooked ISP gel and shrimp-ISP mixture, and (2) the apparently gummy, compacted and softer microstructure of Hercules 30 cooked gel and shrimp-Hercules 30 mixture.

Table 1—Contribution of geographic source of raw shrimp, and binding-matrix agent to the texture of cooked shrimp patties measured by Instron texture profile analysis (TPA)

Treatment		Mean Instron TPA[c] Springiness (mm)	Cohesiveness	Hardness (kg)
Geographic source[a]	Indian	9.31	0.473	51.69
	Gulf of Mexico	6.49	0.365	17.97
Binding matrix agent[b]	ISP	8.40	0.284	26.44
	Hercules-30	2.20	0.076	9.14

[a] Shrimp patties containing 100% of comminuted shrimp
[b] Shrimp patties containing 95% of comminuted Indian shrimp and 5% binding-matrix agent
[c] Mean represents the average five observations. Means within each textural parameter and contribution are significantly different at 1% level.

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AGNES F. ZAMORA and MARION L. FIELDS
Dept. of Food Science & Nutrition
University of Missouri, Columbia, MO 65201

PRODUCTION OF CORN AND LEGUME MALTS FOR USE IN HOME FERMENTATION

ABSTRACT

Aqueous extracts of amylases from germinated corn, cowpeas, chickpeas and Great Northern beans were made to determine the optimum pH, temperature and time of incubation for their activity. Starch hydrolysis, measured by starch-iodine reaction, and amount of glucose liberated from soluble starch and from the seed starches were measured. Alpha-amylase from corn had a lower pH and a higher temperature optima than cowpeas, chickpeas and Great Northern beans. The amylase (total dextrinizing) activities measured at the optimum pH and temperature were 18.4, 14.3 and 2.66 for corn, cowpeas and Great Northern beans respectively. Chickpeas had a very low alpha-amylase activity. Corn and cowpea malts are more practical to use in home fermentation because they converted the starch to dextrin much faster. More reducing sugars were produced from the bean starches by corn and cowpea malts than by chickpea and Great Northern bean malts.

INTRODUCTION

TODAY, a deficiency of animal proteins prevails in many parts of the world and this will become more serious as the growth in world population rapidly increases. Therefore, more plant proteins should be used to meet the required amount of protein as already being done in many developing countries.

Because of availability, low cost and acceptability, corn and legumes are an important source of protein and calories in countries where animal proteins are limited. However, it is well recognized that protein from these sources is of lower quality than those from animals.

Because of the importance of corn and legumes as food sources and with increased emphasis on the nutritive value of foods, better use of legumes for home preparations should be made for the improvement of the diet and nutrition of the people (Patwardhan, 1962).

Current research is being done to improve the amino acid balance in corn and legumes (Hackler and Dickson, 1973). One method which is worthwhile nutritionally and economically is mixing different plant protein such that the resulting combination will give a good balance and recommended levels of amino acid (Bandemer and Evans, 1963).

Another possible method of improving the nutritive quality of corn and legumes is by yeast fermentation of the flour and meals of these materials. The starch from the corn and legumes should be hydrolyzed to sugars which the yeast can metabolize. This yeast fermentation is a means by which the protein quality of corn and some of the legumes can be improved.

Industrially, conversion of starch to fermentable sugars is frequently done by commercially available amylases. But since the emphasis on this research was for home preparation, the corn (*Zea mays*), cowpeas (*Vigna sinensis*), chickpeas (*Cicer arietinum*) and Great Northern beans (*Phaseolus vulgaris*) were germinated for possible sources of alpha-amylase which can be used in home fermentation. Sprouting can be easily carried out at home with minimum utensils and materials. Sprouting does not need sunlight or soil so it can be done indoors in any season.

This work was instituted to determine the optimum pH, temperature and time of incubation for degradation of starch with alpha-amylase produced in germinated corn, cowpeas, chickpeas and Great Northern beans and measure the alpha-amylase (total dextrinizing activity).

EXPERIMENTAL

Production of malts

Corn, cowpeas, chickpeas and Great Northern beans which were purchased from the University of Missouri Central Food Stores were used for this study. They were washed several times in running water and soaked overnight in tap water. The water was drained and the seeds were put in trays covered with wet paper towels. They were incubated at 30°C for 4 days. The seeds were moistened everyday and pans of water were placed in the incubator to prevent the seeds from dehydrating. After 4 days, when the sprouts were about 2 in. long, the seeds were dried at 50°C and ground through 1 mm mesh screens in a Wiley Mill.

Extraction of enzyme from germinated corn and beans

Aqueous enzyme extracts were prepared from ground malt by the procedure of Tauber (1949) with the following modifications: A 10% aqueous enzyme solution was prepared by constant stirring of the water-ground malt mixture for 30 min at room temperature. The solution was centrifuged at 2.5G for 15 min in a refrigerated centrifuge (model B 20A, International Equipment Co., MA). The supernatant was taken as the enzyme extract.

Preparation of buffered soluble starch solutions

A 2% soluble starch (Fisher Certified Soluble Starch for Iodometry) solution was made by boiling the starch in each of the 9 buffers (pH 4 to 12) prepared. For pH 4 to pH 8, the required amounts of 0.1M citric acid and 0.2M Na_2HPO_4 were mixed. To prepare pH 9 buffer, 0.25M borax and 0.1M HCl were used. Buffers of pH 10 and 11 were prepared by mixing 0.05M $NaHCO_3$ and 0.1M NaOH. A 0.2M KCl and 0.2M NaOH were used in preparing buffer of pH 12.

Determination of pH and temperature optima

The pH, temperature and time of incubation at which the activity of the enzymes was optimal were determined. Five ml of aqueous enzyme extract from each type of malted seeds were added to 10 ml of 2% buffered soluble starch solution at pH 5–12. For corn alpha-amylase, pH 4 was also tested. Incubation temperatures of 40°, 50° and 60°C for 30, 60, 90 and 120 min were tested.

Determination of reducing sugars (expressed as glucose)

The concentration of reducing sugars (calculated as glucose) was determined as described by Dubowski (1962).

Determination of starch-iodine reaction

Quantitation of the starch-iodine reaction was performed as described by Schwimmer (1947), with the following modifications: 5 ml of a solution of iodine in potassium iodide (0.005M iodine and 0.23M potassium iodide) was added to the starch enzyme solution after incubating at different temperatures and times. One ml of this was pipetted into 10 ml distilled water. The transmittance was read at 660 nm in a Bausch and Lomb Spectronic 20 using distilled water to set the instrument to 100%.

Production of starches from beans and corn

The ungerminated seeds were ground through 1 mm mesh screen in a Wiley Mill. Water (v/w, 10×) was added to the ground seeds and stirred constantly for 30 min. The mixture was strained through cheesecloth to remove large pieces, and the filtrate was centrifuged at 2.5G for 15 min in a refrigerated centrifuge (Model B 20A, International Equipment Co., MA). The precipitate was dried at 50°C.

Hydrolysis of the seed starches

Two percent buffered starch suspensions from corn, cowpeas, chick-

peas and Great Northern beans were prepared by boiling the seed starch-buffer mixture. Enzyme hydrolysis of the seed starches was tested at the optimum pH, temperature and time of incubation determined from the pure starch-buffer systems.

The starches from the four types of seeds were hydrolyzed by autoclaving at 121°C for 60 min in 0.5% H_2SO_4 to determine the maximum amount of reducing sugars which could be formed. This was used as a control treatment. The starch-water mixtures were boiled to gelatinize the starch so that the starch was made available for hydrolysis with the alpha-amylases. The amount of reducing sugars liberated from the starches in all treatments was determined. The reducing sugars present in the malt extract were measured to correct for the amount determined from the hydrolysis of starch by the enzyme. As another control, the amount of reducing sugars in the nontreated starch was measured.

The percent effectiveness of the malt was calculated as follows: the amount of reducing sugar in the starches was determined. This amount was subtracted from the acid- and enzyme-treated starch. Since it was assumed that the acid-treated starch would yield the maximum amount of reducing sugar, the enzyme-treated starch reducing sugar values were divided by the acid-treated starch reducing sugar values, multiplied by 100 to yield the effectiveness of the malt at its optimum conditions.

Determination of alpha-amylase (total dextrinizing activity) of corn and legume malts

The reagents and procedure followed were that of Tauber (1949), with the following modifications: the 5% aqueous solution of malt was filtered and 5 ml of the filtrate was mixed with 10 ml of 2% soluble starch solution in a test tube. The starch was buffered at pH 5 for corn, pH 7 for chickpeas and pH 8 for cowpeas and Great Northern beans. These were the pH levels found optimum for the corn and legumes. The tubes were incubated at 40°C for chickpeas, cowpeas and Great Northern beans and 50°C for corn. At different intervals of time, 1 ml of the mixture was added to 5 ml of dilute iodine solution. This procedure was repeated until the color of the tube matched the standard.

Calculation. In this experiment, 1 ml of 2% starch was hydrolyzed by 0.5 ml of the 5% malt extract. If hydrolysis of starch to dextrin occurred in c min, 1 ml of malt extract will convert in 1 hr $60/c \times 1/0.5$ ml of starch solution.

RESULTS & DISCUSSION

Effect of pH, temperature and time of incubation on amylase activities

Alpha-amylase from corn showed maximum activity (both by color of iodine-starch and amount of reducing sugar) at pH 5 when incubated at 50°C after 90 min incubation (Fig. 1). This closely agrees with the results of Wahl (1971) who concluded that the maximum activity was at pH 5.2. Greenwood and Milne (1968) also said that the optimum activity of alpha-amylases from higher plants is between 5 and 6. It was observed in this study that the optimum pH for the corn amylase activity was near the pH of the ungerminated corn which was 5.2 and corn malts which was 5.5. There was a slight decrease in activity at pH 4. At 40° and 50°C, the lowest activity also occurred at pH 7–11. The activity at 60°C appeared to be lowest at all pH levels.

The effect of other incubation time was not shown in the graph because it did not seem to affect the enzyme activity. The readings were almost the same at different incubation times at the particular pH and temperature.

Figure 1 also shows the amount of reducing sugar produced at different pH's and temperatures of incubation. The amount of reducing sugar was highest at pH 5. Production of reducing sugar reflected the hydrolyzing power of the amylase at pH values and temperatures tested. The starch-iodine and reducing sugar tests complemented each other.

The activity of cowpea alpha-amylase gradually increased from pH 5 to pH 8 with optimum at pH 8–9 as measured by starch-iodine reaction (Fig. 2). This optimum is above the normal pH of the ungerminated cowpeas and malt which was 6.2. As the temperature was increased from 40° to 60°C, more inactivation of amylase probably occurred. This was reflected more at pH values between 5–9 than at higher pH levels with the starch-iodine reaction. The amount of reducing sugar produced at 40°C, however, was not the maximum. More reducing sugar was detected at 50°C at pH 8 than at other pH values.

The optimum pH for the activity of chickpea alpha-amylase was at pH 7 at 40°C, although there was only a slight decrease in activity at pH 8–12 as measured by the starch-iodine reaction. In contrast, there was a rapid increase in activity from pH 5 to 7 (Fig. 3).

Much more reducing sugar was produced at 40°C regardless of pH than at 50° or 60°C. At 40°C more reducing sugar was produced at pH 7 than at other pH units; the least reducing sugar was produced at pH 5. Quantities of reducing sugar liberated were nearly constant at 50° and 60°C (Fig. 3).

The behavior of alpha-amylase from Great Northern beans was similar to that of chickpeas and cowpeas (being least active at pH 5 and more active at pH above 7). The alpha-amylase from these beans was the only one that was affected by time of incubation. At the temperatures tested, 120 min incubation was found suitable for this enzyme. At 120 min incubation and 40°C, regardless of pH, there was much more

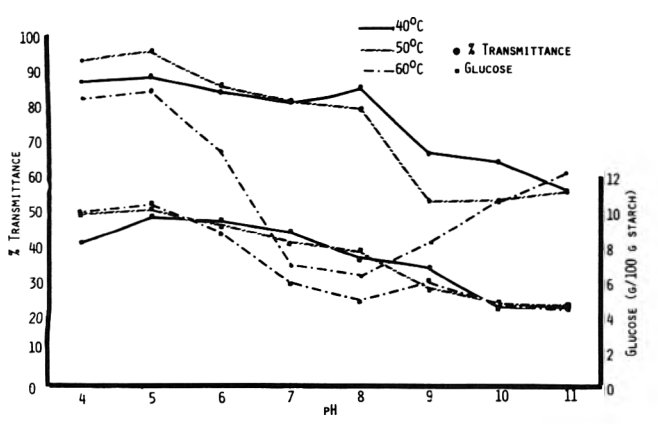

Fig. 1—Starch hydrolysis (blue value-reduction of starch-iodine reaction measured as percent transmittance) and amount of reducing sugars (expressed as glucose) produced by corn alpha-amylase after 90 min incubation at different pH and temperature combinations.

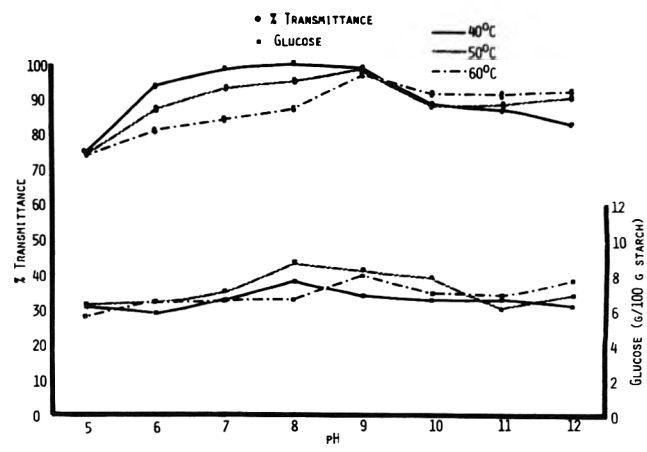

Fig. 2—Starch hydrolysis (blue value-reduction of starch-iodine reaction measured as percent transmittance) and amount of reducing sugars (expressed as glucose) produced by cowpea alpha-amylase after 90 min incubation at different pH and temperature combinations.

starch hydrolysis (by the starch-iodine reaction) and reducing sugar produced than at 50° and 60°C (Fig. 4). Whitaker (1972) stated that protein denaturation is increased 6 to 5000 times for each 10°C change in temperature. This could be occurring at 50° and 60°C.

As in all enzymes, the amylases from corn, cowpeas, chickpeas and Great Northern beans exhibit pH and temperature optima. The decrease in activity below and above the pH optimum is due either to reversible or irreversible effects on protein structure (Allen and Spradlin, 1974). Except for corn, the other alpha-amylases showed more activity above pH 7. This did not follow the conclusion of several authors who stated that the pH optimum for most alpha-amylases is between 5 and 6. Thoma and Spradlin (1971), however, said that the alpha-amylases from higher plants are generally stable from pH 5.5–8.0. Below pH 5, irreversible inactivation occurs. This type of inactivation of alpha-amylases of cowpeas, chickpeas and Great Northern beans might have occurred at pH 5 or 6 since the activity was very low at those pH values.

The anions present in the buffer might have had an effect in shifting the pH optimum to about pH 7–9 as compared to the optimum pH 5–6 of the alpha-amylases found by other workers. Chloride ions are very effective in activating alpha-amylases (Whitaker, 1972; Greenwood and Milne, 1968). Whitaker (1972) mentioned that when monovalent anions were present, the optimum pH of near 6 of alpha-amylase was shifted to near pH 7. Buffer for adjustments to pH 9 contained chloride ions which might have affected the activity of the alpha-amylases. The same reason might be suggested for the observed activity at pH 12, since the buffer contained chloride ions.

Reducing sugars from corn and bean starches

As expected, the amount of reducing sugar in the extracted starch without the enzyme or acid was the lowest for all the seeds. Also, more reducing sugars were formed when the starches were autoclaved with 0.5% H_2SO_4 (Table 1).

Using the percent effectiveness of the enzyme on the starch of the seed from which the malt was made, it was clear that the cowpea malt was the most effective followed by the corn malt. The effectiveness of the amylase from the Great Northern bean and chickpea malts was exceedingly low.

Amylase values of corn and legume amylases

It took 6.5, 9 and 45 min for corn, cowpeas and Great Northern bean amylases respectively, to convert 10 ml of 2% soluble starch to dextrin. The corresponding amylase values for those times were 18.4 for corn, 13.3 for cowpeas and 2.66 for Great Northern beans. It took several hours for the chickpea amylase to act on the soluble starch. The end point was reached after incubating the starch-enzyme solution overnight. The amylase value, therefore, was very low and no units were calculated.

The amylase activities determined for corn and cowpeas were higher than for chickpeas and Great Northern beans. These activities were reflected in the amount of reducing sugars formed when the seed starches were hydrolyzed with their respective enzymes (Table 1). More reducing sugars were produced from corn and cowpea starches than from the starches of chickpeas and Great Northern beans.

The data show that the corn and bean malts can hydrolyze the starch. It would also be feasible to mix the raw grains and malts although studies should be done to determine the proportion of malts and raw grains to be mixed to achieve a good degree of conversion of the seed starches to sugars.

CONCLUSIONS

CORN, COWPEAS, CHICKPEAS and Great Northern bean amylases exhibited pH and temperature optima. The optimum conditions observed for corn amylase were pH 5 at 50°C.

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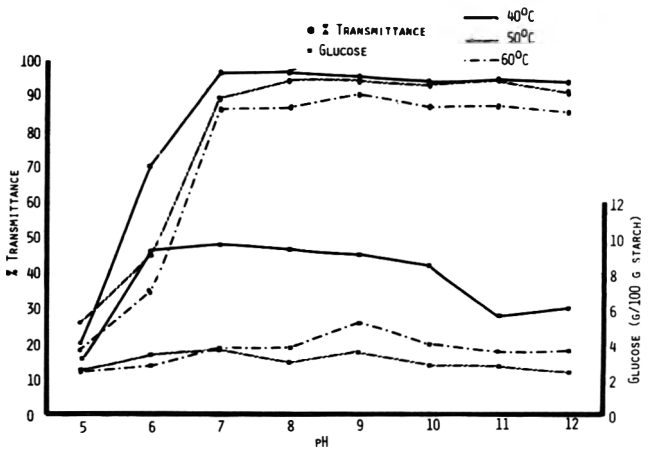

Fig. 3—Starch hydrolysis (blue value-reduction of starch-iodine reaction measured as percent transmittance) and amount of reducing sugars (expressed as glucose) produced by chickpea alpha-amylase after 90 min incubation at different pH and temperature combinations.

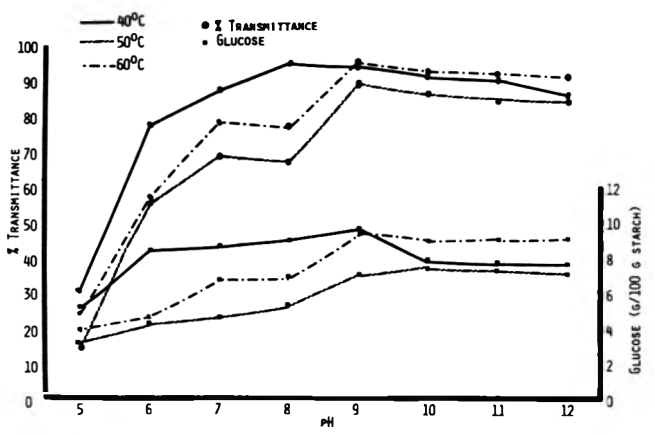

Fig. 4—Starch hydrolysis (blue value-reduction of starch-iodine reaction measured as percent transmittance) and amount of reducing sugars (expressed as glucose) produced by Great Northern bean alpha-amylase after 120 min incubation at different pH and temperature combinations.

Table 1—Reducing sugars produced from corn and bean starches[a]

Substrate and pH	w/0.5% H_2SO_4	w/o enzyme (g/100g starch)	w/enzyme	% effective[b]
Corn starch pH 5	12.6	1.8	3.6	16.6
Cowpea starch pH 8	12.0	1.2	3.2	18.5
Chickpea starch pH 7	7.8	0.7	1.0	4.2
Great Northern bean starch pH 8	11.0	0.7	1.51	7.8

[a] Incubation times and temperatures were: corn, 90 min, 50°C; cowpeas and chickpeas, 90 min, 40°C; Great Northern beans, 120 min, 40°C.

[b] $\frac{\text{Reducing sugar produced by malt — w/o enzyme}}{\text{Reducing sugar produced by acid hydrolysis — w/o enzyme}} \times 100$

VALDEMIRO C. SGARBIERI, RUTH S. GARRUTI, MARIA A. C. MORAES and LEOPOLD HARTMAN
Departamento de Planejamento Alimentar e Nutrição–UNICAMP
Caixa Postal 1170, 13100 Campinas, SP, Brasil

NUTRITIONAL AND SENSORY EVALUATION OF MIXTURES OF SOYBEAN (Glycine max L.) AND COMMON BEAN (Phaseolus vulgaris L.), FOR DIRECT USE AS HUMAN FOOD

ABSTRACT

The objective of this investigation was to study some acceptability parameters and nutritional improvement of common beans by mixing them with different proportions of soybeans. Difference tests showed that mixtures of 20% soybeans and 80% common beans did not differ from 100% common beans. On the other hand, preference tests (Hedonic scale) indicated practically the same mean value for mixtures with 20, 30, 40 and 50% soybeans. Regarding the improvement of the protein quality (PER), 50% soybeans in the mixture increased the PER value by 65%, while 80% soybeans doubled the PER as compared with the common beans alone.

INTRODUCTION

IN LATIN AMERICAN COUNTRIES as in many other developing regions the protein deficiency in the human diet ranks next to the deficiency in the dietary calories. Legume seeds and in particular soybeans constitute an excellent source of protein and energy to improve the cereals and roots based diets of low income countries (Bressani et al., 1973; Bressani, 1973, 1975; Souza and Dutra de Oliveira, 1959; Dutra de Oliveira and Zappelini de Menezes Salata, 1971). The spectacular increse of soybean production in Brazil in the last decade, followed by the increase in the cultivation of this legume in other Latin American countries, has given rise to attempts to include soybeans in the diet of populations with a limited purchasing power. The present low human consumption of soybeans in Latin America and in the Western World as a whole is due to several reasons, according to Bourne (1972). Apart from dietary customs, the low acceptability of soybeans is the result of their poor texture when cooked, their "greasy" feel in the mouth and occasional development of strong "beany" flavors which are undesirable. Defatting of soybeans with hexane after dehulling and splitting in halves reduced their fat content, after which their acceptance when cooked was greatly increased (Bourne, 1972). However, an extraction time of 2–3 wk is needed to reduce the fat content of half split beans to about one-tenth of the original value, which turns a large scale production economically unviable; even the reduction of the fat content to one-half requires several days.

Nevertheless, the idea of substituting soybeans for common beans appears sound, especially in countries in which the consumption of the latter is considerable. This applies in the first place to Brazil, where common beans together with rice form the staple diet of the majority of the population.

Apart from the much greater protein content of soybeans – about 40% against 18–25% of common beans – there is also the price factor to be considered. Soybeans are at least 2–3 times cheaper and their cultivation and harvesting is much easier.

However, the introduction of new alimentary habits is fraught with difficulties which are inversely proportional to the educational level of a population. As already stated, soybeans do not constitute a common food item in the Western world and those social classes who would nutritionally and financially benefit most from their consumption show, as a rule, the greatest resistance against their adoption.

These considerations prompted an attempt to make soybeans acceptable in the form of a mixture with common beans. The present work shows the results of a sensory evaluation of such mixtures containing up to 80% of soybeans, and presents their protein efficiency ratio (PER) obtained from animal experiments. In addition, PER values for mixtures of soybeans, common beans and rice were determined in view of the dietary habits prevailing in Brazil.

MATERIALS & METHODS

SOYBEANS of the variety "Santa Rosa" and common beans variety "Rosinha" of a pink color were obtained from the Agronomical Institute in Campinas, State of São Paulo, Brazil. The rice used was a regular commercial white rice of the variety "Goiano."

For the sensory evaluation soybeans were mixed with common beans in various proportions up to 80% and after soaking in water for 6 hr they were cooked in autoclave (1.5 kg/cm², 121°C) for 15 min. The methods used in the sensory analyses were the Directional Paired Test (Guilford, 1954; Dawson, et al. 1963) for detecting differences and the Hedonic Scale (Peryam and Pilgrim, 1957; Ellis, 1968) for preference. The experimental designs were paired and randomized blocks with six replicates. The laboratory panel was composed of 10 trained members, five men and five women.

Chemical analyses of crude protein, petroleum ether extractable material, fiber, ash and moisture were performed in both soybeans and common beans by procedures described in AOAC (1970).

The amino acid composition of the protein was determined by the method of Spackman et al. (1958) using the Beckman 120C Amino Acid Analyzer and the procedure described by the manufacturer.

Protein Efficiency Ratios (PER) were determined on groups of six weaning rats of the Wistar strain using essentially the method of Osborne and Mendel (1917). The animals weighing an average of 45g were caged individually with diet containing approximately 10% protein and allowed to diet and water "ad libitum". Diet consumption and body weight gain were recorded twice a week during 4 wk. For all the biological assays a group of six rats was maintained on a 10% casein control diet.

RESULTS & DISCUSSION

A SERIES of sensory evaluation tests has been carried out with the purpose of establishing to what extent soybeans could be added to common beans without affecting the acceptability of the product.

Table 1 contains the statistical analyses of sensory evaluation tests for differences among samples using the Directional Paired Test. The results show clearly that a mixture with 20% soybeans could not be distinguished from the common beans. On the other hand, 20% soybeans did not differ from 40% and 40% was not different from 60% soybeans in the mixture. However, when the difference in soybean concentration was greater than 20% of the sample taken as comparison, the panelists were able to find significant differences among samples.

When preference tests (Hedonic scale, 0–9 points) were carried out, the results were quite different and no significant differences were found between the mixture with 20% soybeans, used as a standard and mixtures with 30, 40 and 50% soybeans. The analysis of variance for the preference tests appears in Table 2. On the basis of the results obtained it can

Table 1—Difference in flavor and preference for mixtures of soybeans and common beans (Directional Paired Test)[a]

Comparison of mixtures	Difference		Preference[b]					
	TJ	CJ	1	2	3	4	5	Total
1 X 2	26	16ns	11ns	5				16
1 X 3	25	22***	17**		4			21
1 X 4	24	23***	17*			6		23
1 X 5	16	16***	13*				3	16
2 X 3	15	11ns		6ns	5			11
2 X 4	15	13**		9ns		4		13
2 X 5	15	15***		10ns			5	15
3 X 4	15	11ns			7ns	4		11
3 X 5	16	15***			13**		2	15
4 X 5	16	13*				9	4	13
Total	157	137***	58	30	29	23	14	136
%			76.3	54.5	50.0	38.3	23.7	

[a] ns = not significant; TJ = total judgements; CJ = correct judgements
[b] 1=0%; 2=20; 3=40; 4=60%; 5=80% soybeans
* p = 0.05
** p = 0.01
*** p = 0.001

Table 2—Analysis of variance for preference and mean values of mixtures of soybeans and common beans (Hedonic scale)[a]

S.V.	D.F.	S.S.	M.S.	F.
Total	23	3.36986		
Mixtures	3	0.10341	0.03447	0.22337ns
Blocks	5	0.95166	0.19033	1.23337ns
Error	15	2.31479	0.15432	

% Soybeans	Mean values[b]
20	7.22
30	7.32
40	7.14
50	7.22

[a] ns = not significant
[b] Mean of six replicates

Table 3—Proximate composition of soybeans, common beans and the mixture of soybeans plus common beans (50 + 50%)

Components	Products		
	Soybeans	Common beans	Mixture
Crude protein	36.6	19.4	27.9
Total lipid	22.7	3.5	13.2
Water	7.5	4.9	7.3
Ash	5.3	3.4	4.5
Crude fiber	5.2	4.6	5.1
Carbohydrate	27.9	68.8	47.2

Table 4—Amino acid composition of proteins from different sources (g/16gN)

Amino acid	Protein sources: Beans (A); Soybeans (B); Rice (C)						
	100A + 0B	65A + 35B	50A + 50B	20A + 80B	0A + 100B	20A + 10B + 70C	0A + 0B + 100C
Lys	7.9	8.9	8.5	8.4	8.4	5.6	3.1
His	2.2	2.5	2.8	2.8	2.4	2.1	1.7
NH_3	2.2	2.4	2.7	3.1	2.4	2.4	2.8
Arg	5.6	6.3	6.4	6.6	6.5	5.8	6.0
Asp	16.2	16.1	16.9	15.6	16.3	13.3	10.9
Thr	4.8	4.5	5.0	4.6	4.4	3.8	3.3
Ser	6.8	6.2	6.6	6.4	6.4	5.8	5.5
Glu	23.3	26.0	27.8	25.3	32.5	23.1	27.9
Pro	3.8	4.5	5.3	5.3	5.5	4.4	4.5
Gly	4.3	4.6	5.2	5.2	5.0	4.5	4.6
Ala	4.3	4.6	5.3	5.2	4.9	5.2	5.9
1/2Cys	0.6	0.9	1.2	1.3	1.5	1.3	1.4
Val	5.0	5.3	5.6	5.5	5.1	5.4	5.5
Met	0.7	0.9	1.2	1.3	1.3	1.4	1.9
Ile	4.5	4.8	5.4	5.3	5.1	4.1	3.8
Leu	9.3	9.3	9.3	10.0	9.6	9.2	9.8
Tyr	2.8	3.0	3.3	3.4	3.5	2.7	3.4
Phe	5.5	5.3	5.7	4.9	5.0	5.1	4.9

be stated that the addition of 20% soybeans to common beans did not produce significant difference from the standard (100% common beans). Taken the mixture with 20% soybeans as the new standard and using preference tests for the evaluation of mixtures with 30, 40 and 50% soybeans, the mean values for preference were essentially the same showing no significant difference in preference for the different mixtures (Table 2). One could go perhaps beyond 50% addition of soybeans but this could impair the flavor and weaken the viscosity of the broth. It seems therefore that a mixture of 50% soybeans and 50% common beans is most indicated from the practical point of view.

The proximate composition of precooked, dried and ground soybeans, common beans and the mixture of 50% soybeans and 50% common beans is shown in Table 3. The main characteristics of the mixture in comparison with the common beans are higher protein and lipid contents. Both the protein and the lipid contents were increased by a quantity of approximately 10% in the 1:1 (w/w) mixture in relation to the common beans. The elevation of the lipid content is very important because it would increase the calories of the diet of the low income group in Brazil, but it is also advantageous because of the addition to the diet of essential fatty acids, phospholipids and vitamin E.

The amino acid profiles of soybeans, common beans, rice and some mixtures of these products are shown in Table 4. As could be expected, a mixture of 50% soybeans and 50% common beans (w/w) is an excellent source of protein regarding both quantity and amino acid composition. The addition of soya to common beans increases the proportion of methionine and cystine (limiting amino acids in beans), which improves the biological value of the proteins. Table 4 also includes the amino acid composition of rice and its mixture with beans in the proportion of 2:1 (w/w) as commonly consumed in Brazil. The addition of rice increases the nutritional value of the mixture still further owing to the considerable amount of sulphur-containing amino acids in that cereal. The low lysine content of rice is compensated for by the high proportion of this amino acid in the beans and soybeans.

The results of experiments with rats held on various rations are shown in Table 5 and Figure 1. The examination of data in Table 5 shows clearly that the PER values increase with increasing proportion of soybeans up to 80% in the mixture. For the 1:1 mixture it is 60% higher than the value for 100% common beans and the PER for the mixture with 80% soybeans and 20% common beans reached a value which was the double of that found for common beans alone. It is worth mentioning that our results differ considerable from those of

Bressani (1975) for a mixture of black beans and soybeans. He showed maximum protein quality for a mixture containing 72% black beans and 28% soybeans with a protein distribution of 60% from black beans and 40% from full-fat soybeans. It is also interesting that the mixture of 20% common beans, 10% soybeans and 70% rice had a PER value of 2.14 and thus higher than 2.0 found for 100% soybeans, based on the corrected value 2.5 for casein. The elevation of the PER value of common beans by adding soybeans and rice reflects the improvement of the amino acid profiles particularly in relation to the sulphur-containing amino acids. The improvement of PER correlates well with the improved rate of growth as shown in Figure 1.

As has already been emphasized in the introductory remarks the progressive introduction of soybeans into the diet of lower income groups of Brazil and Latin America would be of great nutritional as well as economical importance. For this reason large scale consumption trials utilizing the above mixture are being carried out and their results will be reported in a separate communication.

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Table 5—Biological values (PER) of proteins from different sources

Protein sources					PER Corrected (casein 2.5)
Beans (%)	+	Soybeans (%)	+	Rice (%)	
100	+	0			1.0
65	+	35			1.3
50	+	50			1.6
20	+	80			2.0
0	+	100			2.0
20	+	10	+	70	2.1

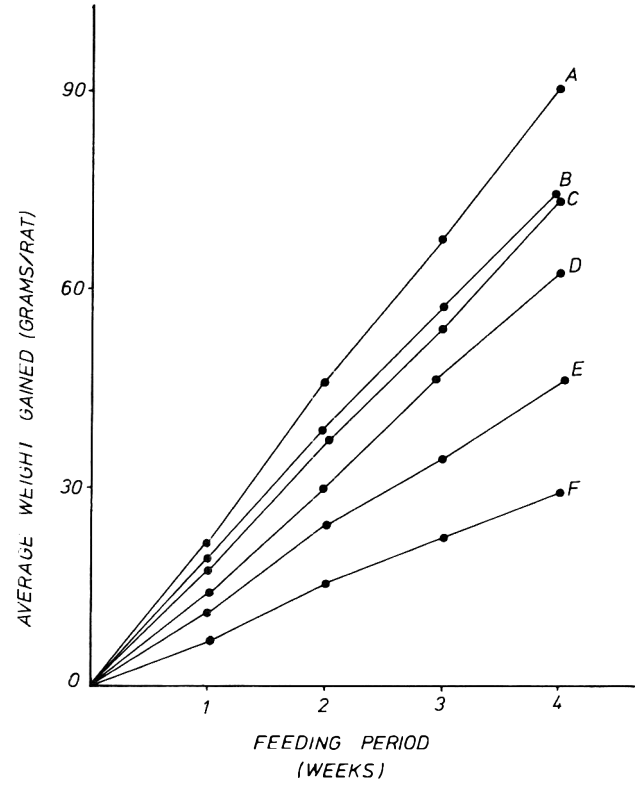

Fig. 1—Growth rate curves for rats (6 per group) on diets containing the following ingredients (%) as source of protein: A, casein; B, common beans 20, soybeans 10 and rice 70; C, soybeans 10; D, common beans 50 and soybeans 50; E, common beans 65 and soybeans 35; and F, common beans 100.

D.W. FREEMAN and W. A. SISTRUNK
Arkansas Agricultural Experiment Station
Fayetteville, AR 72701

EFFECTS OF POST-HARVEST STORAGE ON THE QUALITY OF CANNED SNAP BEANS

ABSTRACT

A study was conducted to simulate conditions that exist in loaded trailers and bulk bins during transport and storage of snap beans to study the effects of post-harvest storage on quality of canned snap beans. Post-harvest variables were 2 air flow rates, 3 storage times, 4 storage temperatures and 2 methods of blanch. Respiration rates were higher in beans that were held under high temperatures and a fast flow rate. Sensory ratings for color and general appearance were higher on beans stored under a slow flow rate. Beans stored under a fast flow rate were higher in % seed, fiber, hemicellulose and cellulose. Browning was more severe in beans stored at a fast flow rate. As storage times and temperatures were increased, beans decreased in greenness ('–a') and sensory scores. Shear press values, % seed, fiber, total sugar and cellulose increased in the canned beans as storage times and temperatures were increased. The beans that were blanched in rotary steam were lighter in color and less firm than those blanched in water.

INTRODUCTION

SNAP BEANS have become a highly mechanized crop in recent years. Since harvesting and processing schedules can be readily controlled, delays in handling and transport should be kept to a minimum. However, the raw product is frequently produced farther from the processing plant and in greater quantities than most other vegetables. Snap beans are commonly loaded into bulk trucks and transported without refrigeration. Holding after harvest for 12–48 hr is not uncommon and these long holding times are detrimental to quality of fresh and canned beans.

During post-harvest holding, plant cells continue to respire and lose nutrients. Snap beans have a high respiration rate, averaging 212 ml CO_2/kg/hr (Parker and Stuart, 1935). One visual sign of quality loss in plant tissue is the decrease in greenness or loss of chlorophyll (James, 1953). Significant losses in chlorophyll have been demonstrated in snap beans that were stored at 10° and 21°C for 10 days (Guyer and Kramer, 1950), and in asparagus that was stored at 10°C for 4 days (Kramer et al., 1949).

Browning of cut ends or damaged pods had a detrimental effect on color of snap beans during post-harvest storage (Groeschel et al., 1966). However controlled atmosphere storage in 3–10% CO_2 atmospheres retarded chlorophyll breakdown and color degradation. When O_2 levels were reduced to 2.5% with N_2 during storage, fermentation occurred (Henderson, 1977). One-day storage in 10, 20 and 30% CO_2 atmospheres greatly reduced the amount of discoloration in fresh snap beans without affecting quality of canned beans. Reitmeier (1975) demonstrated that levels of CO_2 of 10 and 20% reduced browning at 24°C and effectively prevented browning for 48 hr at 16°C. Concurrently, reducing the temperature to 16°C in the air control was as effective in inhibiting browning as 20% CO_2 at 24°C.

Weight losses in snap beans during storage for 2 days were 1.3% at 2° and 10°C and 3.5% at 21°C (Parker and Stuart, 1935). Moisture loss in cellophane packages was insignificant during storage at 21°C, while unpackaged lots lost considerable weight in 3 days (Lieberman et al, 1950).

Changes in quality of snap beans during post-harvest storage are reflected in changes in carbohydrates. Parker and Stuart (1935) found that low temperature storage of snap beans caused an increase in sucrose and reducing sugars. Lutz (1938) indicated that total sugars of snap beans decreased in 4 days at ambient temperature.

According to Guyer and Kramer (1950), there was no change in % fiber and seed in bush snap beans stored at different temperatures for periods up to 10 days. Time and temperature of storage affected the color, drained weight and sloughing in canned snap beans (Sistrunk, 1965a). There was a net loss of Calgon-soluble pectin, hemicellulose and cellulose in the pods and an increase in % seed and hardness of seed in beans that were canned after 3 and 5 days of storage (Sistrunk, 1965b). Concurrently, there were large increases in hemicellulose and cellulose in the seed. Snap beans blanched in steam were comparable in quality attributes to those blanched in water (Freeman and Sistrunk, 1973). Ten second exposure to live steam followed by 2 min at 74°C in a steam-heated chamber was an adequate blanch.

In preliminary studies, bulk loads of snap beans were probed by means of a grain sampler equipped with a thermocouple and an air hose to obtain air samples at different depths and time intervals. While temperatures were taken, a sample of air was pulled into a gas collection chamber by a hand operated pump. A syringe of air was taken from the collection chamber and these were taken immediately to the laboratory for analysis of CO_2 by gas chromatography. Temperatures within the loads ranged from 18–40°C with CO_2 content ranging from 0.5–10%.

The present study was designed to simulate conditions that were found in loaded trailers during transport and storage in order to determine the effects of storage time, storage temperature, air flow rate and method of blanching on quality attributes of canned snap beans.

EXPERIMENTAL

THE CULTIVAR CASCADE used for this study was obtained from commercial processors. The bush-type beans were grown within 100 mi of the processing plants under commercial cultural practices. All beans were mechanically harvested and hauled in bulk trailer trucks to the processing plants where random samples were obtained for processing at our laboratory.

This experiment was designed as a 4-way factorial involving 3 storage times, 4 storage temperatures, 2 air flow rates and 2 methods of blanch. The experiment was replicated 4 times during the spring of 1975.

Post-harvest handling and storage

Snap beans were graded by a commercial grader into sieve sizes. Sieve size 4 beans were dipped into a solution of 1% Botran (2,6-dichloro-4-nitroaniline), to retard mold growth, air dried to remove surface moisture, divided into 1.5-kg lots and placed *into* respiration chambers. The chambers were stored at 13, 21, 29 and 38°C (±1°C) for 0, 24 and 48 hr. Two rates of air flow, low and high, were circulated through the chambers at each temperature. The low flow rate was maintained at 900 cc/hr and the fast flow rate at 20,000 cc/hr. No external adjustments of CO_2 were made. At each sampling time the beans were removed from the chambers and weighed to obtain weight loss.

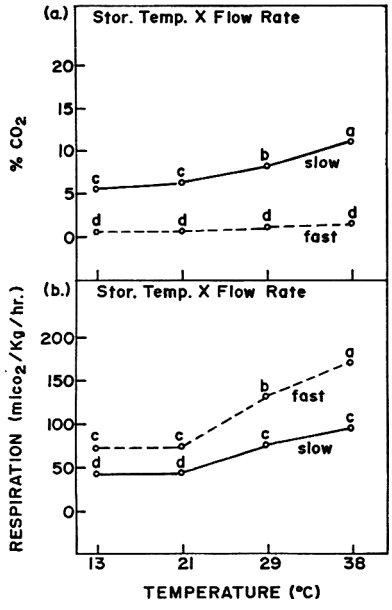

Fig. 1—Interaction of flow rate X storage temperature on CO_2 and respiration of stored snap beans (24 hr).

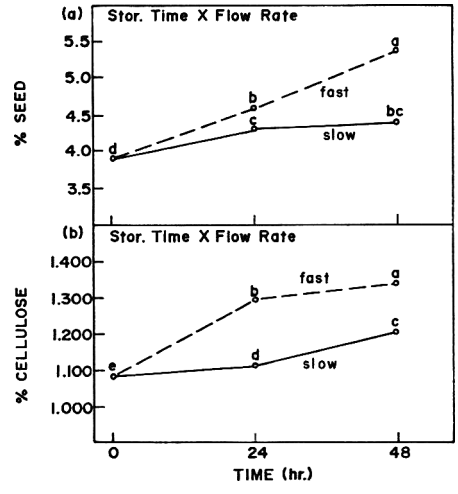

Fig. 2—Interaction of storage time X flow rate on % seed and cellulose of canned snap beans.

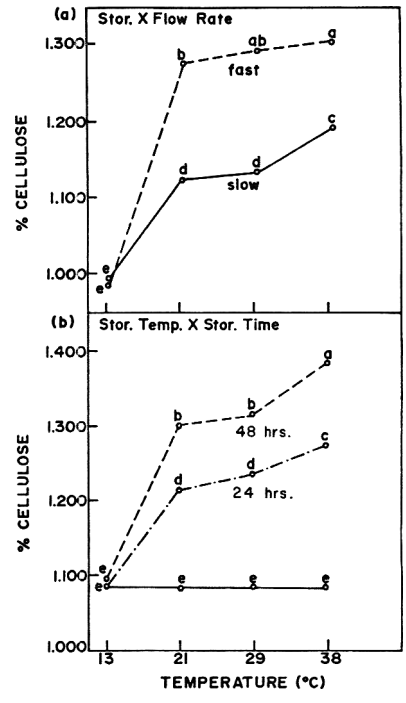

Fig. 3—Interaction of storage temperature X flow rate and storage temperature X storage time on cellulose of canned snap beans.

Processing

The 1.5-kg lots were snipped and cut with commercial-size equipment. Twenty-four lots were blanched in steam at 80°C for 2 min in a perforated stainless steel drum (25 cm diam × 60 cm length), suspended in a steam box. The drum was rotated at approximately 20 rpm. Also, 24 lots were blanched in water at 80°C for 2 min in an automatically controlled steam-jacketed kettle.

After blanching, the beans were cooled and packed in 303 J-enamel cans to a constant fill of 255g. A 50-grain salt tablet was added to each can and the fill completed with boiling water. Cans were closed with a semi-automatic closer, processed 20 min at 116°C, cooled in cold tap water and stored at 24°C until analyses were made.

Analytical procedures

Sensory evaluations were made by a panel of 4 trained judges on a scale of 1 (poor) to 10 (best). Percentages of seed and fiber were obtained by the method described in U.S. Standards for Grades of Canned Green and Wax Beans (USDA, 1972). Resistance to shear was measured by placing 150g of beans in a standard cell of the Allo-Kramer Qualitometer shear press fitted with a 136 kg proving ring.

The sheared beans were recovered and blended for 2 min with 150 ml distilled water. Color was determined on a blend by a Color Difference meter (CDM), using a standard reference plate: 'L'-50.6, '–a' 30.2 and 'b'-7.2.

Total sugars, starch, pectins, hemicellulose and cellulose were determined on the blend by procedures described by Sistrunk (1965b).

The storage chambers at 13, 21, 29 and 38°C (±1°C) were monitored for CO_2 at 12 hr intervals by a gas chromatograph.

Data collected were analyzed as a factorial experiment. Means of main effects of storage time, storage temperature, flow rate and blanch method are recorded in tables. Wherever significant interactions occurred the data were graphed. Duncan's multiple range test (DMRT) was applied to the means when F values were significant.

RESULTS & DISCUSSION

SNAP BEANS stored under fast rates of air flow did not differ in CO_2 at different storage temperatures (Fig. 1a). However, at slow rates of air flow there was an increase in CO_2 at high temperatures. There were no differences in CO_2 beyond 24 hr so no data are given for storage time. Respiration rate (ml CO_2/kg/hr) was lower in beans stored under slow rates of air flow as compared to fast flow rates. Respiration rate increased in beans at both flow rates when storage temperature was increased above 21°C (Fig. 1b). Kidd (1917) reported that storage of vegetables under higher CO_2 decreased respiration rates.

Weight losses increased as storage time was increased, and also as storage temperature was increased above 21°C (Table 1a, 1b). Slow flow rates resulted in smaller losses than fast flow rates (Table 1c). This was attributed to a higher transpiration rate at faster air flow.

Storage of beans for 48 hr prior to processing resulted in a loss of greenness (lower '–a'), and an increase in discoloration (lower 'L') as shown in Table 1a. There was no difference in CDM values between flow rates (Table 1c). The CDM 'L' and 'b' values were higher in beans blanched in rotary steam than those that were blanched in water. (Table 1d).

Percent seed and fiber increased in the beans as storage time was increased (Table 1a). Fiber and seed were lower when beans were stored at 13° as compared to 29°C (Table 1b). These results conflict with those reported by Guyer and Kramer (1950) who found no change in % seed and fiber during storage. Percent seed and fiber were lower in beans stored under slow flow rates (Table 1c). This was attributed to the high levels of CO_2 found in beans stored under slow flow rates. The significant interaction storage time X flow rate (Fig. 2a) illustrates that % seed did not increase after the first 24 hr of storage under slow flow rates but increased at both 24 and 48 hr under fast flow rates.

Beans increased in shear press values as storage time and temperature were increased (Table 1a, 1b). Rotary steam blanching resulted in less firm beans as shown by lower shear press values (Table 1b).

There was an increase in total sugars and cellulose during storage and a decrease in starch and hemicellulose (Table 2a). Pectin content was lower in beans stored for 48 hr. Parker and Stuart (1935) observed a similar decrease in starch in beans during storage.

Table 1—Main effects of storage time, storage temperature, flow rates and blanch method on canned snap beans and weight loss of fresh beans[a]

Main effects	Shear press (1b/150g)	% Fiber	% Seed	CDM 'L'	CDM '–a'	CDM 'b'	% weight[b] loss (fresh)
a. Storage time (hr)							
0	55.3c	0.017c	3.9c	39.7a	7.5a	19.8a	0.0c
24	58.8b	0.024b	4.4b	39.5a	7.4a	19.6b	1.0b
48	62.6a	0.030a	4.9a	38.8b	6.9b	19.4c	1.7a
b. Storage temperature (°C)							
13	56.6b	0.021b	4.2b	39.5a	7.3a	19.6ab	1.0c
21	57.9b	0.022b	4.3ab	39.4a	7.2a	19.5b	1.0c
29	60.0a	0.028a	4.5a	39.1a	7.3a	19.5b	1.4b
38	61.1a	0.024ab	4.5a	39.3a	7.2a	19.8a	1.8a
c. Flow rate							
slow	59.1a	0.020b	4.2b	39.3a	7.2a	19.6a	1.0b
fast	58.7a	0.024a	4.6a	39.2a	7.3a	19.6a	1.6a
d. Blanch method							
water	60.0a	0.025a	4.3a	31.1b	7.2a	19.5b	–
steam (rotary)	57.8b	0.024a	4.4a	34.5a	7.3a	19.7a	–

[a] Means separated within main effects and columns followed by the same letter or letters are not significantly different at the 5% level by Duncan's multip e range test.
[b] Calculated on fresh beans at each storage period

Snap beans stored at 13°C were higher in WS pectin than those stored at 29 and 38°C (Table 2b). Hemicellulose was lower in beans stored at 38°C than those stored at the other temperatures. Cellulose increased with each increase in temperature. The significant interaction of storage temperature X storage time indicates that there was no difference in cellulose between 21 and 29°C at either 24 or 48 hr but there were large differences between 13, 21 and 38°C (Fig. 3b).

Beans stored under slow flow rates were lower in hemicellulose and cellulose than beans stored under fast flow rates (Table 2c). The significant interaction of storage time X flow rate illustrates that there were larger increases in cellulose during storage at the fast flow rate than at the slow flow rate (Fig. 2b). Hemicellulose decreased more at the slow than the fast flow rate between 24 and 48hr. The significant interaction between storage temperature X flow rate was caused by the large increase in cellulose at 21°, 29° and 38°C at the fast flow rate as compared to the slow flow rate (Fig. 3a).

The WS pectin was higher when beans were blanched in rotary steam compared to being blanched in water, otherwise blanch method did not affect the other carbohydrates.

Sensory ratings for color, absence of browning, general appearance and flavor were lower after beans were stored for 48 hr (Table 3a). The differences were not significant for flavor and absence of browning at 24 hr. Ratings for the sensory attributes decreased as the temperature was increased from 13 to 29 and 38°C but ratings for general appearance also decreased between 13 and 21°C (Table 3b).

All sensory attributes were rated lower when beans were stored under slow flow rates as compared to fast flow rates except flavor. The beans stored under slow flow of air were off-flavor probably beacuse of low O_2 levels and anaerobic

Table 2—Main effects of storage time, storage temperature, flow rate and blanch method on carbohydrates of canned snap beans[a]

Main effects	% Total sugars	% Starch	% WS[b] Pectins	% CS[b] Pectins	% Hemi-cellulose	% Cellulose
a. Storage time (hr)						
0	1.469b	1.044a	0.269b	0.258a	0.327a	1.085c
24	1.633a	0.979b	0.280a	0.216b	0.295b	1.204b
48	1.678a	0.913c	0.256b	0.215b	0.258c	1.277a
b. Storage temperature (°C)						
13	1.543a	0.969a	0.282a	0.255a	0.301a	1.089d
21	1.629a	0.982a	0.276ab	0.266a	0.297a	1.202c
29	1.633a	1.001a	0.251b	0.240a	0.295a	1.214b
38	1.568a	0.962a	0.265b	0.232a	0.281b	1.250a
c. Flow rate						
slow	1.581a	0.977a	0.266a	0.225a	0.289b	1.137b
fast	1.605a	0.980a	0.271a	0.234a	0.298a	1.241a
d. Blanch method						
water	1.566a	0.986a	0.257b	0.236a	0.292a	1.184a
steam (rotary)	1.621a	0.980a	0.280a	0.223a	0.295a	1.194a

[a] Means separated within main effects and columns followed by the same letter or letters are not significantly different at the 5% level by Duncan's multiple range test.
[b] WS—Water soluble; CS—Calgon soluble

Table 3—Main effects of storage time, storage temperature, flow rate and blanch methods on sensory quality of canned snap beans[a,b]

Main effects	Color	Absense of browning	General appearance	Flavor
a. Storage time (hr)				
0	8.5a	8.0a	8.6a	7.3a
24	7.6b	7.8a	7.5b	7.2a
48	5.8c	6.7b	5.2c	6.8b
b. Storage temperature (°C)				
13	8.3a	8.2a	8.4a	7.6a
21	8.0a	7.8a	7.8b	7.6a
29	7.2b	7.2b	7.0c	7.0b
38	5.6c	6.7c	5.3d	6.3c
c. Flow rate				
slow	7.8a	7.9a	7.7a	6.3b
fast	6.7b	7.0b	6.5b	7.8a
d. Blanch method				
water	7.4a	7.4a	7.2a	7.1a
steam (rotary)	7.2a	7.5a	7.0a	7.1a

[a] Sensory ratings made on a scale of 1 (poor) to 10 (best) by a trained panel, comparing each sample against a standard reference.
[b] Means separated within main effect and columns followed by the same letter or letters not significantly different at the 5% level by Duncan's multiple range test.

respiration. High CO_2 in beans stored at slow flow rates is an indication of anaerobic conditions (Fig. 1a).

In conclusion, holding snap beans after harvest in loaded trucks for 24 hr or longer is detrimental to quality, especially at temperatures above 21°C. Snap beans held at the higher temperatures under fast flow rates of air had higher respiration rates, shear press values and weight losses as well as higher % seed, fiber, hemicellulose and cellulose. Fast air flow accelerated browning although color was not affected at lower temperatures. Extending the storage time of beans magnified the changes in quality. Beans stored under low air flow, which was comparable to conditions found in loaded trucks, lost less weight during storage and were lower in % fiber, seed, hemicellulose and cellulose but the canned beans were off-flavored. Therefore, movement of air through loaded trucks during long periods of holding is necessary to prevent anaerobic conditions. High CO_2 atmospheres are beneficial in the inhibition of browning when adequate air circulation is provided (Reitmeier and Buescher, 1975). Holding the beans at temperatures below 21°C was less detrimental to quality than those held at higher temperatures.

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Snap beans for the study were graciously supplied by Steele Canning Co. and Allen Canning Co.

Use of commercial brand names does not signify endorsement by the University of Arkansas.

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Higher pH optima were observed for cowpeas, chickpeas and Great Northern bean alpha-amylases. These enzymes were more active at 40°C than at 50° or 60°C. Except for Great Northern bean amylase, the time of incubation did not seem to affect the total activity of the enzyme.

The total dextrinizing activities for corn and cowpeas were much higher than for Great Northern beans and chickpeas, which would make them more suitable in hydrolyzing starches from various sources for use in home fermentation.

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LUANA CASSANDRA BREITENBACH BARROSO COELHO, RUY BASTO de MEDEIROS and HERNANDO FLORES
Departamento de Bioquimica, Centro de Ciencias Biológicas e Departamento de Nutrição
Centro de Ciências da Saúde, Universidade Federal de Pernambuco,
Cidade Universitária, 50.000 Recife, PE, Brasil

EFFECT OF STORAGE ON THE AMINO ACID COMPOSITION AND BIOLOGICAL QUALITY OF IRRADIATED MACAÇAR BEANS Vigna unguiculata (L.) WALP

ABSTRACT

The effects of two doses of gamma radiation (100 and 1,000 krad) upon the stability over a 6-month storage period of the amino acid composition and protein efficiency ratio (PER) of the macáçar bean *Vigna unguiculata* (L.) Walp were investigated. No important differences were noted when the aminograms of irradiated and nonirradiated beans, either raw or cooked were compared. Nevertheless, the losses of lysine, arginine and histidine due to cooking were greater in the irradiated beans. The PER of nonirradiated was higher than that of irradiated beans before and after the 6 months of storage, and was always lowest in the beans subjected to the higher dose of radiation. Qualitatively, an association was observed between the nutritional value (PER) and small decreases in the content of certain amino acids which resulted mainly from increased thermal lability of the irradiated bean protein.

INTRODUCTION

LEGUMINOSAE REPRESENT an important source of vegetable protein for human consumption (Chaves et al., 1952). In the case of beans, preservation methods have been employed in an attempt to reduce the losses that result from deterioration during storage (Zonenschain, 1975). The use of gamma radiation for this purpose has led to some benefits such as preservation of the physical appearance (Massa et al., 1973), reduction of the cooking time (Kiss et al., 1974; Sreenivasan, 1974), destruction of the so-called flatulent factors, and higher digestibility due to partial hydrolysis of the proteins (Sreenivasan, 1974). The chemical composition (Massa et al., 1973), and the protein content of beans (Revetti, 1973; Sreenivasan, 1974; Metta et al., 1957) have been found to be unaffected by gamma radiation. Although the aminogram has not been found altered in some cases (Adrian and Frayssinet, 1974; Sreenivasan, 1974), marked losses of lysine and arginine in lima beans have been reported by Johnson et al. (1958). As to the effects of gamma radiation on the nutritional value of beans, the available data show that the digestibility and the biological value (BV) of lima beans (Metta et al., 1957) were not affected by this kind of treatment. Also, no harmful effects on the nutritional value of cowpeas, have been observed (Adrian and Frayssinet, 1974).

In the present work, we have examined the effects of two doses of gamma radiation (100 and 1,000 krad) upon the chemical composition, amino acid composition, and nutritional value of the macáçar bean, and evaluated its stability on the basis of these parameters. Our results show that losses greater than those found by storage alone can occur as a result of the irradiation with gamma rays.

MATERIALS & METHODS

Sempre Verde (Krutman et al., 1968) macáçar bean seeds Cowpea, *Vigna unguiculata* (L.) Walp, were obtained from Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). After selection and homogenization, 27 kg of seeds were divided into 3 batches of 9 kg each. Two of these batches were placed into plastic containers and irradiated with a ^{60}Co source of 437 Ci by means of a Gamma Cell Irradiator (Radionics Laboratory, New Jersey). Irradiation was conducted at 25°C, at a dose rate of 0.88 krad per minute. The dose rate was calculated according to Fricke and Hart (1966). Two different doses (100 and 1,000 krad) were employed (Goresline, 1973; Hansen, 1966; Hoedaya et al., 1973; Ingram and Rhodes, 1962; Kovalskaya, 1971; Mohyuddin and Skoropad, 1970; Morgan, 1959). The third batch, nonirradiated, was used as control.

Flours were prepared from each batch by 2-hr boiling in distilled water. After this the seeds were oven-dried overnight at 60°C, ground and sieved.

Aliquots of raw seeds from each batch were stored in tightly closed containers with equilibrium hygroscopic solutions (Grinspun, 1974) to ensure constant humidity, around 85%. The temperature was maintained at 28°C, the average temperature of the region.

Water, fat, ashes and fiber were measured according to AOAC (1970) methods. Protein was determined by a Kjeldahl procedure (AOAC, 1970), while carbohydrates were obtained by difference.

The amino acid composition was determined by an ion exchange chromatography method (Beckman Instruments, 1970) using an Unichrom Amino Acid Analyzer (Beckman Instruments, GMBH, Frankfurter, Germany). Tryptophan was measured separately (Villegas and Mertz, 1971), after protein hydrolysis with papain. All the analyses were run in triplicate.

Forty-eight male Sprague-Dawley rats, 23-days old, were used to measure the protein efficiency ratio (PER) over a 28-day period (Allison, 1955; Campbell, 1963).

The diets used in the experiments contained bean protein (10.10–10.48%), vegetable oil (10.08–10.26%), mineral salts (Nutritional Biochemicals Corp., Cleveland, OH) and vitamins (Nutritional Biochemicals Corp., Cleveland, OH) and corn starch. The diets differed as to the source of their protein (casein for the control diet and the different bean flours for the other diets). The control diet also contained 2% cellulose.

At the end of the 28-day bioassay period, the animals were fasted for 10 hr, anesthesized by intra-peritoneal injection of sodium pentobarbital (3 mg/100g body weight) and submitted to bilateral laparotomy for collection of blood from the abdominal aorta and removal of the livers for macroscopic examination. Serum was obtained by centrifugation of the blood previously allowed to clot at room temperature. Total serum protein (Wolfson et al., 1948) and protein fractions (Beckman Instruments, 1965) were also determined.

The amino acid standards were obtained from Beckman Instruments Inc. (Palo Alto, CA). The albumin standard solution was purchased from Sigma Chemical Co. (St. Louis, MO). All the other reagents used were of analytical grade.

RESULTS & DISCUSSION

IT IS APPARENT that both storage and irradiation did not cause substantial alterations in the chemical composition of the raw or cooked seeds of the macáçar bean, when allowance is made for experimental error (Table 1). On a dry-weight basis, there seemed to be a drop in the fiber and an increase of the carbohydrate and lipid contents of the cooked as compared to the raw seeds. Nevertheless, the coefficients of variation were all within acceptable ranges, with the exception of that of the fat (15.3%). This is in keeping with previous results on other types of beans (Revetti, 1973).

Table 1—Chemical composition of the various flours from macáçar *beans' seeds*[a]

Sample	Radiation (krad)	Storage time (months)	Water	Fat	Ash	Fiber	Protein	Carbohydrate
			(g/100g dry beans)					
Raw	0	0	9.25	1.07	3.69	6.03	29.65	59.18
Cooked	0	0	2.56	1.84	4.25	5.32	30.11	58.46
Raw	100	0	10.36	1.47	3.91	6.14	30.27	58.19
Cooked	100	0	3.20	1.75	4.28	5.34	31.85	56.77
Raw	1,000	0	12.93	1.51	4.05	6.26	29.57	58.60
Cooked	1,000	0	6.38	2.06	4.60	5.50	28.86	59.04
Raw	0	6	12.36	1.61	3.81	6.46	29.90	58.22
Cooked	0	6	3.84	1.87	4.22	5.00	29.06	59.85
Raw	100	6	10.99	1.62	3.88	6.13	29.74	58.62
Cooked	100	6	3.41	1.76	4.24	5.79	29.00	59.20
Raw	1,000	6	10.74	1.56	4.15	6.05	29.07	59.17
Cooked	1,000	6	1.68	1.52	3.92	5.62	30.72	58.21

[a] Each value represents the mean of two determinations.

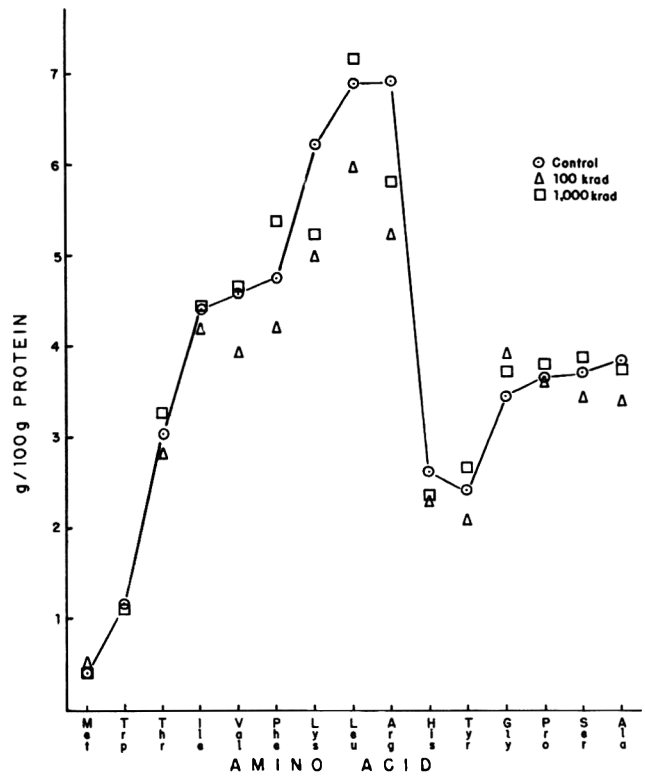

Fig. 1—Effect of gamma radiation upon the aminogram of raw seeds of nonstored macáçar *bean.*

Another approach to evaluating the effects of irradiation upon the nutritional value of the beans would be the analysis of the amino acid pattern. The nonstored beans irradiated with 100 krad had lower valine (3.95 ± 0.17 vs 5.08 ± 0.52), phenylalanine (4.34 ± 0.21 vs 4.80 ± 0.06), lysine (4.91 ± 0.06 vs 5.91 ± 0.33), leucine (6.07 ± 0.06 vs 6.91 ± 0.17) and arginine (5.42 ± 0.32 vs 6.59 ± 0.34) contents as compared to the nonirradiated control. However, this pattern does not apply to the seeds irradiated with 1,000 krad, as shown in Figure 1. The lack of a dose-response association makes the interpretation of these changes difficult. These observations are in keeping with those of Johnson et al. (1958). Leaving apart small variations of discrete importance, irradiation did not affect the stability to storage of the aminogram of the raw seeds (Fig. 2).

The data in Figure 2, suggest that the irradiation increased the lability of some of the amino acids to heat (cooking). Particularly notable are the reductions in lysine and arginine contents, not observed when the aminograms of the nonirradiated raw and cooked beans are compared. Irradiation might affect the structure of the bean protein so as to promote greater exposure of the thermolabile amino acids during cooking. In support of this hypothesis, irradiation of beans with 1,000 krad was found to increase the proportion of free amino acids, possibly as a result of fragmentation of the bean proteins into lower molecular weight species (Sreenivasan, 1974). A similar result was obtained by Srinivas et al., (1972) with wheat proteins submitted to a radiation dose of 1,000 krad. The nutritional value of control and irradiated beans was studied through the efficiency of the bean protein in promoting growth on rats. The protein efficiency ration of the nonirradiated, nonstored beans was in good agreement with previous values for this type of beans (Liener, 1976). The storage

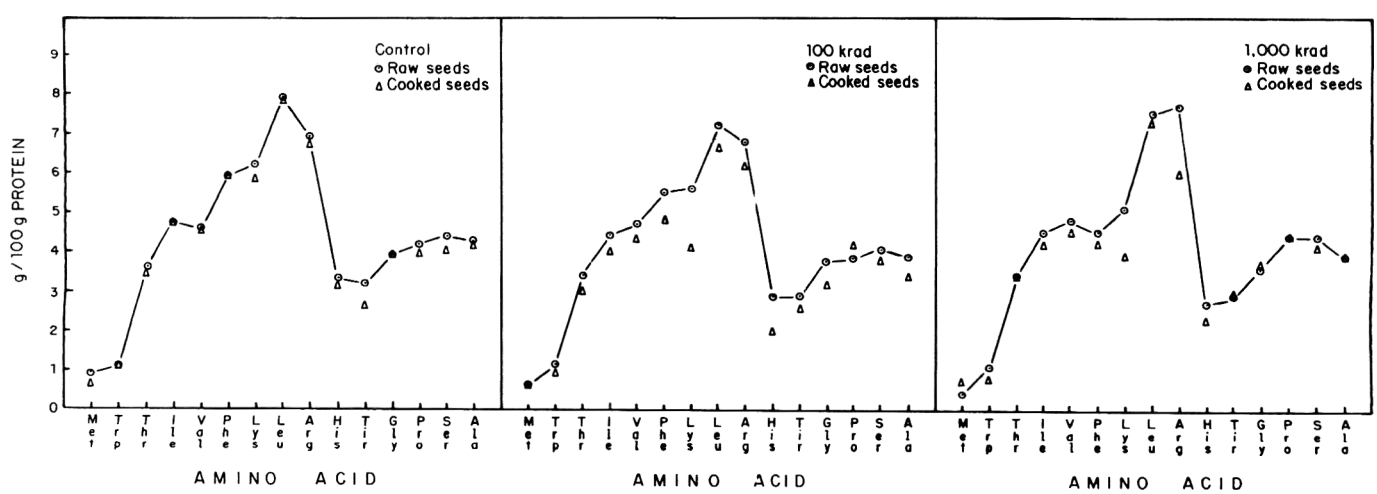

Fig. 2—Effect of gamma radiation upon the stability of the aminogram of the macáçar *bean to cooking. The seeds were stored for 6 months before cooking.*

during 6 months caused a drop in the efficiency ratio of 56%; this drop was more marked in the irradiated beans, bearing some relationship with the radiation dose: 74 and 89% for the 100 and 1,000 krad treatments. The effects of irradiation also showed in the nonstored samples: the PER values were 78.7 and 68% of the nonirradiated control in the beans irradiated with 100 and 1,000 krad respectively (Table 2). Clearly, the irradiation did not bring about any benefits to the stability of the bean protein to storage, but instead, caused an important decrease of the nutritional value of the non-stored seeds. These results strongly support the contention of Revetti (1973) that biological methods should be used for investigating the possible harmful effects of irradiation upon the nutritional value of foods, since these effects are often not detected by using chemical methods alone.

Johnson (1960) and Vidal (1974), when considering irradiation as a method for food preservation, state that, as far as the nutritional value is concerned, it leads to fully satisfactory results. Metta et al. (1957) could not find any significant differences in the biological value (BV) of lima bean proteins caused by irradiation. It is quite possible that the BV had been a less efficient means for evaluating the nutritional value of the foodstuff than the PER. In fact, for a number of foodstuffs in which both the biological value and the protein efficiency ratio have been determined (Organización de las Naciones Unidas, 1970), the biological value fluctuated between 44.5 and 93.5%, a range of variation of 2.1 times. In contrast, the protein efficiency ratio varied between 0.6 and 3.86, a 6.4-fold fluctuation. Furthermore, there is no fixed relation between the BV and the PER (Organización de las Naciones Unidas, 1970).

Table 3 shows that the relative liver weights (g/100g body weight) were essentially the same in all groups of rats used in these experiments. In addition to that, the biochemical parameters studied (proteinemia and albuminemia) did not show any consistent variations. However, this can be explained either by the short period of the experiments (28 days), or by an adaptation of the animals to the poor quality protein.

It is all too clear that chemical analyses alone are not sufficient to evaluate irradiation as a preservation method. In fact, although drops in the contents of some of the amino acids could be detected, caused either by storage, irradiation or both, these drops cannot explain the decrease in the protein efficiency ratio. This was always lower in the beans irradiated with 1,000 krad, while the data in Figure 1 show that the drop in some of the essential amino acids was bigger in the seeds irradiated with 100 krad. Therefore, nothing more than a qualitative association can be claimed to exist between the deterioration of the aminogram and that of the protein efficiency ratio, which is in keeping with previous reports (Liener, 1976). The contrast between the results of our chemical determinations and those of the experiments on animals show that the former can be highly misleading. Losses much greater than those found upon normal storage result from irradiation, thus ruling out this method of preservation, at least for macáçar beans. A word of caution is in order for other foodstuffs of high protein content which, as is the case of beans, constitute significant sources of dietary protein.

Table 2—Protein efficiency ratio (PER) of flours from cooked macáçar *beans' seeds*

Sample	Radiation (krad)	Weight gain (g)	PER
Non stored	0	12.42 ± 2.73[a]	0.94 ± 0.16
Stored 6 months	0	4.82 ± 1.73	0.41 ± 0.13
Non stored	100	10.15 ± 2.20	0.74 ± 0.13
Stored 6 months	100	1.75 ± 1.29	0.16 ± 0.12
Non stored	1,000	7.58 ± 1.40	0.64 ± 0.10
Stored 6 months	1,000	1.35 ± 1.98	0.07 ± 0.19
10% casein standard diet	—	78.40 ± 5.13	2.98 ± 0.13

[a] Mean ± Standard error

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Table 3—Some biochemical parameters of rats fed macáçar *beans flours for the determination of its protein efficiency ratio*

Beans' sample	Radiation (krad)	Liver wt (g/100g body wt)	Proteinemia (g/100ml)	Albuminemia (g/100ml)
Nonstored	0	4.90 ± 0.18[a]	4.77 ± 0.14	1.87 ± 0.08
Stored 6 months	0	5.07 ± 0.17	4.50 ± 0.13	1.90 ± 0.08
Nonstored	100	4.87 ± 0.19	4.50 ± 0.17	1.86 ± 0.12
Stored 6 months	100	5.13 ± 0.30	4.10 ± 0.13	1.70 ± 0.16
Nonstored	1,000	4.72 ± 0.24	4.32 ± 0.12	1.66 ± 0.08
Stored 6 months	1,000	4.55 ± 0.34	5.35 ± 0.31	1.60 ± 0.10
10% casein standard diet	—	4.03 ± 0.28	5.82 ± 0.17	2.58 ± 0.10

[a] Mean ± Standard error

R. TCHALUKOVA and G. KRIVOSHIEV
Canning Research Institute, 21 Bul. Lenin, Plovdiv 4000, Bulgaria

COLOR CHANGES OF HOT-HOUSE GROWN TOMATOES DURING STORAGE

ABSTRACT

Color changes of hot-house grown tomatoes stored for periods up to 8 days at temperatures from 6–20°C were investigated. Color was measured by light transmission at two wave lengths (575 and 675 nm) and characterized by a definite color index. A dependence between the color index change, the temperature and the storage time was established. On the basis of this index an instruction for sorting hot-house grown tomatoes was worked out. By means of this instruction the preferred market maturity of tomatoes can be determined and acceptable differences of tomatoes in one package achieved.

INTRODUCTION

THE AIM of this investigation was to determine the dependence of tomato color on temperature and time of storage. Hot-house grown tomatoes, exported from Bulgaria, reach the consumer after 2–6 days of transport because of the long distance to market.

The ripening of the tomatoes (on the vine and after harvest) depends upon environmental conditions and has been investigated and reported by many authors (Gaffney and Jahn, 1970; Koskitalo and Ormrod, 1972; Ryall and Lipton, 1972; Wu et al., 1972). The ideal means of determining the stage of ripeness of the tomato fruit after harvest is the utilization and application of methods and instruments that allow for nondestructive measurements. This has been achieved by determining the external and internal color of the fruit by colorimetry, spectrophotometry or abridged spectrophotometry (Birth et al., 1957. Birth and Norris, 1958; Dempster, 1974; Kirvoshiev, 1974; Worthington et al., 1974).

A bichromatic index of the internal color is used as an indicator of maturity, since the internal color offers more information on the ripening process than the surface color. Moreover this index represents the basis for the principle of operation of the automatic color sorting machines ASC used for handling of hot-house and field-grown tomatoes as well as in the canning industry (Krivoshiev et al., 1975).

MATERIALS & METHODS

EXPERIMENTS were carried out during the 1973 and 1974 seasons with sound tomatoes (neither bruised nor decayed) of the Extase variety, obtained from hot houses in the Plovdiv district and harvested at different stages of maturity. The hot-house conditions were maintained at 24 ± 2°C, a relative humidity of 65 ± 5% and natural light. Batches of 60 fruit were placed in chambers with constant temperatures (20, 16, 12, 8, 6 and 4°C ± 0.5°C) and a velocity of air flow of 0.1m/sec. The fruit color was measured once a day on tomatoes held at the higher temperatures and once every other day for tomatoes held at the lower temperatures.

The color index was determined by

$$\log P = \log \frac{(p/p_{st}) \text{ at } 675 \text{ nm}}{(p/p_{st}) \text{ at } 575 \text{ nm}}$$

where p/p_{st} is the ratio of the transmittance of the sample to the transmittance of a known standard. This index may characterize the internal color of tomatoes, since it has been shown to correlate with that of juice extracted from the same fruit (Krivoshiev, 1974).

The fruit transmittance was measured by an SFP-1 spectrophotometer, similar to the Refobiospect used by Birth et al. (1957). During the second year, results were confirmed with a larger quantity of tomatoes.

RESULTS & DISCUSSION

FIGURE 1 (a–e) presents the relationship between color index and storage period for individual fruit from each batch investigated. There is a trend toward decreasing the slope of the curve as the temperature decreased in the range investigated (20–4°C). At a temperature lower than 6°C, if color is taken as the index of ripening, the fruit did not ripen and no change of the index, log P, during storage was noted.

At fixed temperatures color changes in each fruit are subject to biological variation whether the fruit are harvested at different maturities or not. The rate of color change or log P = f(t) of a certain fruit is taken as a section of the curve of the following equation:

$$\log P = \log P_{max} \quad (1 - e^{-\frac{t}{\tau}}) \qquad (3.1)$$

where $\log P_{max}$ = maximum color index; τ = time factor, (τ1T) where T = temperature; and t = Δt or ripening period in 24-hr intervals.

The fruit should be harvested when it possesses the optimum chance of ripening during storage, provided the temperature is favorable. This optimum chance of ripening has been designated the minimum maturity stage and is characterized by the index $\log P_{min}$. For various storage temperatures (T), P_{min} will differ and can be determined experimentally. Thus the section of the curve which fits the equation log P = f(t) will indicate color index changes with time (t) at a constant temperature (T) and could be expressed in the following manner:

$$\log P = \log P_{max} - (\log P_{max} - \log P_{min})\, e^{-\frac{t'}{(\tau 1 T)}} \qquad (3.2)$$

where $t' = \Delta t = t - t_{min}$ (t_{min} being the period during which the color index has reached the value $\log P_{min}$).

At harvest, the maturity of the fruit can be characterized by the index $\log P_o$ (where $\log P_{max} \geqslant \log P_o \geqslant \log P_{min}$) and by the period necessary to reach the maturity at harvest time t_o. During the storage period t_s, where $t_s = t - t_o$, the color index will increase according to the equation

$$\log P = \log P_{max} - (\log P_{max} - \log P_o)\, e - \frac{t_s}{\tau 1 T} \qquad (3.3)$$

The time necessary for fruit with a given initial maturity to

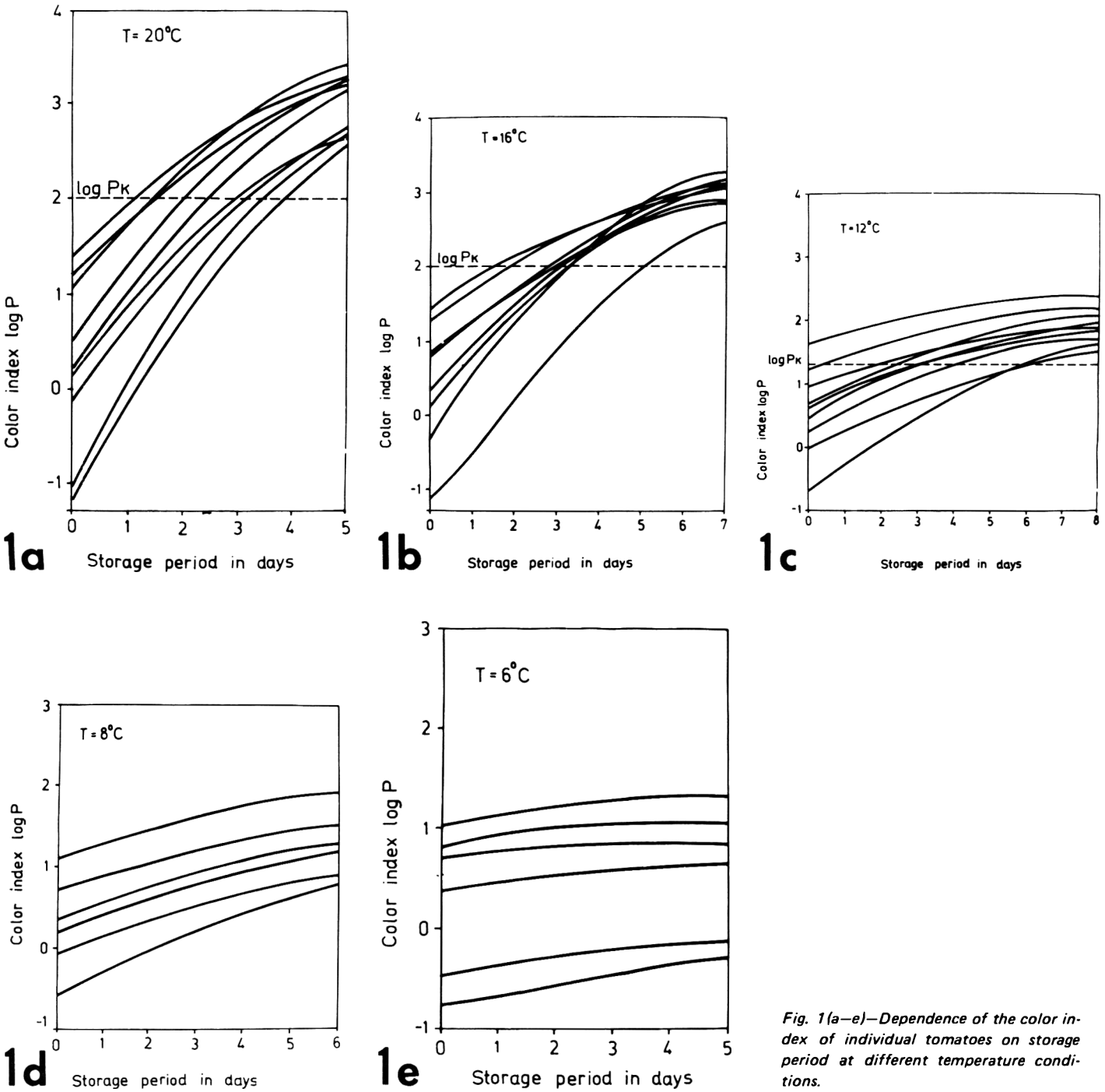

Fig. 1(a–e)—Dependence of the color index of individual tomatoes on storage period at different temperature conditions.

reach a definite color index (e.g. market maturity) could be determined from the equation

$$t_s = 2.303\,(\tau 1 T)\log\left(\frac{\log P_{max} - \log P_o}{\log P_{max} - \log P}\right) \qquad (3.4)$$

$$t_s = (\tau 1 T)\ln\left(\frac{\log P_{max} - \log P_o}{\log P_{max} - \log P}\right) \qquad (3.4a)$$

$$t_s = (\tau 1 T)\ln\left(\frac{\log P_{max}/P_o}{\log P_{max}/P}\right) \qquad (3.4b)$$

The numerical values of log P_{max}, log P_{min} and $\tau 1T$ were determined cn the basis of the experimental data. Table I shows the statistically most probable values of log P_{max} and τ, as well as the values for log P_{min}. It can be seen that the maximum color index, log P_{max}, is decreasing, log P_{min} is increasing, and the time factor τ tends to increase with decreasing temperature T.

The standard deviation of the summed rates of color formation (eq 3.2) for a 3–4 day storage period $\tau_{log\ p}$ = 0.1–0.25. On the basis of the dependence of log P = $f_1(\lambda_D)$, where λ_D = the dominating wave length in nm (Krivoshiev, 1974), it was established that in 95% of the cases the tomato color after

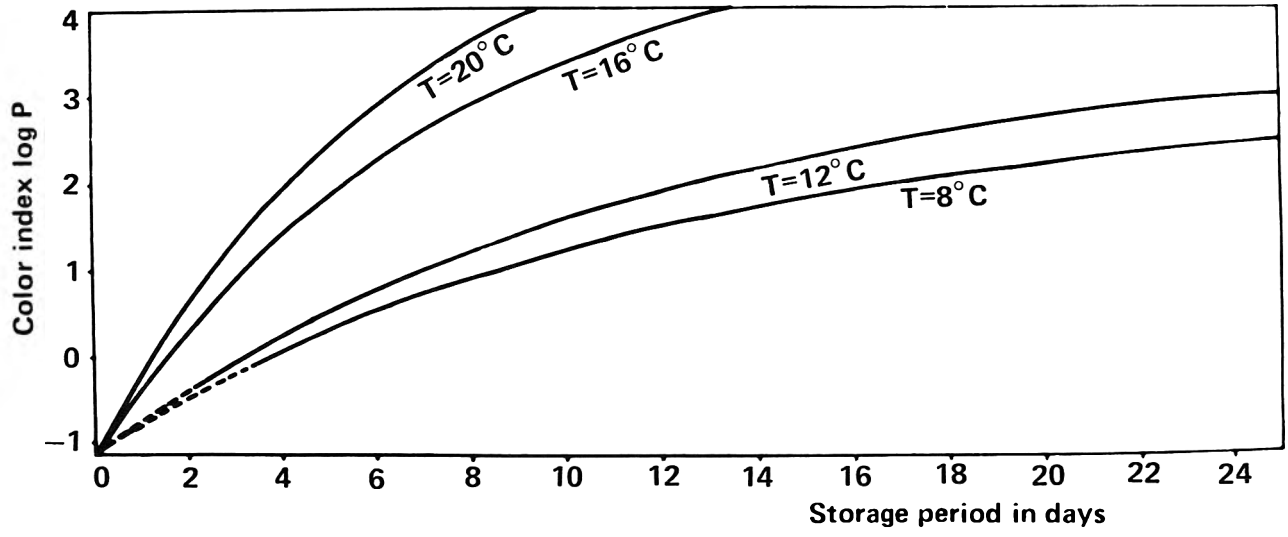

Fig. 2—Summed up dependences of the color index on storage periods.

transport will differ from the predicted (Eq 3.3) by not more than ±3 nm for T = 8°C and ±7 nm for T = 20°C.

In order to reach the appropriate market maturity log P_K, the authors established a linear dependence of 0.76–0.88 (market time t_K, determined by means of individual rates of color formation) between log P_o and $t_s = t_K$. The time t_K necessary for an individual fruit to reach a fixed color index log P_K can be determined accurately beforehand for 95% ± 1 day.

The summed rates of color formation (Fig. 2) based on Eq (3.2) and Table 1 can be used in the formulation of color sorting instructions of hot-house grown tomatoes. An example of such instructions is given in Table 2. Since during handling tomatoes are sorted in a certain number of fractions, the color index log P_K will be taken in a given range. The log P_K range was determined with the aid of research data in such a way that the color differences of tomatoes in a single package would be acceptable to the consumer. On the basis of the limiting value of log P_K for each fraction and the curves in Figure 2, we determined the limiting values of the color index at harvest time and during handling log P_o. Table 2 has been worked out for two stages of market maturity and for 3 and 6 days transport.

The instructions for color sorting of tomatoes could be used in practice in three ways:

1. The workers are furnished with tomato standards, with color chosen by means of the Internal Quality Analyzer, Model 170 (Dempster, 1974) or by the Gardner Colorimeter (Bontovits et al., 1972).
2. The workers are furnished with standard color charts, relying on the correlation between the surface and internal tomato color.
3. The tomatoes are color sorted by means of the ASC-11 instrument.

The authors applied the third method of sorting. After storage at the respective temperature conditions, the color of the

Table 1—Values of log P_{max}, τ/T and log P_{min} in Eq (3.3), characterizing the color change of hot-house tomatoes during transport

Storage temp °C	log P_{max}	τ/T	log P_{min}[a]
20	6.763	6.7	−1.137
16	6.183	7.6	−1.0
12	4.680	11.5	−0.32
8	3.888	10.85	−0.1

[a] log $P_{max} \geq$ log $P_o \geq$ log P_{min}

Table 2—Example of the instruction for color sorting of hot-house tomatoes—before (log P_o) and after (log P_K) storage—giving acceptable color differences to tomatoes in one package after transport at different temperatures

Storage time days	Temperature conditions															
	20°C				16°C				12°C				8°C			
	log P_K[a]		log P_o[b]		log P_K		log P_o		log P_K		log P_o		log P_K		log P_o	
	from	to	from	to	from	to	from	to	from	to	from	to	from	to	from	to
3	1.1	1.92	−1.14	−0.32	0.85	1.58	−1.0	−0.19	0.85	1.58	0.09	0.73	0.85	1.58	0.4	1.04
6	2.62	3.3	−1.14	−0.14	1.92	2.87	−1.0	−0.19	1.24	2.06	−0.28	0.52	0.85	1.58	−0.1	0.62
3	1.45	2.48	−0.49	0.51	1.16	2.05	−0.45	0.24	1.24	2.06	0.56	1.37	1.18	2.03	0.85	1.72
6	–	–	–	–	2.26	3.16	−0.41	0.52	1.54	2.51	0.24	1.44	1.3	2.13	0.46	1.44

[a] Range of color index preferred by the consumer
[b] Necessary range of color index during handling

tomatoes sorted according to the instructions was close to the predicted one.

If temperature conditions differ from the above mentioned are used in practice, the values of log P_{max} τ1T and log P_{min} can be determined from the graphs using the data contained in Table 1. Table 3 shows the sorting instructions worked out on the basis of the present investigation and recommended for Bulgarian hot-house grown tomatoes.

Table 3—Sorting instruction for Bulgarian hot-house grown tomatoes, recommended for transport temperature 9 ± 1°C

Color fraction	Range of color index during handling log P_o		Destination of fractions	Duration of cooling and refrigerated transport days
	from	to		
I	–	−0.23	Not exported	–
			Export by refrig. cars to	
			Basel, Switzerland	4
			Malmö, Sweden	5
II	−0.23	0.4	Stockholm, Sweden	6
			Moscow, USSR	5
			Leningrad, USSR	6
			Export by refrig. cars to	
			Munich, FRG	3
III	0.3	0.91	Hamburg, FRG	4
			Vienna, Austria	3
IV	0.87	1.80	Export by airplane	1–2

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J. SAVANI[1] and N.D. HARRIS[2]
Dept. of Food & Nutrition, Florida State University, Tallahassee, FL 32306
and W.A. GOULD
Dept. of Horticulture, Ohio State University, Columbus, OH 43210

SURVIVAL OF CLOSTRIDIUM SPOROGENES PA 3679 IN HOME-CANNED TOMATOES

ABSTRACT

C. sporogenes PA 3679 and various acids including ReaLemon (a reconstituted lemon juice), cream of tartar, and citric acid were added to high pH tomatoes (pH > 4.6) prior to canning. Quart tomato jars were processed for 20 min to determine the effectiveness of different acids on inhibiting the growth of PA 3679 and for 45 min to determine the safety of using the procedures recommended in USDA Home and Garden Bulletin No. 8. ReaLemon prevented and acids inhibited the growth of PA 3679 in the 20-min heat treatment. Since the recommended procedures in Home and Garden Bulletin No. 8 for home-canning raw pack tomatoes in quart jars were modified, this study did not prove or disprove the safety of using the USDA recommendations for home-canning tomatoes.

INTRODUCTION

IN THE UNITED STATES from 1899 to 1973, 89% of food-borne botulism outbreaks of known food sources were due to home-processed foods (Lynt et al., 1975).

The water-bath method has been considered safe and practical for preserving home-canned high-acid food (Extension Home Economics 58, 1975; Heriteau, 1975; USDA Home and Garden Bulletin No. 8, 1976). Nevertheless, recent outbreaks of botulism in high-acid food (pH $\leqslant$ 4.6) indicate that the conventional methods of home-canning may need to be reevaluated (MMWR, 1967; 1969; 1973; 1974a, b). Tomatoes are considered a high-acid food with an average pH of 4.3 (Farrow, 1963; Lehninger, 1975). Tomatoes were involved in two of the botulism outbreaks (MMWR, 1973; 1974b). The lowest pH levels for germination and for growth of *C. botulinum* vary from 4.8 to greater than 5.0 (Hauschild et al., 1975; Baird-Parker and Freame, 1967; Ingram and Robinson, 1951; Ingram and Handford, 1957; Ohye and Christian, 1966; Wagenaar and Dack, 1954; Townsend et al., 1954). As a result of unpublished data by the National Canners Association Western Branch Laboratory in 1927–1931 from studies on acidification to prevent the growth of *C. botulinum*, the maximum pH for control purposes was agreed to be 4.6 (Townsend et al., 1954).

A study concerning the home canning of high-acid food preserved by the water-bath method appeared warranted. In using the water-bath method for home-canning foods in a questionable pH range, the most effective and practical methods for preventing the growth of *C. botulinum* spores are to lower the pH or to increase the processing time. This study investigated the safety of using the USDA recommendations in Home and Garden Bull. No. 8 for tomatoes and the effectiveness of adding various acids to decrease the pH to inhibit the growth of *C. sporogenes* PA 3679.

EXPERIMENTAL

THE NATIONAL CANNERS ASSOCIATION (NCA) strain of Putrefactive Anaerobe 3679 obtained from the National Canners Association was used in this study because PA 3679 does not produce a lethal toxin. With respect to the American Type Culture (ATCC), the organism is classified as *C. sporogenes* ATCC 7955. Spores were prepared and enumerated (1×10^6 spores of PA 3679 per 10 ml of sterile distilled water) by the National Canners Association. For production of spores, the organism was grown in pork broth and the spores harvested May 24, 1966. Spores had a D_{250} value of 0.98 in phosphate buffer.

C. sporogenes PA 3679 has been found to have similar cultural, biochemical, heat resistance, nutritional and seriological characteristics to *C. botulinum* type A (CRC Handbook of Microbiology, 1973; Smith and Holdeman, 1968; Kinder et al., 1956).

Tomatoes selected were Ohio 7584, a high pH breeding line variety not available commercially. The mean pH of comminuted Ohio 7584 tomatoes was 4.6 and the pH was measured with a Beckman Zeromatic pH Meter.

Canning procedures

The canning procedure was the method recommended by the United States Department of Agriculture in Home and Garden Bulletin No. 8 (1976). Modifications to the recommended procedure included using firm but overripe tomatoes to obtain higher pH tomatoes, using a water-lye solution to remove tomato skins, and removing the tomato cores mechanically to decrease preparation time for processing. Whole peeled tomatoes were raw packed into clean, quart, Ball jars. The tomatoes were gently pressed by hand into the jars and tomato juice was added leaving a 0.5-inch headspace. One teaspoon of salt was added to each quart jar.

The experimental design is shown in Table 1. One treatment had no acid added which served as control for acid treatments. The quantities of acids used were determined by the amount of ReaLemon or cream of tartar needed to decrease the pH approximately 0.3 pH units in commercially canned tomato juice. Six ml (one teaspoon) of reconstituted lemon juice and 0.9g (one-half teaspoon) of cream of tartar were added to the quart tomato jars. Two other acid treatments were prepared by adding citric acid or citric acid plus 3.4g sucrose (one teaspoon). The citric acid added was in the form of two tomato canning tablets marketed by the Morton Salt Company, Chicago, IL 60606. The tomato canning tablet contained sodium chloride, calcium sulfate, anhydrous citric acid, microcrystalline cellulose, sorbitol solution, magnesium stearate and silicon dioxide. The reconstituted lemon juice. ReaLemon, marketed by Borden, Inc., Chicago, IL, contained water, lemon juice, concentrated lemon oil, 3/100ths of 1% sodium benzoate and 1/40th of 1% sodium bisulfite.

Eight jars in each acid treatment and no acid treatment with a 20 and 45 min heat treatment were inoculated with 10^4 spores of PA 3679. For controls, eight jars in each acid treatment and no acid treatment with a 45-min heat treatment were not inoculated with PA 3679. After the tomatoes had been packed and acid and spores added for certain treatments, the jars were closed with a two-piece Ball lid.

Quart jars were heated in an industrial canner containing boiling water. The canned tomatoes were boiled gently for 20 and 45 min. Twenty minutes were selected because this is the processing time used in a reported botulism case (MMWR, 1974b). Also, 20 min were selected for a processing time to determine the effectiveness of different acids in inhibiting bacterial growth when using a marginal heat treatment. The 45-min process was selected because this is the processing time recommended for home-canning quart jars of tomatoes by the USDA. Jars were transported in a non air-conditioned automobile from Columbus, OH to Tallahassee, FL where they were stored at ambient temperature for 3–7 months.

Bacteriological tests

Three to seven months after canning at various time intervals, 10 ml of tomato brine from inoculated jars were subcultured into Schaedler

[1] Present address: American Home Foods, Milton, PA 17847
[2] To whom requests for reprints should be directed

agar (BBL) and were analyzed for vegetative cells of PA 3679. Schaedler agar was recommended by Abramson (1975) for the enumeration of vegetative cells of PA 3679. Schaedler agar contains trypticase soy broth, polypeptone peptone, dextrose, yeast extract, Tris (hydroxymethyl) aminomethane, hemin, L-cysteine and agar.

To minimize the effect of long-chain fatty acids in inhibiting spore germination, 0.2% corn starch was added to the medium (Smith, 1975). Quantitative plate counts were determined from the samples of tomato brine. Plates were incubated at 30°C for 72 ± 2 hr in anaerobic jars (BBL). Plating was done in duplicate.

All colonies from the tomato samples resembling the morphological characteristics of a pure culture of *C. sporogenes* were identified as the same. Gram stains were taken periodically from surface and subsurface colonies resembling the morphological characteristics of a pure culture of *C. sporogenes* and gram positive rods grown anaerobically were presumed to be *C. sporogenes*. Randomly selected organisms grown in an anaerobic environment on Schaedler agar resembling the morphological characteristics of a pure culture of *C. sporogenes* were for tentative identification for *C. sporogenes*.

pH determinations

Three to seven months after canning, three pH determinations were determined using a Beckman Century pH Meter for each unspoiled tomato jar and the average pH per jar and the average pH per acid treatment calculated.

Statistical analysis

A one-way analysis of variance was used to determine significant differences. Duncan's Multiple Range Test was used to compare treatments for the effect of various acids on growth inhibition of the organism (Steel and Torrie, 1960).

RESULTS & DISCUSSION

FIGURE 1 summarizes average colony counts per ml for *C. sporogenes* PA 3679 for the 8 jars in each acid and no acid treatment for a 20-min heat treatment. Data in Figure 1 show growth of vegetative cells of PA 3679 when subcultured from inoculated jars stored at ambient temperature for 3–7 months into Schaedler agar in an anaerobic environment.

Additional acids had an inhibitory effect on growth of the organism and canned lemon juice prevented growth. The lemon juice treatment was expected to give a low bacterial count since it contained sodium benzoate and sodium bisulfite. The lemon juice treatment was significantly different at the 0.05 level from the other acid treatments and from the no acid treatment in inhibiting the growth of PA 3679. Cream of tartar was found to be the second most inhibitory to PA 3679 and to be significantly more inhibitory at the 0.05 level than citric acid, citric acid with sugar, and no acid added. Citric acid was not as inhibitory to growth of the organism as ReaLemon or cream of tartar, but was significantly more inhibitory at the 0.05 level than citric acid with sugar or no acid treatments. The number of organisms of PA 3679 which grew in the citric acid with sugar treatment and no acid added treatment were significantly greater at the 0.05 level than other treatments, but no significant differences at the 0.05 level existed between the citric acid with sugar treatment and the no acid added treatment. Since all jars with added sugar in the 20 and 45-min heat treatments were spoiled, sugar should not be added to home-canned tomatoes.

In the 45-min heat treatment no colonies of PA 3679 were detected after subculturing from the stored tomato jars into Schaedler agar. Perhaps the longer heat treatment with a decrease in pH during processing. prevented the growth of PA 3679.

Many jars in the 20-min heat treatment and 45-min heat treatments with and without PA 3679 added to the quart tomato jars showed liquid spurting from the jars, gas bubbles, soft, slimy tomatoes, cloudy liquid, sediment, malodorous tomatoes, leaking jars, and bulging tops. Spoilage was visually observed in the quart jars while transporting the jars from Ohio to Florida.

Spoilage was most likely the result of transporting the jars from Ohio to Florida in a warm (>43°C) environment which allowed for the growth of thermophilic anaerobes. No thermophilic anaerobes were recovered from spoiled tomato jars when subcultured into Schaedler agar and incubated anaerobically at 45°C and 55°C for 3 days. The thermophilic anaerobes prob-

Table 1—Experimental design

	Acid group	No. of jars
Samples inoculated with 10^4 spores of PA 3679		
20 min		
Heat treatment	citric acid	8
	citric acid and sugar	8
	lemon juice	8
	cream of tartar	8
	no acid added	8
45 min		
Heat treatment	citric acid	8
	citric acid and sugar	8
	lemon juice	8
	cream of tartar	8
	no acid added	8
Samples to which no organism was added		
45 min		
Heat treatment	citric acid	8
	citric acid and sugar	8
	lemon juice	8
	cream of tartar	8
	no acid added	8

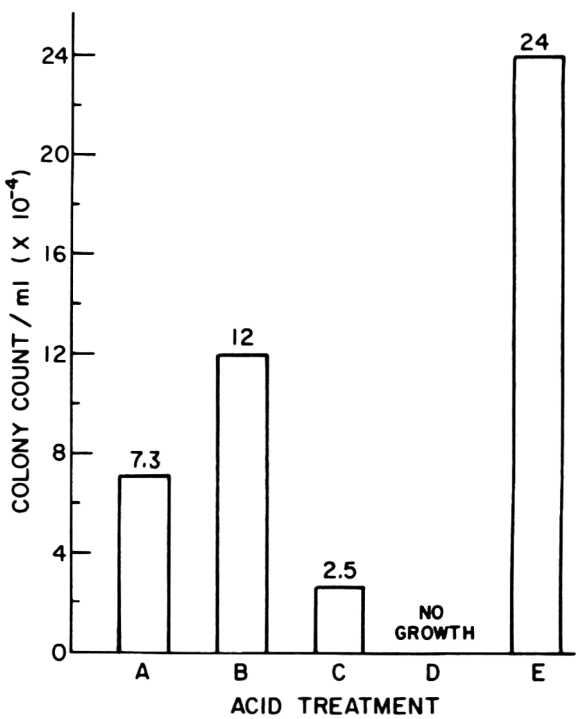

Fig. 1—Effect of adding acid to Ohio 7584 tomatoes to inhibit C. sporogenes PA 3679 with a 20-min heat treatment. Acid treatments (Range): (A) citric acid (8.6 X 10^4), (b) citric acid with sugar (2.1 X 10^5), (C) cream of tartar (4.6 X 10^4), (D) ReaLemon (0.0), and (E) no acid added (1.0 X 10^5).

Table 2—Average pH per acid treatment in unspoiled tomato jars after 3—7 months' storage

Acid treatment	Avg pH (Range)
Citric acid	4.8 (0.1)
Cream of tartar	4.8 (0.1)
ReaLemon	4.8 (0.2)
No acid	4.8 (0.1)

ably died while stored at ambient temperature for 3—7 months.

Randomly selected organisms grown on Schaedler agar resembling the morphological characteristics of a pure culture of *C. sporogenes* PA 3679 were tentatively identified as *C. sporogenes* PA 3679. All organisms examined were hemolytic, actively fermented glucose and maltose, were highly proteolytic, and produced lipase; but none produced indole, or lecinthinase (Smith and Holdeman, 1968).

The mean pH of comminuted Ohio 7584 tomatoes was 4.6. After treating the overripe tomatoes with a water-lye solution to remove tomato skins, the average pH of comminuted Ohio 7584 tomatoes was 5.1. In Table 2 are shown average pH readings for acid and no acid treatments in unspoiled tomato jars after 3 to 7 months storage at ambient temperature.

In the no acid and acid treatments the average pH was 4.8. Processing most likely accounts for the lower pH in the no acid and acid treatments (Adams, 1961). The added acids may have had little or no effect on Ohio 7584 tomatoes because of the addition of a water-lye solution to remove tomato skins or the buffering capacity of the tomatoes. Data in Table 2 show the quantities of acids added were not sufficient to decrease the pH to an acceptable level of 4.6 for processing acid foods to prevent the growth of *C. botulinum*.

Since comminuted Ohio 7584 tomatoes had an average pH of 5.1 after preparation for processing and were overripe and tomato jars had an insufficient quantity of acid added to decrease the pH to an acceptable level of 4.6 for processing acid foods, this study did not prove or disprove the safety of using the USDA recommendations in Home and Garden Bulletin No. 8 for tomatoes or the effectiveness of adding acid to decrease the pH in tomatoes.

This study does show ReaLemon is effective in preventing the growth of PA 3679. Possibly, a canning tablet could be developed and marketed for tomatoes and other acid foods processed by the water-bath method which contains citric acid and the preservatives sodium benzoate and sodium bisulfite (all GRAS) to prevent the occurrence of a public health problem in home-canned foods. Since sulfite can cleave and inactivate the thiamine molecule, a canning tablet for home-canned foods most likely should not contain sodium bisulfite (De Ritter, 1976).

All known cases of botulism from 1967 to 1974 involving home-canned high-acid food occurred when people failed to follow the procedures recommended in Home and Garden Bulletin No. 8 (MMWR, 1967 1969; 1973; 1974a, b). Since 80% of the outbreaks of botulism between 1967 and 1974 occurred in rural areas, the goverment agencies should make available in grocery stores, general stores and seed stores their publications concerning home-canning. These publications should be widely distributed and readily available to all parts of the country.

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J. M. AGUILERA[1] and F. V. KOSIKOWSKI
Dept. of Food Science, Cornell University, Ithaca, NY 14853

EXTRUSION AND ROLL-COOKING OF CORN-SOY-WHEY MIXTURES

ABSTRACT

A partially demineralized, delactosed whey product containing about 36% protein and 56% lactose was used to supplement corn meal together with soy flour and soy isolate. All four mixtures prepared contained 75% corn meal. Mixtures A and B had 5% and 22% lactose-hydrolyzed whey respectively, mixture D, 5% unhydrolyzed whey and mixture C used skim milk as in CSM. Protein content was standardized to 20% protein. Amino acid analysis revealed an increase in all amino acids except leucine with respect to corn. Processing, either roll-cooking or extrusion reduced the quantity of essential amino acids. PER values of all mixtures were not significantly different ($P > 0.05$) from casein. Functionally, the whey product reduces water absorption capacity but increases water and nitrogen solubility. Products from hydrolyzed whey, suitable for snacks or breakfast cereals, showed increased sweetness.

INTRODUCTION

CORN is the second world supplier of vegetable protein. Yet, the quality of corn protein is poor mainly due to deficiencies in lysine and tryptophan.

Supplementation, or the addition of the deficient amino acids as protein, has been successfully accomplished in corn by blending heat-processed cornmeal, toasted soy flour and nonfat dry milk. The resulting blend is the base of CSM (Blended Food Products, Formula No. 2) a high protein food for preschool children used in international programs (Senti, 1969). The combination gives a minimum protein level of 19% with an adjusted protein efficiency ratio of 2.42–2.48, comparable to that of casein. Recent shortages of nonfat dry milk available to the U.S. Department of Agriculture together with a two-fold price increase since 1972 have stimulated the search for a substitute to be used in the U.S. Food-for-Peace Program (Pallansch, 1974; Anonymous, 1975).

A partially demineralized, delactosed (PPD) whey product similar to nonfat dry milk in protein and lactose contents, ForeTein 35, (Formost Co., San Francisco, CA) is claimed to be particularly high in lysine and tryptophan and to have a PER value of 3.2, adjusted to casein 2.5, better than the 3.13 value of nonfat dry milk. Also, since it can be produced at a lower price than nonfat dry milk its use as a replacement is apparently justified.

Lactose intolerance in members of non-Caucasian races severely limit the use of milk and some dairy products such as whey. Hydrolysis of lactose into digestible mono-sugars (glucose and galactose) employing lactase from microbial sources would allow the utilization of higher levels of whey products and at the same time increase sweetness (Nickerson, 1974).

Gelatinization of corn by passing it between heated steam rolls (roll-cooking) is the most economical commercial method according to Anderson et al. (1969). Recently, cooking extruders have become popular and are extensively used in achieving controlled starch gelatinization of cereal products. Anderson et al. (1969) studied the processing of corn grits by roll- and extrusion-cooking under different operating conditions, including those for producing corn meal suitable for CSM. The cooked corn was dry blended with the other ingredients of the instant CSM food blend.

There is a need for high-protein foods other than floury products. Snacks and breakfast cereal-type products can be very efficient nutritional carriers but they should withstand heat-treatments that may damage heat-labile proteins.

The objective of this study was to evaluate the nutritional and functional properties of three mixtures, similar in composition to CSM, that contained the PDD whey product and that were roll- and extrusion-cooked.

MATERIALS & METHODS

Lactose hydrolysis in PDD whey

A commercial lactase (β-D-galactosidase, EC 3.2.1.23) derived from *Saccharomyces lactis*–Mailact (Enzyme Development Corp., New York, NY)–was used for lactose hydrolysis of PDD whey solutions. Since the objective was not to optimize enzymatic conditions but to obtain a low-lactose product, only 30 and 50% PDD whey dispersions were enzyme-treated. Enzyme concentrations of 80 and 120 mg/100g were used for the 50% dispersions and 20, 40 and 60 mg/100g for the 30% dispersions.

One hundred ml of each PDD whey solution were placed in 250 ml Erlenmeyer flasks. The pH was raised from about 5.0 to approximately 6.4 using a 5% NaOH solution. Enzyme was added and incubation was performed in an incubator shaker (Gyrotory Model R 25, New Brunswick Scientific Co., Inc., New Brunswick, NJ) at 35°C and 200 rpm for 4 hr. After incubation samples were standardized to 10% solids for lactose hydrolysis analysis. The enzyme was inactivated by heating for 2 min at 80°C.

Incorporation of materials

Composition of the four mixtures is presented in Table 1. Mixtures provided about 20% protein.

Cornmeal was sifted to pass 18 mesh. Ingredients were dry-blended in a Hobart mixer for about 10 min. A 50% hydrolyzed PDD whey solution was sprayed onto the other ingredients with an electric sprayer to assure uniform distribution. Prepared mixtures were stored in Cryovac S bags at 5°C until needed.

Processing methods

Extrusion was performed in a 8-head Wenger X-5 extruder (Wenger Mfg., Sabetha, KS) under the following conditions: screw speed–600 rpm; feed rate–220–240 g/min; die–5/32 in. (0.4 cm); moisture content of the feed–35%. The two heads of the feed section were water-cooled while the rest were maintained at 135°C. Product temperature

Table 1–Composition of corn-soy-whey mixtures

	Mixture components (%)						
Mixture	Corn meal	PDD Whey hydrolyzed	PDD whey	Soyafluff 200T	Promine -D	Nonfat dry milk	Protein content
A	70	5	–	25	–	–	19.6[a]
B	70	22	–	–	8	–	21.0
C	70	–	–	25	–	5	20.4
D	70	–	5	25	–	–	18.3

[a] Average value for the three prepared A mixtures: A–uncooked (A-UC) = 19.3% protein; A–extruded (A-E) = 19.7% protein; A–roll-cooked (A-RC) = 19.9% protein.

[1] Present Address: J.M. Aguilera, Instituto Tecnologico de Chile INTEC, Cadilla 667, Santiago, Chile

Table 2—Composition of corn-soy-whey diets

	Diet[a]						
	Casein	A-UC	A-E	A-BC	B-E	C-E	D-E
				%			
Protein source	11.6	51.5	50.7	50.3	47.6	49.0	54.4
Alphacel[b]	1.0	0.3	0.3	0.3	0.3	0.3	0.3
Corn oil[c]	7.7	6.5	6.5	6.5	6.5	6.5	6.5
Vitamin mix[d]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mix[e]	5.0	3.8	3.8	3.8	4.2	3.8	3.8
Corn starch[f]	73.7	36.9	37.7	38.1	40.4	39.4	34.0

[a] Vitamin-free casein (General Biochemicals, Chagrin Falls, OH)
[b] A cellulose product (Nutritional Biochemicals Corp., Cleveland, OH)
[c] Mazola (Corn Products, NY)
[d] Vitamin mix AOAC, Cat. No. 40055 (Teklad, Test Diets, Madison, WI)
[e] Mineral Salt mix, Roger-Harper (Teklad Test Diets, Madison, WI)
[f] Standard type

Table 3—Analyses of ingredients for corn-soy-whey mixtures

Ingredients	Moisture (%)	Protein (%)	Fat (%)	Lactose (%)	Ash (%)
Corn[a]	9.3	9.8	4.1	–	1.3
Soyafluff 200T[b]	6.3	53.2	3.2	–	6.5
Promine-D[c]	7.2	93.4	0.2	–	4.1
ForeTein 35[d]	3.8	36.5	3.4	56.6	2.2
Nonfat dry milk[e]	8.8	35.9	0.8	52.3	7.7

[a] Local store (Ithaca, NY)
[b] Central Soya (Chicago, IL)
[c] Central Soya (Chicago, IL)
[d] Foremost Foods (San Franciso, CA)
[e] Dairy Plant, Cornell Univ. (Ithaca, NY)

Table 4—Amino acid composition of raw materials for corn-soy-whey mixtures (g/16 gN)

Amino acid	Corn	PDD whey	Promine-D	Soyafluff 200T	Nonfat dry milk
			Essential amino acids		
Lysine	3.02	10.45	5.40	5.72	6.79
Threonine	3.67	7.98	3.50	4.48	4.10
Valine	4.10	5.22	4.67	4.80	6.15
Methionine	1.80	2.56	1.30	1.47	3.34
Isoleucine	3.02	7.81	4.66	4.59	4.99
Leucine	13.45	11.61	8.11	8.12	9.97
Phenylalanine	4.55	3.29	5.32	5.05	4.63
Tryptophan	0.58	1.60	1.50	1.10	1.75
			Nonessential amino acids		
Histidine	2.76	1.81	2.17	2.27	2.36
Arginine	4.42	2.61	6.96	6.74	1.96
Aspartic acid	6.92	12.61	12.03	12.63	7.54
Serine	5.18	6.68	5.31	3.87	5.20
Glutamic acid	20.32	17.56	20.10	19.30	21.48
Proline	8.74	5.38	5.18	4.89	9.03
Glycine	3.68	2.24	4.00	3.92	1.74
Alanine	7.37	5.30	3.92	4.40	3.04
Tyrosine[b]	3.35	3.20	3.92	3.88	4.50
1/2 Cystine[b]	1.34	1.91	1.20	1.92	0.79
Ammonia	1.73	1.32	0.41	N.D.[a]	0.85

[a] Not determined
[b] Cystine and tyrosine have a sparing effect on methionine and phenylalanine, respectively, and thus can be considered as semiessential (FAO, 1970).

varied between 85 and 90°C. Total residence time of the product in the extruder was about 30 sec. Since this is a laboratory extruder, extrapolation of data to larger extruders should be carefully analyzed.

For roll-cooking, a laboratory atmospheric double drum-drier with two 6 in. (15.24 cm) diam × 5 5/8 in. (14.3 cm) length chrome plated drums (Blaw-Know Co., Buffalo, NY) was used. It was operated at a steam pressure of 40 psig (2.9 kg/cm²) and drum speed of 4 rpm that exposed the material to heat for 10–15 sec. Feed moisture content was adjusted to 35%.

After processing, mixtures were dried to approximately 8% mositure in a 45°C oven and ground into a fine powder for analysis.

Only mixture A was extruded and roll-cook, (Mixtures A-E and A-RC). Mixtures B, C and D were only extruded (B-E, C-E and D-E). Uncooked mixture A (A-UC) was used as reference for evaluating processing effects.

Analytical tests

Moisture, nitrogen, fat and ash analyses were performed according to AOAC (1975). The factor 6.25 was used to convert Kjeldahl nitrogen to protein in the mixtures.

Lactose and its products from hydrolysis were analyzed by thin-layer chromatography following a procedure by Wierzbicki and Kosikowski (1971). Direct reading of the optical density of the spots was obtained from a spectrodensitometer model SD 3000 (Schoefell Instrument Corp., Westwood, NJ).

All mixtures and raw materials were analyzed for their amino acid composition. The amino acids were obtained from acid hydrolysis and analyzed according to the method of Moore and Stein (1957) using a Beckman Model 120 C amino acid analyzer (Beckman Instruments, Palo Alto, CA). Tryptophan was determined according to Spies and Chambers (1948, 1949)—procedure N.

Functionality tests

Water solubility and water absorption indexes were determined by the method of Anderson et al. (1959). Nigrogen solubility index (NSI) was determined according to method BA 11-65 of the AOCS (1970).

Nutritional evaluations

To determine the nutritional value of the mixtures, a rat feeding experiment was performed.

Seven groups of six weanling male rats of the Sprague-Dawley strain (Holtzman Lab. Co., Madison, WI) with an average body weight of 50g were assigned randomly to experimental diets after feeding them for 3 days on a commercial stock diet. Each rat was housed separately in wire-bottomed cages and fed from porcelain food cups covered with a wire screen to prevent spillage. Drinking water was available from bottles attached to the cages.

Semi-purified diets contained approximately 10% protein. The composition of the experimental diets and the reference standard casein diet are presented, Table 2. Diets were fed to the rats *ad libitum* during 28 days. Individual comsumption and body weights were recorded at 2- or 3-day intervals.

Protein efficiency ratio (PER) was calculated from the formula:

$$\text{PER} = \frac{\text{Gain in body weight}}{\text{Weight of protein consumed}}$$

Data on PER was treated statistically by an analysis of variance and a Tukey test of significance.

RESULTS

HIGHEST LACTOSE HYDROLYSIS was achieved in a 50% PDD whey solution using 80 mg of enzyme/100g sample. Fifty-six percent conversion of lactose into glucose and galactose was obtained under these conditions. This material was used in the mixtures.

Statistical analysis of data of a 10 member sensory evaluation panel showed that this sample of hydrolyzed PDD whey was not significantly different ($P > 0.05$) from an unhydrolyzed sample that had 7% of the whey replaced by sugar.

Proximate analysis of the ingredients is shown in Table 3. Nonfat dry milk and PDD whey have very similar lactose and protein content although they differ markedly in their ash and fat content.

Amino acid analyses of raw materials are presented in Table 4. As expected, corn is particularly deficient in lysine

and tryptophan. PDD whey shows a very high lysine content. Its tryptophan, although much higher than corn, is lower than the 3.3g/16g N value listed for the product.

Soybean products present a better balanced amino acid pattern than corn, but are still deficient in sulfur amino acids. Mixtures of corn and soy should be complementary in this regard. Nonfat dry milk has a well balanced amino acid pattern favored in this case by an abnormally high methionine content that commonly constitutes its limiting amino acid together with cystine.

Amino acid composition of the mixtures is shown in Table 5 together with the amino acid pattern recommended by the FAO-WHO expert group (FAO, 1973). A significant improvement in the level of all the essential amino acids except leucine can be observed, based on the essential amino acid profile of corn. Lysine content was raised from a value of 3.02g/16g N in mixture A-E. As a consequence, sulfur amino acids became the limiting amino acids in all the mixtures except B-E where lysine is limiting. The excessively high leucine content of corn was reduced to 10.88g/16g N or less.

Extrusion or roll-cooking of mixture A reduced the quantity of essential amino acids as observed in Table 5. Roll-cooking was less deleterious than extrusion. Results from statistical analysis on PER values, shown in Table 6, demonstrated no significant difference ($P > 0.05$) between the seven samples. All were equally satisfactory as casein (PER = 2.5).

Water absorption (WA), water solubility (WS), and nitrogen solubility (NS), indexes are presented in Table 7. Extrusion of mixture A increased WA three times while roll-cooking doubled it. Water solubility was less affected by processing and sample values showed only small variations. As expected, processing of mixture A reduced the nitrogen solubility index in the raw mixture by almost half.

Table 5—Amino acid composition of raw and processed corn-soy-whey mixtures (g/16g N)

Amino acid	A-UC	A-E	A-RC	B-E	C-E		FAO[a]
Essential amino acids							
Lysine	5.07	4.54	4.81	4.32	5.08	4.52	5.44
Threonine	4.21	4.27	4.36	4.86	3.90	4.10	4.00
Valine	5.02	4.56	4.83	5.36	4.85	4.53	4.96
Methionine	1.71	1.51	1.46	1.95	1.60	1.76	(3.52)[b]
Isoleucine	4.75	4.04	4.65	4.91	4.50	3.91	4.00
Leucine	10.45	9.80	10.03	10.88	9.85	9.61	7.04
Phenylalanine	5.02	4.64	4.99	4.34	5.04	4.59	(6.08)[b]
Tryptophan	0.87	0.85	0.87	0.92	1.18	1.13	0.96
Nonesential amino acids							
Histidine	2.48	2.25	2.36	1.88	2.42	2.18	
Arginine	6.05	6.41	6.18	4.50	5.85	6.25	
Aspartic acid	10.79	10.80	10.92	12.46	10.26	10.39	
Serine	5.34	5.41	5.19	4.89	5.24	5.42	
Glutamic acid	16.56	20.16	20.16	19.80	19.94	19.72	
Proline	6.16	6.05	5.14	6.00	4.65	6.41	
Glycine	3.92	3.77	3.77	3.11	3.84	3.92	
Alanine	5.80	5.45	4.98	5.38	5.32	5.46	
Tyrosine[c]	3.94	3.73	3.83	3.02	3.67	3.55	
1/2 Cystine[c]	1.26	1.27	1.11	1.30	1.08	1.06	
Ammonia	0.83	1.04	1.18	1.04	1.11	1.01	

[a] FAO (1973)
[b] (Methionine + cystine) and (phenylalanine + tyrosine)
[c] Cystine and tyrosine have a sparing effect on methionine and phenylalanine, respectively, and this can be considered as semiessential (FAO, 1970).

Table 6—Biological response of rats fed various corn-soy-whey diets[a]

	Experimental diet	Weight gain (g)	Protein intake (g)	PER
I	Casein	129.9	44.5	2.50
II	Mixture A—uncooked	111.3	39.8	2.41
III	Mixture A—extruded	108.6	34.9	2.66
IV	Mixture A—roll-cooked	138.4	48.0	2.47
V	Mixture B—extruded	110.7	39.7	2.39
VI	Mixture C—extruded	116.9	39.3	2.54
VII	Mixture D—extruded	146.4	46.5	2.70

[a] Six rats per d etary treatment for 28 days

Table 7—Water absorption (WA), Water (WS), and Nitrogen (NSI) solubility indexes of corn-soy-whey mixtures

Mixture	WA Gel weight, g/g dry weight	WS % of dry sample in supernatiant	NSI % soluble nitrogen
A-UC	1.55	12.8	24.9
A-E	5.05	10.2	14.2
A-RC	2.86	15.4	16.5
B-E	3.16	14.9	25.0
C-E	4.66	13.2	13.5
D-E	4.45	12.2	15.0

DISCUSSION

LACTOSE HYDROLYSIS with Maxilact lactase was affected by pH and neutralization with sodium hydroxide. Higher conversion to monosaccharides could be acieved by adjusting the pH closer to 7.0 and by using potassium hydroxide (Bouvy, 1975).

Increase in sweetness through lactose hydrolysis is highly desirable since the acceptability of the mixtures can be improved if sweetened (Senti, 1969). At present sugar prices, lactose hydrolysis in whey followed by demineralization and concentration procedures may be proven economically feasible. The absence of this type of whey product in the market required that hydrolysis be preformed on redispersed PDD whey.

Not only deficiencies but also excesses of amino acids may be detrimental besides being wasteful. Leucine, present in large amounts in corn (Table 4), increases the requirements for valine or isoleucine. Thus, mixtures are not only beneficial because they increase the levels of deficient amino acids but also decrease total leucine content.

Examination of the amino acid patterns of the mixtures and their comparison to the FAO/WHO pattern assures a significant improvement in the nutritional value of corn. The PER of corn varies between 1.40 and 1.60 (FAO, 1970) while all the mixtures prepared, regardless of processing and raw material combinations, have a PER similar to that of casein. Since sulfur amino acids are limiting in all samples but mixture B-E, the reduction in lysine content shown in Table 5 is not followed by a corresponding lower PER. The nutritional evaluation also showed that the levels of whey products used in diets did not induce any abnormality in the growth pattern of the rats, such as diarrhea (Womack and Vaughan, 1974).

Functionality is only slightly affected by lactose hydrolysis as can be inferred by comparing mixtures A-E and D-E in Table 7. Use of higher levels of hydrolzed PDD whey (mixture B, 22%, whey product), reduces the water absorption capacity but improves water solubility and nitrogen solubility. This occurs because PDD whey has minimal water absorption, is exceptionally water soluble, and has a high NSI.

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LEONARD STOLOFF and MARY TRUCKSESS
Bureau of Foods, Food & Drug Administration, Washington, DC 20204
PERRY W. ANDERSON and ELMER F. GLABE
Food Technology Laboratory, Chicago, IL 60631
and JAMES G. ALDRIDGE
Buffalo Testing Laboratories, Buffalo, NY 14216

DETERMINATION OF THE POTENTIAL FOR MYCOTOXIN CONTAMINATION OF PASTA PRODUCTS

ABSTRACT

Freshly extruded macaroni and noodle doughs, dusted with flour to which spores of the toxigenic molds *Aspergillus flavus, A. clavatus, A. versicolor, Penicillium urticae, P. cyclopium* and *P. citrinum* had been added, were held at temperature-humidity combinations [30, 35, 40°C and 85, 90, 95% relative humidity (RH)] expected to simulate conditions that might prevail in a fully loaded pasta drier shut down by a power failure. A sour odor at the highest RH (95%) suggested initial bacterial action. Mold growth was observed on all products under all conditions; the extent of growth after 5 days was directly related to both temperature and RH. Under all conditions, *A. flavus* was the dominant mold; of the mycotoxins (aflatoxins, sterigmatocystin, patulin, penicillic acid, citrinin) expected from the species included in the inoculum, only aflatoxins were detected. At the selected condition of 30°C and 95% RH, mold growth was evident after 24 hr and aflatoxins could be detected after 48 hr. The level of aflatoxins was low for the extent of mold growth observed, probably as a result of the presence of the mixed culture. Because of the souring and obvious mold growth, none of the pasta could be considered usable even before aflatoxin was detected. When the aflatoxin-contaminated pasta was cooked 10 min in boiling water, approximately 1/3 of the aflatoxin was found in the water. Based on these studies and the rarity of encountering aflatoxin in wheat, the possibility of finding aflatoxin in commercial pasta products is highly remote.

INTRODUCTION

EXTRUDED WET PASTA on trays or hung from racks in a drying oven forms a natural filter for the air forced through the system, adding to the normal load of mold spores carried by the pasta ingredients. Mold profile studies of pasta products (Christensen and Kennedy, 1971; van Walbeek et al., 1968; Mislivec, 1976b) have demonstrated the presence of propagules of various toxigenic molds, including the mold species, *Aspergillus flavus*, that produces aflatoxins. Given the normal low moisture levels of the flour and of the dried pasta product (<12% moisture), and the relatively short residence time (<12 hr) of the pasta at high moisture levels, growth of the mold propagules would not be expected. In fact no aflatoxins have been found in any of 101 samples of wheat paste products analyzed for these toxins by the Food & Drug Administration (1975–1976). There is, however, a theoretical possibility that mold growth with toxin production could occur should a power failure take place while a fresh load of wet pasta dough is in a drying oven.

EXPERIMENTAL

Phase I

To explore this theoretical potential, extruded macaroni (durum semolina and water) and egg noodle (durum semolina plus egg yolk solids and water) doughs, dusted with spores of toxigenic molds, were held at all combinations of 30, 35 and 40°C and 85, 90 and 95% relative humidity (RH).

The water content of each dough was adjusted for best extrusion consistency (about 30%) and the dough was extruded in the form of both spaghetti and elbow macaroni. Portions (250g) of each extruded product were spread on stainless steel wire trays (11 in. × 15 in.) and dusted as evenly as possible with 0.1g of flour containing the following approximate numbers of viable mold spores: *A. flavus*, 3.5×10^6; *A. clavatus*, 9.8×10^4; *A. versicolor* 2.9×10^4; *Penicillium urticae*, 6.5×10^3; *P. cyclopium*, 6.1×10^4; and *P. citrinum*, 3.6×10^4. These molds were demonstrated producers of aflatoxins, patulin, penicillic acid and citrinin, respectively. Loaded, dusted trays were transferred to controlled atmosphere cabinets (Aire-Regulator, Food Technology, Inc., Chicago, IL 60631) set for the desired temperature-humidity conditions. Daily observations were made for 5 days for gross appearance and evidence of mold growth or decomposition; on the fifth day all samples were removed for mycotoxin analysis.

Phase II

From the foregoing experiments, a temperature of 30°C and RH of 95% were chosen as likely to result in extensive mold growth on the pasta products and representing, according to practicing experts in the field (Hoskins, 1976; Skinner, 1976), a set of conditions likely to be encountered in a pasta pre-dryer a short time after a power failure. Macaroni and noodle doughs were prepared as before but the shape was confined to "elbows," the spore inoculum density was 1/3 of that used in the initial experiments and the monolayer load per tray was increased to 1.5 kg. One tray of each type of dough was immediately dried to a moisture content of approximately 10%; the remaining trays were placed in the controlled atmosphere cabinet set at the selected temperature and humidity. After 24, 48, 72 and 96 hr exposure to the selected conditions, one tray of each type of pasta dough was removed and dried to approximately 10% moisture in the same manner as the first set of trays of pasta. Before drying, each tray of pasta was observed for mold growth and a portion removed for moisture determination; after drying a portion was removed for mycotoxin analysis. The remaining product was used for Phase III.

Phase III

To determine the effect of cooking on any aflatoxins that had been produced, 400g of dried pasta was added to approximately 3L of boiling tap water and briefly stirred. The pot of boiling water was removed from the heat 10 min after boiling resumed and the contents were drained through a collander. The cooked pasta was weighed and the volume of cooking water measured after it had cooled to room temperature. Portions of each were taken for aflatoxin analysis.

Analytical methods

The wet pasta products from Phase I were analyzed for the presence of aflatoxins, sterigmatocystin, patulin, penicillic acid and citrinin by extraction with water/chloroform and thin-layer chromatography of the concentrated extract. Portions of each chloroform extract were spotted on separate silica gel thin-layer plates, together with appropriate reference standards, for qualitative determination of the presence of aflatoxins (AOAC, 1975, Section 26.019(b)), sterigmatocystin (Stack and Rodricks, 1971), patulin (Scott and Kennedy, 1973), penicillic acid (Ciegler and Kurtzman, 1970) and citrinin (Hald and Krogh, 1973), following only the thin-layer chromatographic steps of the referenced methods.

Because aflatoxins were the only mycotoxins detected, the Phase II and III quantitative analyses were confined to this group. The method used was one originally adopted for peanut products (AOAC, 1975, Sections 26.014–26.019) but found to be generally applicable to grains. It was modified by grinding the sample with the extracting chloroform in a Waring Blender before addition of the specified

amount of water. Prior addition of the water had resulted in a gummy mass that could not be ground. This modification also eliminated the need to dry grind each sample.

The cooking waters were extracted by liquid/liquid partition with chloroform in a separatory funnel, followed by silica gel column clean-up of the chloroform extracts in the same manner as with the extracts from the pasta.

Mycological examination

When mold growth was encountered, typical portions of pasta were examined microscopically for determination of the identities of the molds present.

RESULTS & DISCUSSION

Phase I

The results observed were independent of dough shape and composition, and were related only to temperature and humidity. The observations are therefore presented for the four combinations of shape and composition as if they were one (Table 1). Mold growth was observed after 5 days under all test conditions, ranging from that barely visible to the naked eye at the lowest humidity-temperature combination to prolific growth at the highest humidity-temperature combination. Although *Rhizopus* sp., *Mucor* sp., *A. niger*, *P. urticae* and *P. viridicatum* were identified in some samples, they were clearly dominated in all cases by *A. flavus*. These observations are consistent with the published temperature and water activity optima and limits for the growth of *A. flavus* (Diener and Davis, 1967; Northolt et al., 1976; Schindler et al., 1967; Schroeder and Hein, 1967; Sorenson et al., 1967) and its competitive reputation. The production of detectable amounts of aflatoxins is related to the extent of mold growth, except at the highest temperature (40°C) where abundant mold growth is associated with a trace of aflatoxins or no detectable aflatoxins. This is in agreement with the reports that 40°C exceeds the temperature optimum for aflatoxin production, but not for mycelium production. These observations plus the technological considerations given in the introduction were the basis for selecting 30°C and 95% RH for Phase II of the study.

A strong, sour odor was noticed with an RH of 95% at all temperatures after 24 hr incubation. The sour odor was less intense with an RH of 90% and was barely noticeable with 85% RH. Bacterial activity was suspected but not confirmed. At 95% RH the pasta lost moisture (Table 2). Thus, the initial water activity was greater than 0.95, adequate for the growth of most bacteria (Duckworth, 1975). Souring of this magnitude, without visible mold growth, should provide a strong deterrent to the use of pasta dough held undried for a prolonged period.

Phases II and III

The progression of souring and mold development followed the same course as in Phase I for the temperature-humidity condition selected. The quantitative determination of aflatoxins, however, showed surprisingly low levels for the magnitude of mold growth observed. This could have been caused by failure of the analytical technique or by the mixture of molds present as observed by Christensen et al. (1973) in studies of stored grain with naturally mixed mold populations.

The analytical technique was eliminated as a cause by culturing *A. flavus* alone on wet pasta; aflatoxins in the high μg/g range could be recovered from the pasta after 72 hr incubation. Subsequent studies (Mislivec, 1976a) showed a marked interaction between the mold species used for this work, resulting in a reduction of detectable aflatoxins.

Although pasta products this badly damaged would not conceivably be used as food, the cooking experiments were carried through to obtain information on aflatoxin stability and distribution under conditions of food preparation. There was an obvious distribution of aflatoxin between the cooked pasta and drained water (Table 3); about 1/3 of the aflatoxin was found in the drained water. Given the analytical uncer-

Table 1—Relative mold growth and aflatoxin production on mold-inoculated, extruded pasta dough held 5 days at various temperature and RH combinations

Temp (°C)	RH (%)	Mold growth[a]	Aflatoxin production[b]	Molds detected
30	85	1+	ND	*A. flavus*,[c] *A. urticae*, *P. viridicatum*
30	90	2+	ND	*A. flavus*[c]
30	95	5+	+	*A. flavus*[c]
35	85	1+	ND	*A. flavus*[c]
35	90	2+	ND	*A. flavus*[c]
35	95	5+	+	*A. flavus*[c]c
40	85	3+	ND	*A. flavus*,[c] *Rhizopus* sp.
40	90	5+	TR	*A. flavus*,[c] *A. niger*, *Mucor* sp.
40	95	5+	ND	*A. flavus*,[c] *Rhizopus* sp.

[a] Barely visible mold growth = 1+; abundant mold growth = 5+
[b] TR = trace; ND = not detectable; + = easily detectable quantities
[c] Dominant mold

Table 2—Relative mold growth on and moisture level of mold-inoculated, extruded macaroni and noodle doughs held for various times at 30°C and 95% HR and level of aflatoxins produced in dried products

Dough type	Incubation time (hr)	Moisture (%)	Mold growth[a]	Aflatoxins[b] in dried product (ng/g)
Macaroni	0	31.3	–	ND[c]
	24	28.2	2+	ND
	48	27.8	2+	1.0
	72	26.9	3+	1.5
	96	26.3	4+	2.4
Noodle	0	26.2	–	ND
	24	23.8	2+	ND
	48	23.4	3+	ND
	72	23.2	4+	2.2
	96	22.6	5+	2.6

[a] Barely visible mold growth = 1+; abundant mold growth = 5+
[b] Primarily aflatoxin B_1
[c] ND = not detectable

Table 3—Distribution of aflatoxins between cooked pasta and cooking water after holding 400g dried, extruded macaroni and noodle doughs 10 min in 3L boiling water

Dough type	Pasta			Drained water	
	Dried (μg)	Cooked (μg)	Cooked (%)	(μg)	(%)
Macaroni	0.37	0.14	38	0.16	43
	0.58	0.34	59	0.20	34
	0.95	0.47	49	0.34	36
Avg			49		38
Noodle	0.90	0.53	59	0.30	33
	1.03	0.75	73	0.25	24
Avg			66		29

tainty (a coefficient of variation of about 30%) of the method used (Schuller et al., 1976), the apparent difference between macaroni and noodle doughs in retaining aflatoxins may not be real, nor is the apparent loss of aflatoxin outside the possibility of analytical error.

Since aflatoxin contamination of marketable pasta products appears unlikely as a result of faulty manufacture and since aflatoxin contamination of wheat is so rarely encountered (Shotwell et al., 1977; Stoloff, 1976), the possibility of finding aflatoxin in pasta products is highly remote.

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EXTRUSION/ROLL-COOKING CORN-SOY-WHEY MIXTURES . . .From page 227

A decrease in nitrogen solubiity index is often used as a measurement of the amount of heat treatment a product received. Extrusion and roll-cooking of mixture A lowered the NSI of the raw mixture accordingly. The similarity of NSI values for processed mixtures A, C and D indicates that heat treatments were alike and thus, comparisons made between all products are sound. As already shown for NSI, variations in functional properties are due to the different makeup of the mixtures.

Processing caused a slight darkening of the mixtures with no differences observed between roll- and extrusion-cooking. This browning, however, may be related to a reduction in lysine.

Fully-cooked mixtures that use different proportions of corn, soy and whey can be developed to meet required functional properties by using Response Surface Analysis as demonstrated by Aguilera and Kosikowski (1976). Such mixtures are particularly suited to be used in formed products such as snacks or breakfast cereals and can also be exploited as ingredients in its floury condition.

Lactose-hydrolyzed whey products were shown to be good nutritional supplements for corn and to increase the sweetness of the mintures. Roll- and extrusion-cooking did not impair the nutritional value of the high protein quality raw materials and both contributed to the development of specific functional characteristics.

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M. A. HANNA[1], L. D. SATTERLEE[2] and K. W. THAYER[3]
Food Protein Research Group, University of Nebraska, Lincoln, NE 68583

SENSORY AND SELECTED TEXTURAL PROPERTIES OF PASTA FORTIFIED WITH PLANT PROTEINS AND WHEY

ABSTRACT

Sensory and mechanical properties evaluation were run on pasta products fortified with eight different high protein sources. Some mechanical and compositional properties were analyzed for the expressed purpose of using them as predictors of texture as evaluated by a trained sensory panel. The combination of the rupture force and a creep recovery factor proved to be a relatively good predictor of the textural quality of the cooked products. An analysis of variance indicated that in order to obtain an acceptable protein fortified pasta product, attention should be given to the source of protein. The level of substitution of fortification was found to be of little significance as far as texture is concerned.

INTRODUCTION

PASTA, whether it be in the form of a flat noodle, elbow macaroni or spaghetti, is a food product which is consumed world-wide. Pasta, which is based upon durum wheat semolina as its main ingredient, can also have as ingredients materials such as hard and/or soft wheat flour and various protein sources. To improve the nutritive quality of the final pasta product through the addition of high quality protein sources (milk powder, dried milk whey, or fish flour) and sources rich in protein (soy flour, soy concentrate, yeast protein) has been the goal of much research both within the United States and around the world.

After investigating several food systems which are designed to deliver protein in both quantity and quality, Clausi (1971) chose elbow macaroni as the food system since it is considered to be a food with universal appeal and is a fabricated food which can be manufactured from materials grown and/or processed locally. The pasta product he tested was based upon wheat semolina, corn flour and soy flour. Other researchers, Glabe et al. (1967) and Paulsen (1961) have produced fortified pasta products using nonfat dry milk and soy flour, respectively. Both of the above authurs stated that the fortified pasta products tended to yield a higher solids-in-cooking water value than did unfortified pasta and that their overall acceptability and texture were not as good as evidenced in the unfortified product.

McCormick (1975) described the use of a commercially produced yeast protein (52% protein) and soy isolate to enhance the nutritional value of elbow macaroni. The combination of 85% semolina, 9% yeast protein and 6% soy isolate gave a final product with a corrected PER of 2.23 and a protein content of 24%, whereas a product made from 94% semolina and 6% yeast protein had a PER of 2.0 and an 18% protein content. Sensory evaluation of the two products after cooking indicated the products were slightly elastic and had a favorable cream color and slightly "meaty" taste. The product containing yeast and soy protein absorbed more water upon cooking than did the control or the yeast protein product. Using an Instron Universal Tester with a back extrusion cell, the "strength" of the control and yeast protein product were similar and the yeast plus soy fortified pasta was tougher.

Matsuo et al. (1972) found that for a number of durum wheat varieties, protein quantity had a pronounced effect of the cooking quality of spaghetti. In all cases the cooking quality improved with higher protein content. This is substantiated by Dahle and Muenchow (1968) who removed various amounts of lipid and protein from spaghetti before cooking. This removal resulted in increased amounts of amylose in the cooking water and impaired cooking quality.

Molina et al. (1975) investigated the influence of thermal processing on the quality of a semolina based pasta containing corn flour. A pasta product containing 60% corn flour, 40% semolina and 0.3% lysine possessed the best amino acid profile and the best PER (1.91). Maximum heat treatment of the corn flour prior to incorporating it into the pasta lowered the solids lost during pasta cooking and enhanced the organoleptic evaluation score of the cooked product.

This project studied the effects of the previously mentioned proteins on the sensory and physical characteristics of pasta products in the form of elbow macaroni. Only limited research has been reported on the modeling of physical characteristics of pasta products.

Binnington et al. (1939) determined a "tenderness score" for macaroni products using a compression/creep-type test. Karacsonyi and Boros (1961) developed a torsionmeter to mechanically measure macaroni and spaghetti quality. Holliger (1963) developed an apparatus to measure the stretching and bending properties of cooked and uncooked spaghetti. A "tenderness index" was developed by Matsuo and Irvine (1969) to describe the tenderness of cooked spaghetti. Their tenderness was an indication of the length of time it took a loaded cutting edge to cut through the specimen. Shimizu et al. (1958) studied some physical properties of noodles made from Japanese domestic wheat flour. They found that the elastic modulus, breaking strength and stress relaxation increased with increased crude protein content and boiling time.

Walsh et al. (1971) used linear programming to study the interrelationship among extruding operations and finished spaghetti quality. Processing constraints and quality equations were used to construct an L.P. matrix to describe the extrusion-quality system. Further refinements were recommended for improved accuracy.

In this project, elbow macaroni fortified with a number of different proteins was subjected to a taste panel evaluation and various mechanical tests to determine if there were some characteristic sensitive to the protein source and/or quanity of protein added. An investigation was made to determine a relationship between the taste panel evaluation of texture and certain mechanical and ingredient properties.

MATERIALS & METHODS

Protein sources used

Both commercial and experimental protein sources were used in this study. The commercial protein sources were: spray-dried sweet whey (19% protein), nonfat dry milk (33% protein), soy concentrate (70% protein), soy grits (50% protein), and soy isolate (91% protein). The soy grits had a protein digestibility index (PDI) of 60 and were only moderately heat treated (hexane extracted). The soy isolate was Pomine D from Central Soya, Chicago, IL and the soy concentrate was from Griffith Laboratories, Chicago, IL. The experimental protein sources used were a dry edible bean protein concentrate (84% protein) as described by Satterlee et al. (1975), cottonseed meal (56% protein) supplied by the Oilseed Products Laboratory, Texas A&M University and a yeast protein concentrate (70% protein). The cottonseed meal had a nitrogen solublity index (NSI) of 28.54%. The solvent used to remove the oil from the cottonseed to produce the meal was hexane. The yeast protein concentrate used was produced by extracting ham-

[1] Agricultural Engineering Dept., University of Nebraska-Lincoln.
[2] Food Science and Technology Dept., University of Nebraska-Lincoln.
[3] Agricultural Engineering Dept., University of Nebraska-Lincoln.

Fig. 1—La Parmigina laboratory model pasta extruder.

mer-milled brewers yeast with aqueous sodium hydroxide pH 12.5, then precipitating the solubilized yeast protein with heat (90°C) at pH 4.0. The protein was spray dried.

Pasta production

The pasta was produced in the form of elbow macaroni, using durum semolina as the base, The semolina was a U.S. source from North Dakota durum wheat milled in Minneapolis, MN. The control pasta (100% semolina) was produced by adding 47 ml water to 150g of semolina (14% moisture), mixing in a Hobart Kitchen Aid Mixer Model K45 for 15 min and then extruded in a La Parmigina laboratory model pasta extruder (Fig. 1). The extrusion die was brass and the individual nozzles had inner diameters of 3.81 mm and outer diameters of 5.08 mm. While the pasta was being extruded from the die, room air was blown over the product to surface dry the product. The macaroni was then placed in a cabinet equilibrated to 33°C and 95% relative humidity. Over a time period of 24 hr the relative humidity was slowly lowered to 65%. After 24 hr, the product was removed from the cabinet and allowed to equilibrate to room temperature (23°C) and relative humidity (45–55%) for an additional 24 hr. After drying, the product was stored in plastic bags at room temperature. The final moisture content was of the order of 7–8% on the dry basis.

Fortified pasta products were made using semolina as the base and replacing a portion of the semolina with the protein source. Each protein sources was added at three replacement levels (6.66, 13.33, 20%). The amount of water added to the semolina-protein mixture was also varied so that all doughs would have the same consistency prior to extrusion. This consistency is best described as "soft dough particles" 2–3 mm in diameter that were moist throughout. The appearance and texture of the macaroni being extruded from the die was also noted for each protein source at each level tested.

Moisture and protein analyses

All moisture and Kjeldahl protein analyses were performed according to the AOAC (1975) procedures. The Kjeldahl factor of 5.7 was used for control pasta, a factor of 6.38 was used for nonfat dry milk and whey and a factor of 6.25 was used for all pastas containing added protein as well as the yeast protein concentrate, the soy protein products and the dry edible bean protein concentrate.

Sensory evaluation

The sensory properties of color, aroma, texture and flavor were measured on all pasta products using a five member trained panel. The sensory panel consisted of two males, ages 22 and 33, and three females, ages 22, 23 and 32. All panelists were considered average consumers of pasta. Two training sessions were held where "pasta color," texture, flavor and aroma were discussed and quantified using known samples. Two panelists were eliminated from an initial seven during the training sessions. The five remaining panelists were used on all successive panels and were considered good pasta judges. Four samples were served hot on a warming tray to the panelists at one time. The taste panel room consisted of individual booths for the panelists. The ballot used for sensory evaluation was based on a seven-point hedonic scale, with 1 being poor and 7 being excellent for color, aroma and flavor. A score of 7 indicated a bright amber color of pasta. Texture also used a seven-point scale with 1 being a mushy and 7 being a rubbery texture. An analysis of variance was used to determine the significance ($P < 0.05$) of any differences noted in the sensory scores.

Textural properties

Several different tests were run on each of the pasta products both in the cooked and uncooked form. The specimen preparation consisted of boiling the pasta for 12 min just prior to testing.

Tests were run on macaroni by placing a single macaroni on the load cell of an Instron testing machine and cutting with both a ¼ inch square metal bar indenter and a blunted 60° wedge-type metal indenter. The loading rate was 10 mm/min. The force-deformation curves were recorded and the toughness of each specimen was determined with an integrator. A series of creep tests were also run on the different pasta products. This test consisted of loading 5 macaronis, each 15 mm in length along the neutral axis, with a 750 gram dead load for 4 min and then unloading. The loading and approximately 1 min of the unloading curves were recorded.

The macaroni was tested immediately after 12 min of boiling; however, during the actual tests no attempt was made to keep the product warm or moist. Each test was repeated five times and all data were subjected to Chauvenet's criterion for rejection of data (Hetenyi, 1950).

Data and Analysis

The rupture force and toughness values were obtained from the force-deformation curves. The rupture force was taken as the peak force on the force-deformation curve and toughness was the work required to cause rupture. i.e., the area under the force-deformation curve up to the point of rupture. The rupture forces are reported in Newtons (N) and toughness is given in kilogram-meters (kg-m). The creep test gave a deformation versus time diagram from which actual creep curves were drawn. Three different values were taken from these curves, the percent recovery 15 and 30 sec after the load was removed and the strain-axis intercept corresponding to a tangent at the point of constant creep.

This analysis used multiple regression and the analysis of variance (Barr and Goodnight, 1972). An analysis of variance was used to determine whether the taste panel's evaluation or any of the mechanical measures of texture were sensitive enough to detect differences between protein sources and levels of substitution. Multiple regression analyses were run on various combinations of the physical and ingredient properties with the sensory panel's evaluation of texture as the dependent variable. Besides the rupture, toughness and creep parameters, the amount of water added in the pasta mixes, the protein content, quadratic and certain interactions and the common logarithm of the mechanical properties were considered in the regression. After some initial analyses and then careful consideration of the properties, the parameters considered in the final analysis were as given in Table 1. To compliment the regression analysis an analysis of variance was performed on the parameters considered in the regression.

RESULTS & DISCUSSION

THE ADDITION of the various protein sources to the basic pasta formulation yielded dry elbow macaroni products ranging in protein content from 14.1 to 29.4% (Table 2), as compared to a 13.5% protein content for the control pasta. The addition of the protein sources to the durum semolina caused drastic changes in water needed to form the dough

Table 1—Parameters considered in the final regression analysis

Parameter[a]	Description
Texture score	Sensory panel evaluation of texture
F_{rcb} (N), F^2_{rcb}, F^3_{rcb}, log F_{rcb}	Rupture force of cooked macaroni loaded with square bar
T_{cb} (kg - m X 10^{-4}), T^2_{cb}, log T_{cb}	Toughness of cooked macaroni loaded with square bar
$C_{r1\,5}$ (decimal), $C^2_{r1\,5}$, log $C_{r1\,5}$	Percent recovery 15 sec after creep load was removed
H_2O (gs H_2O/150g of pasta mix)	H_2O added to pasta mix
PP (percent protein)	Protein content of macaroni

[a] The superscripts indicate that the initial parameter, i.e., F_{rcb} was squared (2) or cubed (3) and log is an abbreviation for the common logarithm of the respective parameter.

prior to extrusion. When soy grits at the 20% level and soy concentrate at all three levels were added to the semolina, extra water was required to form the dough. All of the other added proteins required equal or lower amounts of water to form dough, when compared to the control. Neither the protein level of the final macaroni product, at the levels tested, nor the amount of water in the dough prior to extrusion significantly affected the color, aroma, flavor or texture of the final macaroni products. Yet the addition of various protein sources did have a significant effect on one or more of the sensory properties of the pasta in that the other contituents present in the protein sources, i.e., carbohydrates, pigments, lipid, had an effect on final product color, aroma, flavor and texture (Tables 3 and 4).

The protein sources that had the greatest detrimental effect on cooked product color were YPC, which imparted a gold-brown color and whey, cottonseed and BPC, which bleached the product. Aroma and flavor of the cooked product were drastically affected by YPC and BPC (Table 3).

Texture of the cooked macaroni, as measured by the sensory panel, was greatly affected by YPC, BPC, and soy grits, creating a softer macaroni and cottonseed which toughened the macaroni (Table 3, Fig. 2).

The variables in Table 1 were entered into a stepwise regression analysis. The regression equation for the prediction of a sensory panel's evaluation of texture of protein fortified pasta products was

$$\text{Texture Score} = 1.821 + 0.4218\ F_{rcb} + 21.79\ C^3_{r1\,5}$$

The mean values of F_{rcb} and $C_{r1\,5}$ and a comparison of the actual versus predicted texture scores are shown in Table 5. The F values for the entering values showed that after the first two variables were in solution the rest of the variables did not contribute significantly to explaining the variation in the texture score. The value of R^2 was 0.67 and the standard error of the estimate was 0.543. Figure 3 shows the texture response surface as a function of F_{rcb} and $C_{r1\,5}$. The surface was plotted using a FORTRAN program called PLOT3D (Schwarz and Heikes).

A look at the residuals of the regression equation and the plots of texture score, F_{rcb} and $C_{r1\,5}$ versus level of protein substitution in Figures 2, 4 and 5 led to an analysis of variance. The first analysis of variance considered only the protein source, quantity of protein added and the source-quantity interaction. This analysis showed that the source and not the quantity of protein generally reflected more of the differences in the sensory panel's evaluation of texture. The sensory panel's response to quantity depended on the source. Some significant interactions indicated that the quantity of protein added may or may not effect the sensory panel's evaluation and this increase in the level may or may not increase the taste panel score. Reviewing Table 4 shows that the taste panel was

Table 2—Level of replacement, quantity of water needed and the final protein content of control and protein fortified pasta

Protein source	Level of replacement (%)	Water added per 150g semolina-protein mixture (ml)	Protein content of dry pasta (%)
Control	–	47	13.5
BPC—Bean protein conc	6.7	30	18.2
	13.3	26	23.6
	20.0	23	28.4
YPC—Yeast protein conc	6.7	44	16.9
	13.3	39	19.9
	20.0	36	22.0
Cottonseed	6.7	45	16.8
	13.3	40	19.8
	20.0	35	22.7
Whey	6.7	40	14.1
	13.3	35	14.4
	20.0	32	14.7
NFDM—Nonfat dry milk	6.7	30	15.4
	13.3	29	16.8
	20.0	31	18.3
Soy grits	6.7	47	16.5
	13.3	46	19.1
	20.0	50	21.7
Soy conc	6.7	60	17.8
	13.3	65	21.6
	20.0	68	25.5
Soy isolate	6.7	35	19.0
	13.3	40	24.2
	20.0	45	29.4

Table 3—Sensory scores for control and fortified pasta products

Protein source	Level of replacement (%)	Color	Aroma	Texture	Flavor
Control	–	5.2	4.7	4.2	4.0
BPC	6.7	4.6	4.8	4.6	5.0
	13.3	4.8	4.2	3.0	3.4
	20.0	3.2	4.4	2.4	2.8
YPC	6.7	2.2	2.8	2.8	3.0
	13.3	2.0	2.2	3.0	2.0
	20.0	1.2	1.3	1.8	1.8
Cottonseed	6.7	5.0	5.4	4.2	5.0
	13.3	3.4	4.4	5.0	4.2
	20.0	2.0	4.4	5.6	3.8
Whey	6.7	3.8	5.0	4.6	4.0
	13.3	3.8	5.0	4.4	5.0
	20.0	3.8	5.4	4.6	5.8
NFDM	6.7	5.2	4.7	4.0	3.8
	13.3	5.2	4.7	3.0	4.0
	20.0	5.2	4.7	3.2	5.0
Soy grits	6.7	5.2	4.8	3.7	4.8
	13.3	4.5	4.7	3.7	4.3
	20.0	4.7	4.2	3.0	3.8
Soy conc	6.7	5.3	5.0	4.0	4.2
	13.3	4.7	5.3	4.7	3.8
	20.0	5.0	5.3	4.2	4.0
Soy isolate	6.7	5.0	5.0	4.3	4.8
	13.3	5.9	5.7	4.7	5.2
	20.0	5.8	4.7	4.7	4.7

Table 4—Analysis of variance table

Source of Variance	Texture Score d.f.	Texture Score M.S.	F_{rcb} d.f.	F_{rcb} M.S.	C_{ris} d.f.	C_{ris} M.S.
Total	135	–	109	–	73	–
Source	7	9.67*	7	0.348*	7	391.6*
Quantity	2	0.770	2	0.024*	2	122.5*
Source x Quantity	14	1.56	14	0.051*	14	26.73*
Q/BPC[a]	2	6.47*	2	0.0028	2	1.37
Q/COTT	2	2.47	2	0.062*	2	7.67
Q/GRIT	2	0.889	2	0.0040	2	1.32
Q/ISO	1	0.167	2	0.111*	2	94.4*
Q/NFDM	2	1.72	2	0.0013	2	76.0*
Q/SOYC	2	0.722	2	0.0057	2	0.35
Q/WHEY	2	0.067	2	0.168*	2	110.3*
Q/YPC	2	0.948	2	0.023*	2	18.2
Residual	112	1.09	86	0.0046	50	14.56
Grand Mean	3.85		0.455		14.6	

[a] Q/BPC denotes "quanity of BPC"
* Significant at the 0.05 level.

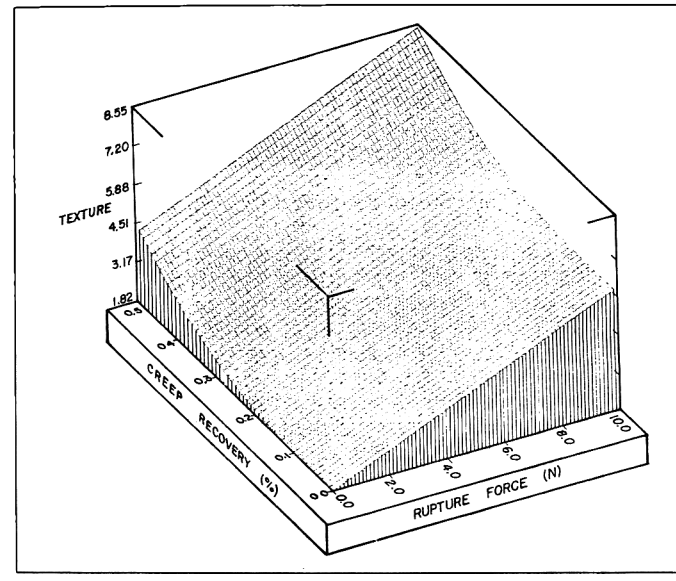

Fig. 3—Texture response surface in terms of rupture force and a creep recovery factor.

generally unable to detect differences due to the quantity of protein added. However, the rupture force and creep recovery both were significantly sensitive to the quantity added. Because of this, a second analysis of variance was conducted to determine the sensitivity of the measured parameters to the three levels of protein within each of the eight different sources.

As has been found many times in texture studies, the differential threshhold for the mechanical properties was found to be lower than that of the taste panel. These results are also included in Table 4. The taste panel was able to detect differences due to the level of BPC added but was not sensitive to the levels of the other seven sources. The rupture force was sensitive to the amounts of cottonseed, soy isolate, whey and YPC added while the creep recovery factor was sensitive to the levels of soy isolate, NFDM and whey. The sensitivity of both

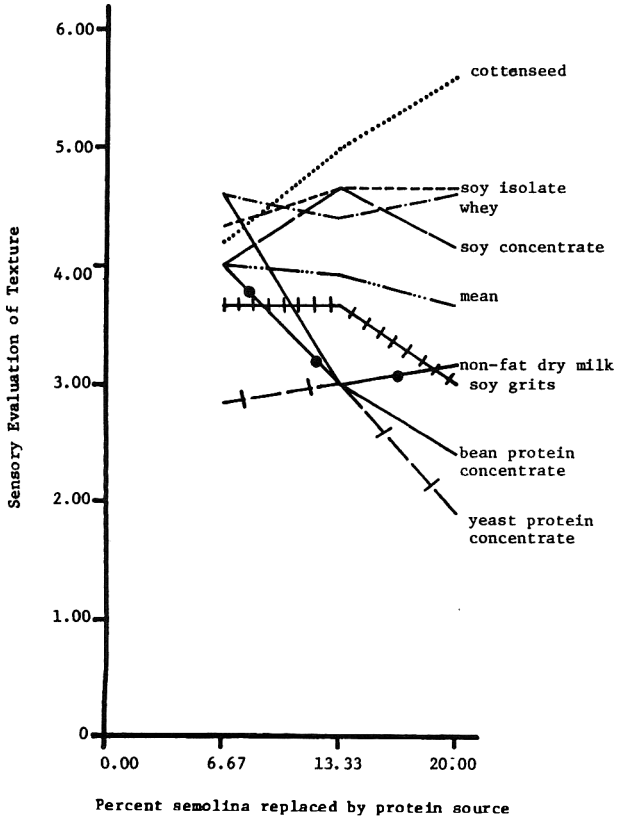

Fig. 2—Effect of quantity of protein source present on the sensory panel's evaluation of texture.

Table 5—Rupture force, creep recovery, observed and calculated texture scores for protein fortified elbow macaroni

Protein source	Semolina replaced by protein source (%)	Obs texture score	Est texture score	F_{rcb} (N)	C_{ris} (%)
Cottonseed	6.67	4.20	4.31	5.74	14.46
	13.33	5.00	4.65	6.62	11.34
	20.00	5.60	5.26	8.05	12.29
Whey	6.67	4.60	4.83	6.91	16.29
	13.33	4.40	3.83	4.41	18.96
	20.00	4.60	3.62	3.13	27.97
NFDM	6.67	4.00	3.37	3.53	13.84
	13.33	3.00	3.48	3.41	21.54
	20.00	3.16	3.66	3.73	22.94
Soy conc	6.67	4.00	4.26	5.55	16.77
	13.33	4.66	4.58	6.25	18.07
	20.00	4.16	4.40	5.87	16.60
Soy grits	6.67	3.67	3.74	4.79	17.34
	13.33	3.67	3.49	3.72	16.76
	20.00	3.00	3.68	4.20	16.02
Soy isolate	6.67	4.33	3.72	4.33	14.73
	13.33	4.66	5.24	7.60	21.39
	20.00	4.66	4.77	6.10	25.88
YPC	6.67	2.83	3.00	2.79	3.88
	13.33	3.00	2.95	2.68	5.75
	20.00	1.80	2.40	1.36	2.65
BPC	6.67	4.60	3.30	3.49	7.13
	13.33	3.00	3.26	3.37	8.10
	20.00	2.40	3.12	3.04	8.90
Control	–	4.16	4.25	2.41	40.19

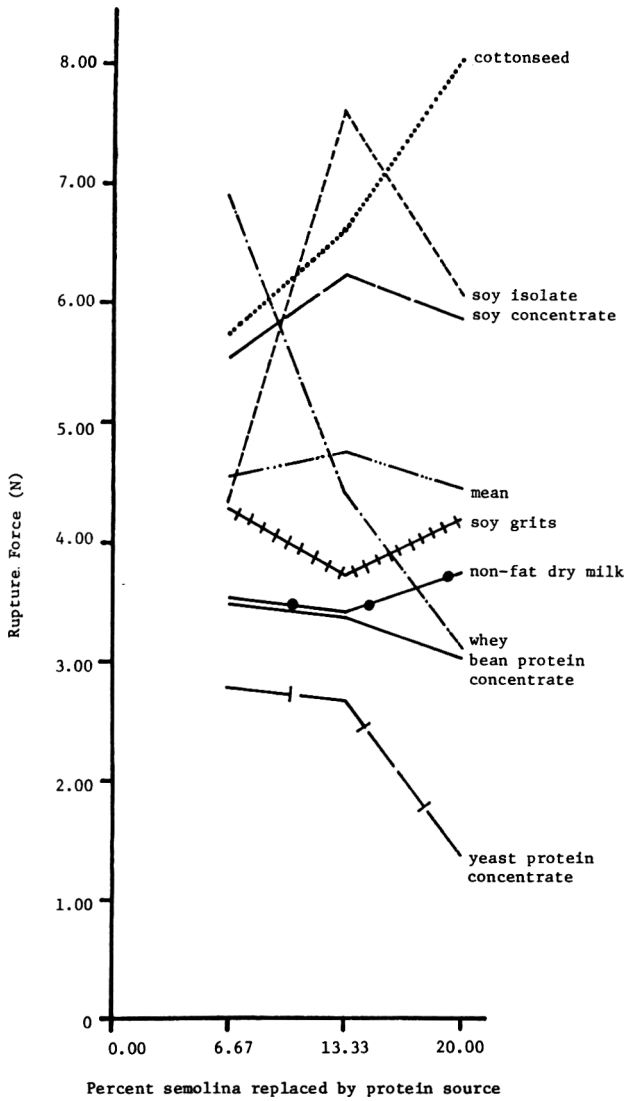

Fig. 4—Rupture force sensitivity to the amount of protein source added to the semolina.

the rupture force and creep recovery factor to the levels of soy isolate and whey is what made the overall quantities sensitive. Other than showing which sources of protein contributed to the overall significance of the quantity of protein added, little can be derived from the analysis because it does not necessarily support the trends previously discussed.

CONCLUSIONS

THE FORTIFICATION of durum wheat semolina with high quality protein sources and sources rich in protein resulted in pasta products exhibiting differences in texture as evaluated by a trained sensory panel. A fairly good correlation was obtained when two mechnical properties were used to predict the sensory panel's evaluation of texture. A regression analysis and then an analysis of vaiance indicated that the source of protein was more detectable than the level of fortification to the sensory panel. The mechanical properties were generally sensitive to both source and level. Evidently the sensory panel was more sensitive to substances in the protein source other than the protein components.

These results indicate that to obtain an acceptable protein fortified pasta product, the greatest concern should be the selection of the source, and not the detrimental effects due to the level of fortification, for the latter are insignificant.

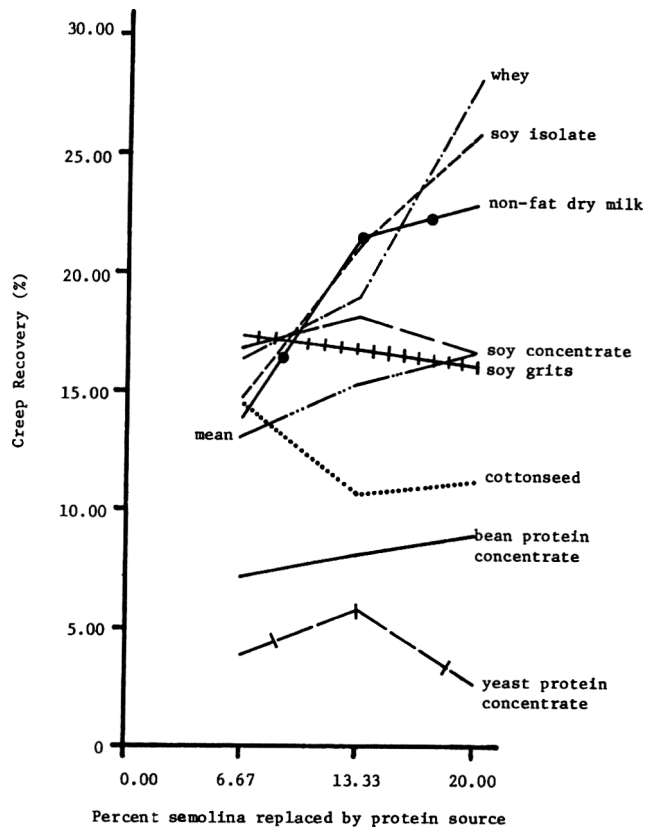

Fig. 5—Creep factor sensitivity to the amount of protein source added to the semolina.

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LUIS M. VACCAREZZA and JORGE CHIRIFE
Departamento de Industrias, Facultad de Ciencias Exactas y Naturales
Universidad de Buenos Aires, Ciudad Universitaria, Núñez, Buenos Aires, Argentina

ON THE APPLICATION OF FICK'S LAW FOR THE KINETIC ANALYSIS OF AIR DRYING OF FOODS

ABSTRACT

Analysis of literature data on air drying of foods during the first falling rate period, showed that the thickness dependence of drying rate is somewhat lower than is predicted by Fick's law. An explanation based on heat transfer effects is proposed to explain this "anomaly" and a quantitative model was accordingly developed. The model was tested with experimental data on sugar beet root drying and an excellent agreement was now found between theoretical (Fick's law) and experimental thickness dependence of drying rate.

INTRODUCTION

RECENTLY, we have shown that Fick's law of diffusion, in terms of moisture gradient, may be used to predict average drying times and moisture distributions during the first falling rate period of sugar beet root drying (Vaccarezza et al., 1974a). It was also shown (Vaccarezza et al., 1974b) that an improved analysis through Fick's law was possible when the effect of changing sample temperature was considered. Finally, a simplified water transport mechanism based on liquid diffusion was proposed for the first falling rate period of sugar beet root drying (Vaccarezza and Chirife, 1975). This work presents the results of further analysis of drying data in the first falling rate period of sugar beet root drying, and of foods in general. The anlaysis is concerned with the ability of Fick's law for predicting the influence of sample thickness on drying rate of food dehydration.

THEORY

SOLUTION of Fick's law for one-dimensional transport considering initial uniform moisture distribution and negligible external mass transfer resistance, yields the well known expression,

$$\bar{W}^* = \frac{\bar{W} - W_e}{W_o - W_e} = \frac{8}{\pi^2} \sum_{i=0}^{\infty} \frac{1}{(2i+1)^2} \exp\left\{-(2i+1)^2 \pi^2 \frac{D\theta}{L^2}\right\} \quad (1)$$

where, L = thickness of the slab; $\bar{W}$ = moisture content, dry basis; W_o = initial moisture content, dry basis; W_e = equilibrium moisture content, dry basis; θ = drying time; and D = diffusion coefficient.

For $\bar{W}^* < 0.6$ Eq (1) reduces to:

$$\bar{W}^* = \frac{8}{\pi^2} \exp(-\pi^2 D\theta / L^2) \quad (2)$$

Consequently, a straight line should be obtained when plotting the experimental data as, ln $\bar{W}^*$ versus time. Further, the slope, s, of the experimental straight lines should be a function of the square of the thickness, for Eq (2) to be satisfied.

Although several authors have utilized Eq (2) for calculating diffusion coefficients from drying data in the first falling rate period of foods drying (Jason, 1958; Saravacos and Charm, 1962; Salas and Labuza, 1968; Labuza and Simon, 1970; Chirife, 1971; Vaccarezza et al., 1974a), the thickness dependence of the experimental slope, s, was not always investigated. Instead a (thickness)^2 relationship was assumed. If we suppose a thickness dependence of the type,

$$s \propto L^{-n} \quad (3)$$

by plotting log s versus log L the value of n may be adequately calculated and compared to the expected theoretical value, n = 2. Vaccarezza (1975) for sugar beet root drying, and Jason (1958) for fish muscle, utilized this procedure for evaluating n from expression (3). Both authors reported values, which although close to 2 where somewhat lower, being 1.80 for sugar beet root and 1.85 for fish muscle. Nevertheless, the value n = 2 was adopted due to the lack of an adequate theoretical explanation at that time and since the difference was not relevant. Saravacos and Charm (1962) using a somewhat less rigorous statistical procedure, reported a (thickness)^2 dependence for their experiments on potato slab drying. They plotted θ (time to dry between specified moisture values) versus $(L)^2$, and found a straight line which was considered a proof of the validity of the (thickness)^2 dependence predicted by Eq (2). However, the straight line so obtained should pass through the origin according to this way of plotting. By inspecting the plot reported by them, it can be seen that it is not so. Consequently, we reprocessed their data according to expression (3) for calculating the correct value of n. Linear regression analysis was employed and a value of n = 1.41 was obtained, which is significantly lower than the reported value of 2. The most usual reason given in the literature for explaining an n value lower than 2 is the existence of external mass transfer resistances (King, 1968). This may account—at least partially—for the data of Saravacos and Charm (1962). The relatively low air velocity (2 m/sec) and thickness range (0.14–0.59 cm) used by these authors strongly suggest the possibility of external mass transfer resistances (Vaccarezza et al., 1974a). External mass transfer resistances are not likely, however, to explain the data reported by Vaccarezza et al. (1974) for sugar beet root. In these experiments the absence of external mass transfer resistances was theoretically (Vaccarezza et al., 1974b) and experimentally verified. Further, recent results on avocado slab drying (Alzamora, 1977) performed with very high air velocities also yielded an n value again somewhat lower than 2. Consequently, a different explanation is proposed which is based on the heat transfer effects which accompanies mass transfer during drying. For this purpose a quantitative analysis is made to account for the observed differences in the n values. Jason (1958), also suggested—qualitatively—that a heat effect may be responsible for the lower value of n.

The results reported by Vaccarezza et al. (1974a, b) and Vaccarezza (1975) will be utilized to develop a model which is based on heat transfer effects and will explain the aforementioned "anomaly." Although the model is tested only with experimental data on sugar beet root drying, it may also be valid for air drying of other foodstuffs. Vaccarezza et al. (1974a) did not find significant internal temperature gradients during sugar beet root drying, so the slab temperature may be considered to vary with time but not with position within the slab. The sample temperature was found to increase rapidly at the beginning of drying towards the air dry bulb temperature. However, the difference between the material and dry bulb temperature becomes negligible only when about 90% of the initial water has been evaporated. By this reason, during the time interval in which the slope, s, is experimentally determined, the food temperature is varying continuously being always lower than the air dry bulb temperature. If, in a first approximation, it is accepted that the slope, s, depends on (thickness)^2, it can be demonstrated (Vaccarezza et al., 1974b) that for most of the first falling rate period the following relationship is valid:

$$T^* = \frac{\pi^2 \gamma}{\beta} \frac{\bar{W}}{W_o} \quad (4)$$

where, $T^* = (T - T_\infty)/(T_o - T_\infty)$; $\beta = h2AL^2/m_{w_o} C_{p_w} D_\infty$; and $\gamma = \lambda/[C_{p_w}(T_\infty - T_o)]$.

Substituting in Eq (4),

$$T = T_\infty - \frac{\lambda D_\infty \pi^2 m_{w_o} \bar{W}}{2h A L^2 W_o}$$

However,

$$AL = \text{Volume} = \frac{(m_{w_o} + m_s)}{\rho_o}$$

Substituting results,

$$T = T_\infty - \frac{\lambda D_\infty \pi^2 \rho_o \bar{W}}{2h(W_o + 1) L} \quad (5)$$

Eq (5) indicates that for a given air dry bulb temperature, T_∞, the sample temperature—at a given moisutre content $\bar{W}$—of a thin sample will be lower than that of a thick one.

RESULTS & DISCUSSION

FIGURE 1 shows the predicted [using Eq (5)] sample temperature of two sugar beet root slabs of different thickness while being dried at an air dry bulb temperature of 67°C. It can be seen that the temperature of the thicker sample is always higher—in the moisture range considered—than that of the thinner one. This fact allows us to propose the following explanation for the "anomalous" n values reported. The thickness dependence of the drying slope, s, is calculated from drying rate data of food samples of different thickness dried at the same air dry bulb temperature. However, the time-average temperature of the thicker samples will be higher than that of the thinner ones. By this reason, even if the air dry bulb temperature is kept constant, the material temperature of the different samples will not be the same. As it will be shown below, this phenomenon may be responsible for the decrease in the n values.

The energy balance may be written in a simplified form neglecting the sensible heat (Vaccarezza et al., 1974b) as:

$$Ah(T_\infty - T) = -\lambda \frac{dm_w}{d\theta} \quad (6)$$

Vaccarezza et al. (1974a) showed that,

$$m_w = m_{w_o} \sigma e^{-s\theta}$$

where $\sigma \approx 1$. Then it follows that,

$$T = T_\infty - \frac{\lambda m_{w_o} s\, e^{-s\theta}}{2hA}$$

Considering that,

$$A = \frac{(m_{w_o} + m_s)}{\rho_o L}$$

it results,

$$T = T_\infty - \frac{\lambda \rho_o L W_o s\, e^{-s\theta}}{2h(W_o + 1)} \quad (7)$$

Eq (7) allows calculation of the sample temperature as a function of drying time.

As mentioned before, s is proportional to the diffusion coefficient which in turn is related to temperature (in a first approximation) to (Vaccarezza et al., 1974a)

$$D = D_o \exp(-E/RT')$$

Then, it follows that s is not only affected by L but also by T. This may be accounted for, by defining a factor, f,

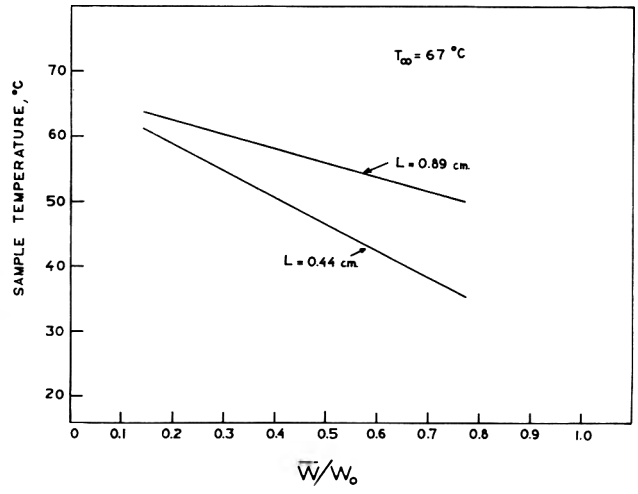

Fig. 1—Prediction of sample temperature of two sugar beet root slabs of different thickness dried at the same air dry bulb temperature.

$$f = \frac{D(\text{at } T = T_\infty)}{\bar{D}} \quad (8)$$

where,

$$\bar{D} = \frac{\int_{\theta_1}^{\theta_2} D(T = g(\theta))d\theta}{\theta_2 - \theta_1} \quad (9)$$

where, $\theta_2 - \theta_1$ is the time interval in which the slope s is experimentally determined. With this procedure it is possible to account for the different temperatures which prevailed in the slabs of different thickness. Consequently, in order to adequately evaluate the thickness dependence of s, we should plot log (s f) versus log L. This will be done by using the experimental data reported by Vaccarezza et al. (1974a) and Vaccarezza (1975) for sugar beet root, which may be summarized as follows:

T_∞	= 67°C
h	= 1.2 10^{-3} cal/sec cm^2 °C
E	= 6,900 cal/g mole
A	= 40 cm^2
ρ_o	= 1.1 g/cm^3
W_o	= 3.1

Table 1 shows the experimental values of s for sugar beet root slabs of different thickness (Vaccarezza, 1975). In the same Table are shown the corresponding values of the correction factors, f, computed through Eq (8) and (9). If the experimental slopes—without any correction for heat effects—are correlated with thickness, the following expression is obtained (Vaccarezza, 1975):

$$s = 9.98\ 10^{-5}\ L^{-1.80}$$

However, when the correction factor, f, is introduced a linear regression analysis yields the following expression:

$$s = 1.73\ 10^{-4}\ L^{-1.96}$$

The exponent, $n = 1.96$ is now almost identical to the theoretical value of 2 predicted by Fick's law.

CONCLUSIONS

IN A SERIES of previous papers (Vaccarezza et al., 1974a, b; Vaccarezza and Chirife, 1975) we have investigated the validity of Fick's law for describing the drying behavior of sugar beet root in the first falling rate period. Some "apparent" experimental discrepancies with Fick's law, like the value of the intercept of the drying curves (Vaccarezza et al., 1974b) and the thickness dependence of the drying rate (present work) have been adequately explained by taking into account the heat transfer which accompanies mass transfer.

It may be concluded that despite the complicated microscopic nature of the drying mechanism of foods, Fick's law—in terms of moisture gradient—constitutes a very good model for describing the drying behaviour of sugar beet root during the first falling rate period.

Table 1—Correction factors, f, for sugar beet root slabs of different thickness. Air dry bulb temperature: 67°C

Thickness (cm)	s (10^4 1/sec)	$s = D_{(67°C)}/\bar{D}$ (dimensionless)
0.359	6.59	1.57
0.376	5.10	1.57
0.428	4.83	1.76
0.437	4.79	1.59
0.496	3.26	1.54
0.638	2.41	1.53
0.671	1.90	1.55
0.681	1.94	1.41
0.891	1.25	1.36
0.926	1.14	1.41

NOMENCLATURE

- A = area of the slab, cm^2
- Cp_w = specific heat of water, cal/g °C
- $\bar{D}$ = diffusion coefficient defined by Eq (9), cm^2/sec
- D_o = constant, cm^2/sec
- D_∞ = diffusion coefficient evaluated at the air dry bulb temperature, cm^2/sec
- E = activation energy, cal/g mole
- h = heat transfer coefficient, cal/sec cm^2 °C
- m_s = dry mass, g
- m_w = mass of water, g
- s = slope of the experimental straight lines, ln $\bar{W}^*$ versus θ, 1/sec
- T' = absolute temperature, °K
- T_∞ = air dry bulb temperature, °C
- T = sample temperature, °C

Greek letters

- λ = latent heat of vaporization, cal/g
- ρ = density of the food, g/cm^3

Subscripts

- o = initial

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L. REBELLATO
I Laboratorio per la Tecnica del Freddo del C.N.R., Corso Stati Uniti, 35100 Padova, Italy
S. DEL GIUDICE
Facoltá di Ingegneria dell'Universitá, Istituto di Fisica Tecnica, Via Marzolo 9, 35100 Padova, Italy
and G. COMINI
Facoltá di Ingegneria dell'Universitá, Istitutodi Fisica Tecnica, Via A. Valerio 10, 34127 Trieste, Italy

FINITE ELEMENT ANALYSIS OF FREEZING PROCESSES IN FOODSTUFFS

ABSTRACT

Finite element analysis concerning freezing processes of interest to food engineering allows the solution of practically any phase-change problem described in terms of two-dimensional geometries. Special features of the program used include isoparametric elements, control of numerical oscillations and an accurate procedure for the estimation of thermal properties. In the examples of applications presented in the paper, reference is made to air-blast freezing of foodstuffs having irregular shapes.

INTRODUCTION

FREEZING PROCESSES enter many aspects of refrigeration technology and it is generally recognized that the manner in which phase-change takes place is of prime importance both with respect to the correct design of refrigeration plants and to the quality of the products. Therefore freezing times, rates of freezing and, if possible, temperature distributions in material being processed, must be predicted with fair accuracy.

Exact and approximate analytical solutions to phase-change problems exist but are limited to geometrically regular shapes and to very special boundary conditions (Luikov, 1968; IIF/IIR, 1972). Finite difference approximations work quite well if simple geometries are considered, but flexibility of problem description with finite difference codes is very poor when complicated shapes are considered (Comini and Bonacina, 1974).

Accordingly, Comini et al. (1974) suggested the finite element method as the most practical way to simulate freezing processes.

In this paper we describe applications of the method to the computation of temperature distributions in foodstuffs of irregular shape during freezing in an air-blast tunnel.

PHYSICAL ASPECTS

IN FOOD MATERIALS, water is the major component. Thus when foods are cooled below 0°C, ice formation occurs, starting at a temperature between 0 and −3°C which depends on the molar concentration of soluble cell components. As the temperature is progressively reduced, more and more water is turned into ice and the latent heat of ice formation adds to the sensible heat involved in cooling both ice and the unfrozen part. This leads to large variations in heat capacities while thermal conductivities too change considerably, mainly because the thermal conductivity coefficient of ice is almost four times greater than that of water. For most biological materials the largest part of the freezing process takes place in a temperature interval between −1 and −8°C, while largest variations of heat capacity occur between −1 and −3°C. Only at temperatures ranging from −20 to −40°C and below there is no more measurable change with temperature in the amount of ice present, and the remaining water, if any, can be considered as non-freezable (IIF/IIR, 1972). However, for practical purposes, a lower limit to the phase-change interval can be defined on the basis of a ratio of ice to total water content of, say, 90%. This choice, in addition to providing an easily applicable criterion, allows one to approximate heat capacity and thermal conductivity curves, above and below the phase-change zone, by means of constant values. In the phase-change zone a triangle and a straight line can be used to interpolate heat capacity and thermal conductivity as shown in Figure 1 (Bonacina et al., 1974).

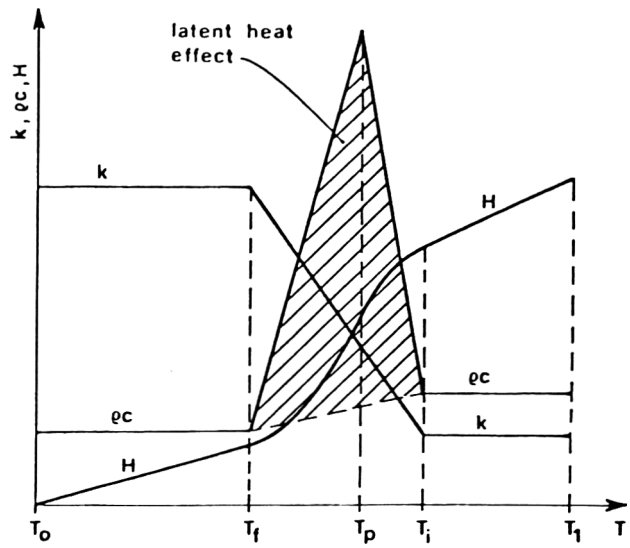

Fig. 1—Estimation of heat capacity and thermal conductivity in freezing problems.

For homogeneous food materials approximate values of thermal properties above and below freezing are given by the formulae (Comini et al., 1974):

$$c_\varrho = p\, c_{w\varrho} + (1 - p)\, c_d \quad (1)$$

$$k_\varrho = p\, k_{w\varrho} + (1 - p)\, k_d \quad (2)$$

$$c_s = p\, c_{w\varrho} + (1 - p)\, c_d \quad (3)$$

and

$$k_s = p\, k_{w\varrho} + (1 - p)\, k_d \quad (4)$$

where c_d and k_d represent specific heat capacity and thermal conductivity of the dry solid, while $c_{w\varrho}$, $k_{w\varrho}$ and c_{ws}, k_{ws} indicate thermal properties of (liquid) water and ice and p is the mass fraction of water. A good choice of values for the above mentioned set of properties is (Comini et al., 1974): $c_{w\varrho}$ = 4187 J/kg·K; c_{ws} = 2093 J/kg·K; c_d = 1256 J/kg·K; $k_{w\varrho}$ = 0.59 W/m·K; k_{ws} = 2.44 W/m·K; and k_d = 0.26 W/m·K.

The latent heat effect can be evaluated as:

$$\lambda = p\, \lambda_w \quad (5)$$

where λ_w = 335 kJ/kg is the heat of fusion/solidification of water. Simple algebra can then be used to compute the value of the peak of heat capacity if T_i, T_p and T_f are known.

Usually, a fairly safe course is to assume $T_i = -1°C$, $T_p = -3°C$ and to estimate T_f as the value that gives the best fit between experimental and computed values of temperatures. Different shapes for the interpolating curves and different values of the reference temperatures have also been tested but improvements obtained, if any, do not justify additional complications (Comini et al., 1974).

FORMULATION OF THE PROBLEM

FORMULATIONS of the finite element method for nonlinear heat conduction problems have been reported by Comini et al. (1974) and Del Giudice et al. (1976). The formulation utilized for the present research follows the usual pattern.

The problem considered in this paper is governed, in a two-dimensional region Ω, by the nonlinear parabolic equation:

$$\rho c \frac{\partial T}{\partial t} = \frac{\partial}{\partial x}\left(k_x \frac{\partial T}{\partial x}\right) + \frac{\partial}{\partial y}\left(k_y \frac{\partial T}{\partial y}\right) \tag{6}$$

subjected to boundary conditions:

$$T = T_b \tag{7}$$

on part of the boundary Γ_1 and:

$$\left(k_x \frac{\partial T}{\partial x} \ell_x + k_y \frac{\partial T}{\partial y} \ell_y\right) + q + \alpha(T - T_a) = 0 \tag{8}$$

on part of the boundary Γ_2.

Eq (6), (7) and (8) refer to unsteady thermal fields in biological materials where ρc is the volumetric heat capacity and k is the thermal conductivity. The terms ℓ_x, ℓ_y are the direction cosines of the outward normal to the boundary surface, while q represents the imposed heat flux per unit area and α is the convective heat transfer coefficient.

Physical reasons for taking into account an imposed heat flux include the possibility of representing radiation boundary conditions (Comini et al., 1974). If no boundary conditions are specified on a given portion of an external surface, then an insulated surface condition is assumed automatically, i.e., $q \equiv \alpha \equiv 0$.

The spacewise discretization of Eq (6), subjected to boundary conditions (7) and (8), can be accomplished using Galerkin's method as shown by Zienkiewicz and Parekh (1970).

Let the unknown function T be approximated, throughout the solution domain at any time t, by the relatioship:

$$T = \sum_{j=1}^{n} N_j(x,y)\, T_j(t) = \underset{\sim}{N}\underset{\sim}{T} \tag{9}$$

where N_j is the usual shape function defined piecewise element by element, and T_j or $\underset{\sim}{T}$ is the nodal parameter. The simultaneous equations, allowing the solution for n values of T_j, are obtained typically, for point j by equating to zero the integral, over the domain Ω, of the product of the weighting function N_j by the residual resulting from substitution into Eq (6) of Eq (9). After making use of Green's theorem, in order to avoid second derivates in the integrals imposing unnecessary continuity conditions between elements, the n equations can be written down in matrix form as:

$$\underset{\sim}{k}\underset{\sim}{T} + \underset{\sim}{C}\dot{\underset{\sim}{T}} + \underset{\sim}{F} = 0 \tag{10}$$

Typical matrix elements are:

$$k_{j\ell} = \sum \int_{\Omega^e} \left(k_x \frac{\partial N_j}{\partial x}\frac{\partial N_\ell}{\partial x} + k_y \frac{\partial N_j}{\partial y}\frac{\partial N_\ell}{\partial y}\right) d\Omega + \sum \int_{\Gamma_2^e} \alpha N_j N_\ell \, d\Gamma \tag{11}$$

$$C_{j\ell} = \sum \int_{\Omega^e} \rho c\, N_j\, N_e \, d\Omega \tag{12}$$

$$F_j = \sum \int_{\Gamma_2^e} N_j (q - \alpha T_a)\, d\Gamma \tag{13}$$

where $(j,\ell = 1,n)$.

In the above, the summations are taken over the contributions of each element, Ω^e is the element region and Γ_2^e refers only to elements with external boundaries for which Eq (8) specifies the condition.

It must be noted that the set of Eq (10) is highly nonlinear since thermal properties are strongly dependent on T.

The set of ordinary differential Eq (10), which defines the descretized problem, can be solved using the three-level scheme described by Comini et al., (1974).

SAMPLE PROBLEMS

IN THIS PAPER parabolic isoparametric elements have been used (Zienkiewicz and Parekh, 1970).

In order to check the program, comparisons have been made with existing analytical solutions for solidification of infinite slabs and corner regions. Finite elements and analytical solutions correlated to within 1% or better in all the test problems run (Comini and Del Giudice, 1976).

The accuracy of the solutions obtained has also been evaluated experimentally. Several freezing tests have been carried out on samples of different biological materials where one-dimensional thermal fields have been realized. The center and the end surface temperatures of the samples have been recorded during each test (Bonacina et al., 1974). Then, with reference to known initial conditions, temperature-time curves have been computed by means of the finite element program.

Experimental, as well as approximate values of thermal parameters estimated from formulae (1) to (5), have been used in the calculations. The agreement between measured and computed values is of the order of 1% when experimental values of thermal parameters are utilized and of the order of 2% if approximate values are employed.

Results that are typical of freezing tests on "Tylose," the water and methylcellulose mixture whose thermal properties are about the same as those of lean beef (Bonacina et al., 1974), are reported in Table 1.

Table 1—Measured and calculated values of temperatures for a typical freezing test of a "Tylose" sample. Computations are made for experimental and approximate values of thermal properties

		Center T (°C)		
			Computed	
t(s)	Surface T (°C)	Measured	Exp properties	Approx properties
0	20.8	20.8	20.8	20.8
500	−1.14	19.8	19.6	19.7
1000	−5.39	13.7	14.2	14.3
1500	−9.25	7.4	7.7	8.0
2000	−12.5	2.0	2.2	2.5
2500	−14.9	−0.8	−0.6	−0.9
3000	−17.2	−1.1	−1.7	−1.6
3500	−19.4	−1.7	−2.7	−2.3
4000	−21.6	−7.0	−6.6	−4.1
4500	−24.4	−17.3	−17.1	−18.8
5000	−28.3	−25.9	−25.1	−25.9
5500	−32.2	−30.7	−29.8	−30.1
6000	−35.2	−34.1	−33.3	−33.5
6500	−37.3	−36.6	−36.1	−36.2
7000	−38.8	−38.2	−38.1	−38.1

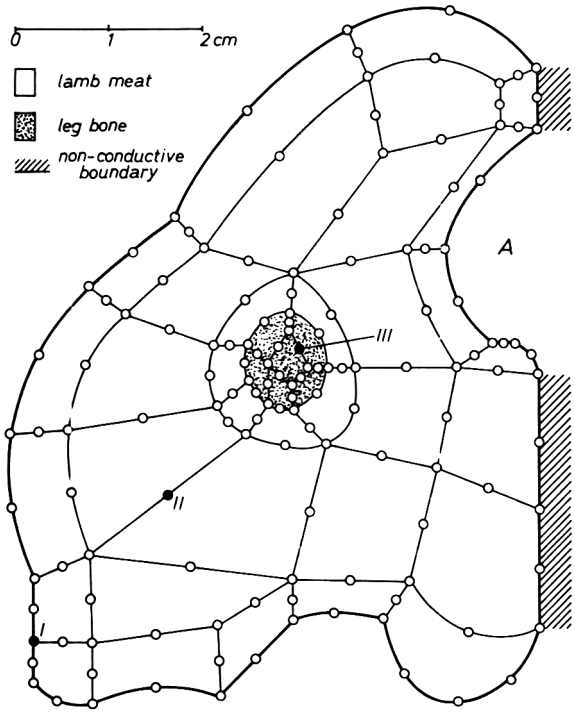

Fig. 2—Finite element mesh used for studying air-blast freezing of a lamb carcass.

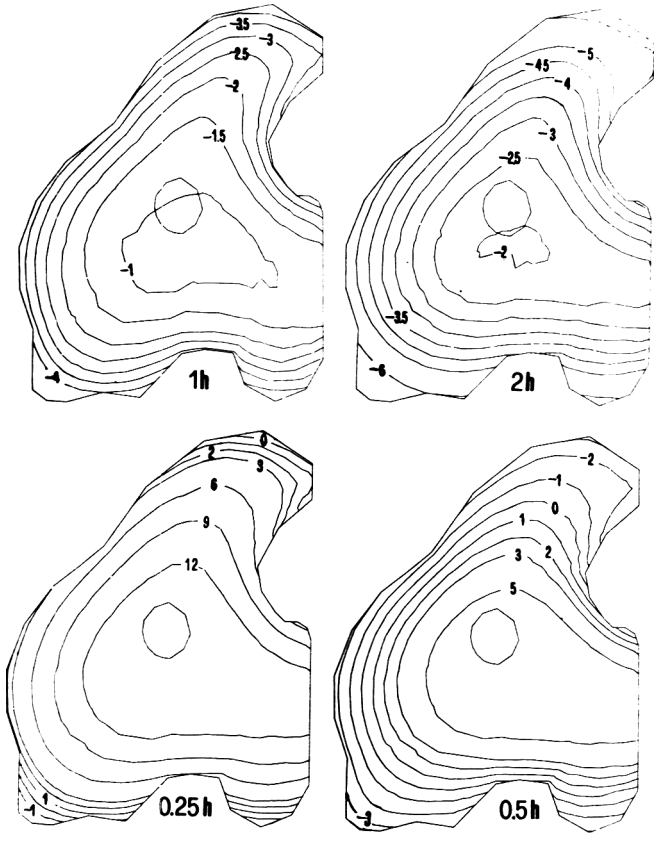

Fig. 3—Isotherm fields during air-blast freezing of a lamb carcass.

In the following examples we illustrate some applications of the program which are of considerable significance to food engineering.

Freezing of a lamb carcass

In the meat industry it is important to understand the physical factors which govern freezing processes. The rate of freezing and the final temperature of products are important since they affect operations such as cutting and may have effect on the quality of the meat. Freezing rates are also important for the design of equipment to freeze meat in the most economical way.

In the present case the problem is the investigation of temperature distributions in a lamb carcass during air-blast freezing. A cross section at the base of a hind leg was considered and, because of the existing symmetry, calculations were made only for the left leg.

The mesh utilized is represented in Figure 2: 40 parabolic elements and 141 nodal points were used. Boundary conditions at the exposed surface were defined by a constant air temperature $T_a = -18°C$ with a convective heat transfer coefficient $\alpha = 23\ W/m^2K$.

A lower value of the convective heat transfer coefficient $\alpha = 14\ W/m^2K$ was used for the anal cavity (zone A in Fig. 2).

An initial value of temperature of 20°C, constant throughout the domain, was assumed since it is common practice to hold lamb carcasses at about 20°C for 12–15 hr before freezing, in order to avoid "cold shortening."

Thermal properties of lamb meat have been computed from formulae (1–5) assuming (Fleming, 1971): $p = 0.70$; $\rho = 1006\ kg/m^3$; $T_i = -1°C$; $T_p = -3°C$; $T_f = -8°C$.

Thermal properties of the leg bone have been estimated as follows (Fleming, 1971):

$$k = 0.26\ W/m \cdot K; \quad \rho c = 8.8 \cdot 10^5\ KJ/m^3K.$$

The isotherm fields during freezing are drawn directly by the computer and are shown in Figure 3. Time-temperature curves concerning representative points of the domain, such as I, II and III in Figure 2, are shown in Figure 4.

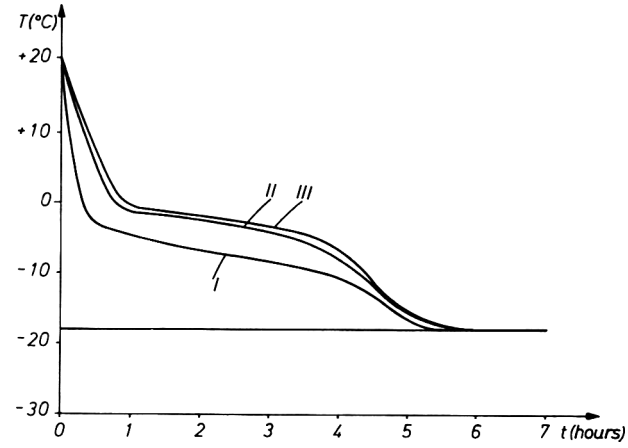

Fig. 4—Freezing rates in lamb carcasses: Time-temperature curves for representative points I, II and III in Fig. 3.

Computing time was the order of 140 sec with 141 total time steps on a CDC 7600 machine.

An analysis for a problem which is very similar to the one discussed here is presented by Fleming (1971). In his work a finite difference model, with 314 mesh points and 256 time steps, is utilized for the solution.

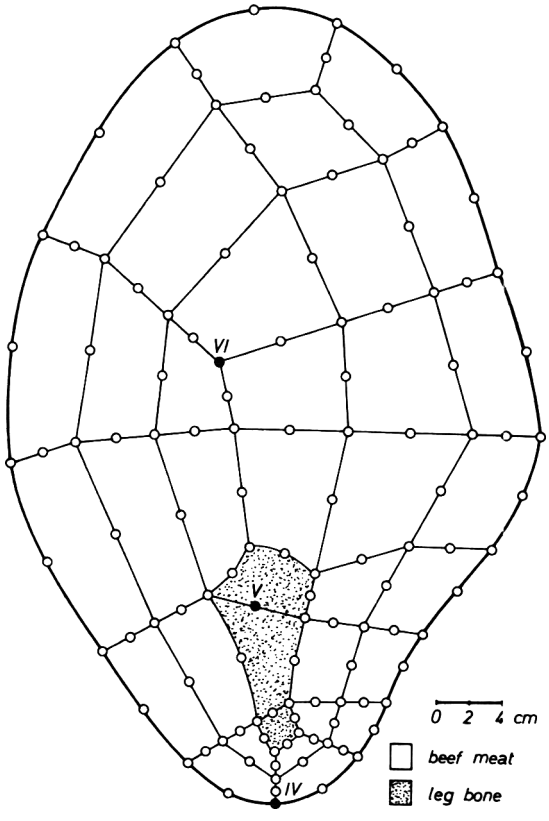

Fig. 5—Finite element mesh used for studying air-blast freezing of a beef side.

Agreement between the finite element and the finite difference model can be defined as good, although a proper quantitative comparison with Fleming's result was not possible because the geometrical data used by Fleming were not available to us. Slight discrepancies in computed temperature distributions are more than justified, in the authors' opinion, by differences in the representation of thermal properties and boundary conditions.

Freezing of a beef side

Because of the different sizes of carcasses, the freezing cycle for beef sides is different from the freezing cycle utilized for lamb carcasses.

The prevailing method in Italy is to chill "hot" beef sides for 1–3 days after slaughter. To avoid toughening due to cold shortening, the air temperature is maintained above 8°C during the first 20 hr of chilling. Afterwards the air tieperature is slowly reduced to 0–1°C.

A cross section of the hind leg was again considered for the finite element analysis. The mesh utilized is represented in Figure 5: 35 parabolic elements and 120 nodal points were used.

Convective boundary conditions with $T_a = -40°C$ and $\alpha = 18\ W/m^2K$ were assumed, together with an initial temperature value of 1°C, constant throughout the domain.

Thermal properties of beef were evaluated from formulae (1–5) assuming (IIF/IIR, 1972):

$$p = 0.77;\ \rho = 1006\ kg/m^3;\ T_i = -1°C;\ T_p = -3°C;\ T_f = -8°C;$$

while for the leg bone the same properties reported in the previous section were used.

The isotherm fields obtained directly as a computer output are shown in Figure 6. Time-temperature curves for the

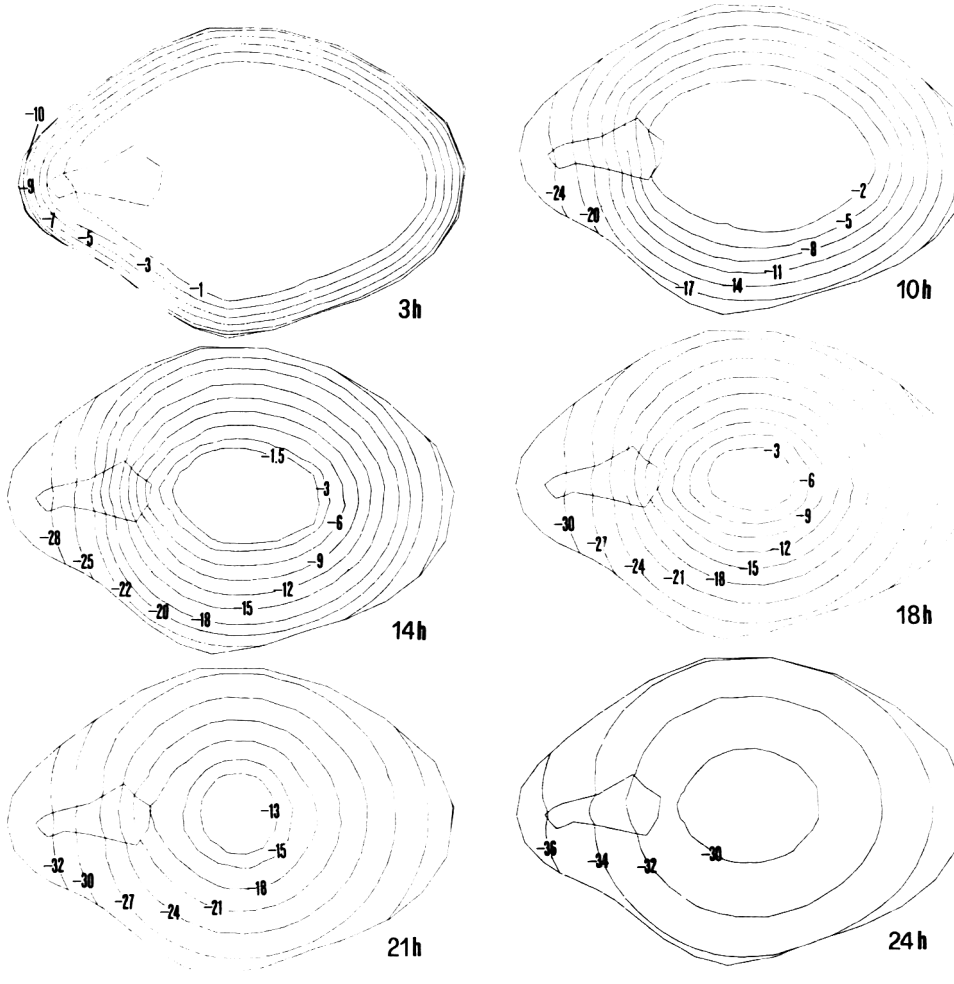

Fig. 6—Isotherm fields during air-blast freezing of a beef side.

representative points of the domain indicated in Figure 5, are reported in Figure 7.

On a CDC 7600 machine computing time was of the order of 184 sec with 192 total time steps.

CONCLUSIONS

THE FINITE ELEMENT method formulated for nonlinear heat conduction allows the solution of a great number of freezing problems in food engineering.

The procedure used in this paper is fast, accurate and sufficiently flexible to accomodate practically any problem described in terms of two-dimensional geometries.

NOMENCLATURE

- c = Specific heat capacity (J/kg K)
- $\underset{\sim}{C}$ = Heat capacity matrix
- $\underset{\sim}{F}$ = Heat load vector
- $\underset{\sim}{H}$ = Enthalpy per unit volume (J/m³)
- k = Thermal conductivity (W/m·K)
- $\underset{\sim}{K}$ = Thermal conductivity matrix
- ℓ_x, ℓ_y = Direction cosines of the outward normal to the boundary surface
- $\underset{\sim}{N}$ = Shape function vector
- p = Mass fraction of water
- q = Heat flux density (W/m²)
- s = Distance in the direction of the temperature gradient (m)
- t = Time (s)
- T = Temperature (°C)
- $\underset{\sim}{T}$ = Vector of nodal temperatures
- x,y = Cartesian coordinates (m)
- α = Convective heat transfer coefficient (W/m²·K)
- Γ = Boundary surface (m²)
- λ = Latent heat effect (J/kg)
- ρ = Density (kg/m³)
- Ω = Domain of definition (m³)

Subscripts and superscripts

- a = Air
- b = Boudary
- d = Dry
- e = Element
- f = Final
- i = Initial
- l = Liquid
- p = Peak
- s = Solid
- t = At the time instant t
- w = Water
- x, y = In the x, y direction
- $\sim$ = Vector quantity
- $< >$ = Average value

APPENDIX–SPECIAL FEATURES OF THE NUMERICAL MODEL

If it is assumed that the temperature varies linearly in the small time interval between $t - \Delta t$ and $t + \Delta t$, Eq (10) can be approximated as:

$$\underset{\sim}{K}_t(\underset{\sim}{T}_{t+\Delta t} + \underset{\sim}{T}_t + \underset{\sim}{T}_{t-\Delta t})/3 + \underset{\sim}{C}_t(\underset{\sim}{T}_{t+\Delta t} - \underset{\sim}{T}_{t-\Delta t})/(2\Delta t) + (\underset{\sim}{F}_{t+\Delta t} + \underset{\sim}{F}_t + \underset{\sim}{F}_{t-\Delta t})/3 = 0 \quad (14)$$

This, after some algebra, results in the following recurrence formula for final integration:

$$\underset{\sim}{T}_{t+\Delta t} = -[\underset{\sim}{K}_t + 3\underset{\sim}{C}_t/(2\Delta t)]^{-1}\,[\underset{\sim}{K}_t\underset{\sim}{T}_t + \underset{\sim}{K}_t\underset{\sim}{T}_{t-\Delta t}/(2\Delta t) + \underset{\sim}{F}_{t+\Delta t} + \underset{\sim}{F}_t + \underset{\sim}{F}_{t-\Delta t}] \quad (15)$$

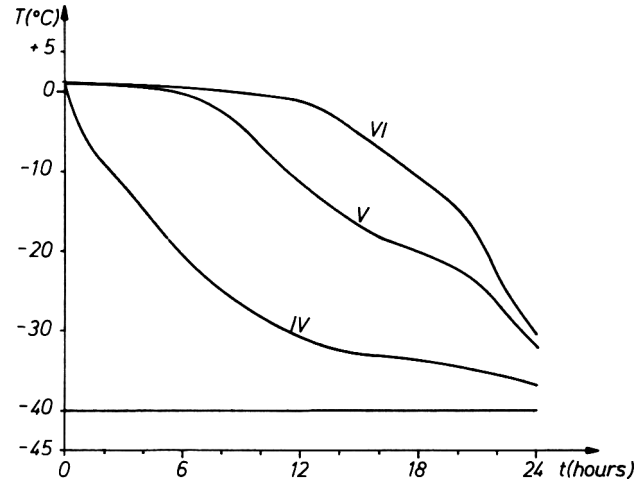

Fig. 7—Freezing rates in beef sides: Time-temperature curves for representative points IV, V and VI in Fig. 5.

in which iterations are avoided since only central values of matrices $\underset{\sim}{K}$ and $\underset{\sim}{C}$ occur. The scheme is apparently not self starting, but as we shall always refer to known stationary conditions, two starting values can be easily assumed.

Algorithm (15) has been found to be unconditionally stable. Oscillations arising from sudden variations in boundary conditions can be kept under control, for example, by adopting the automatic time step adjustment feature described by Comini et al. (1974). Undulations arising from the "numerical noise" inherent in the approximation made can be greatly reduced if, at each time step after the first one, instead of letting simply:

$$\underset{\sim}{T}_{t-\Delta t} = \underset{\sim}{T}_t \quad (16)$$

vector $\underset{\sim}{T}_{t-\Delta t}$ is redefined as:

$$\underset{\sim}{T}_{t-\Delta t} = (\underset{\sim}{T}_{t+\Delta t} + \underset{\sim}{T}_t + \underset{\sim}{T}_{t-\Delta t})/3 \quad (17)$$

before starting again the calculations indicated in formula (15) (Wood and Lewis, 1975).

The program for implementing algorithm (15) follows the usual pattern (Zienkiewicz, 1971). However, matrices $\underset{\sim}{K}$ and $\underset{\sim}{C}$ are now time dependent, through the variations of coefficients with temperature, and a completely new solution has to be obtained at each stage.

The evaluation of temperature dependent quantities in integrals (11) and (12) requires special care, particularly if a rather coarse mesh is employed and spatial variation of the quantities is abrupt. Numerical integration has obviously to be adopted here and therefore ρc and k must be estimated at integrating points in Ω^e.

In the program, a new variable H (enthalpy), is defined as an integral of the heat capacity vs temperature curve (see Fig. 1):

$$H = \int_{T_o}^{T} \rho c\, dT \quad (18)$$

since, in the phase change zone, enthalpy is a much smoother function of temperature than heat capacity. Thus it is reasonable to interpolate enthalpy rather than heat capacity directly, writing the relationship:

$$H = \sum_{j=1} N_j(x,y)\, H_j(t) = \underset{\sim}{N}\underset{\sim}{H} \quad (19)$$

—Continued on page 250

P. P. LEWICKI,[1] G. C. BUSK, P. L. PETERSON and T. P. LABUZA
Dept. of Food Science & Nutrition, University of Minnesota
1334 Eckles Ave., St. Paul, MN 55108

DETERMINATION OF FACTORS CONTROLLING ACCURATE MEASUREMENT OF A_W BY THE VAPOR PRESSURE MANOMETRIC TECHNIQUE

ABSTRACT

The precision and reproducibility of the VPM method has been improved by the simultaneous measurement of water vapor pressure and temperature of the food sample. It has been shown that evacuation of the system substantially lowers food temperature due to evaporation of water. The drop in sample temperature is dependent on water activity of the material and is large at high water activities. Even at steady readings of the manometer, the temperature of the sample is not equal to room temperature. Therefore, calculation of water activity based on room temperature gives erroneous results which are significantly lower than the results based on the measured sample temperature.

INTRODUCTION

THE MEASUREMENT and control of water activity (a_w) of food products is extremely important to many phases of the food industry. As more foods are developed using control of a_w as a technique for preservation, and as more regulations are passed setting a_w limits on food products, the need for an accurate reproducible standard method becomes essential.

A collaborative study conducted between labs at the University of Minnesota, Massachusetts Institute of Technology and Armour and Co. (Labuza et al., 1976) showed that measurement of a_w by the vapor pressure manometric (VPM) technique was the best and most accurate of seven methods evaluated. The report also stated that more accurate control of the temperature during measurement by the VPM should increase the precision reported as ±0.01 as well as the accuracy.

The fact that temperature control is critical to the method has been recognized since its initial publication (Makower and Myers, 1943) and throughout its subsequent development (Taylor, 1961; Karel and Nickerson, 1964; Labuza, 1974). However, there has never been a complete evaluation of the factors which influence the limits of the VPM method. This study was conducted to determine what factors contribute to inaccuracy and how those factors may best be controlled using currently available technology.

MATERIALS & METHODS

REAGENT GRADE SALTS and selected food products were used in this study. The reagent grade salts were dissolved in hot distilled deionized water and left at room temperature for crystallization to form a saturated solution. The food products were ground before the a_w measurement.

The VPM device used in this study and described by Labuza (1974), was modified in the following way. The sample flask was equipped with a side-arm through which a thermocouple was inserted. This was sealed with a rubber stopper to prevent air leakage into the vacuum. A second thermocouple was inserted into the leg of the manometer, and a third thermocouple was hung in the vicinity of the sample flask (Fig. 1). ISA-T thermocouples were used (Thermo Electric, Saddle Brook, NJ) and a digimite (Thermo Electric, Saddle Brook, NJ) was used to display temperatures in Centigrade to ±0.1°C.

[1] Dept. of Food Technology, Warsaw Agricultural University (S.G.G.W.), Warszawa ul. Rakowiecka 26/30, Poland

Measurement of water activity

A 10–50g sample was put in the sample flask and sealed onto the apparatus. The air-space in the apparatus was evacuated to less than 200 microns with the sample flask excluded from the system. Then, the temperatures measured by thermocouples were recorded and the sample flask was connected with the evacuated air-space. The space in the sample flask was evacuated for 1 min. Thereafter the stopcock across the manometer was closed, and the temperatures were read. The level of the oil in the manometer, as well as temperatures were read at 10-min intervals until the difference in height of the legs of the manometer was constant. The final reading is ΔH_1. The stopcock over the sample was closed and the air-space was connected with the desiccant flask. The adsorption process was conducted for 10 min and the difference in levels of the oil in the manometers legs was read to give ΔH_2.

Calculation of water activity

Water activity of the sample was calculated from the following formula:

$$a_w = \frac{(\Delta H_1 - \Delta H_2) \cdot \frac{273.16 + T_S}{273.16 + T_O}}{P_O} \quad (1)$$

where: ΔH_1 = manometer reading with the sample flask connected in centimeters of Apiezon B oil; ΔH_2 = manometer reading with the desiccator flask connected in centimeters of Apiezon B oil; T_S = temperature of the sample, °C; T_O = temperature of the manometer leg, °C; P_O = saturated water vapor pressure at the sample temperature in centimeters of Apiezon B oil.

If no temperature is measured other than that of the room temperature, T_R, then Eq 1 becomes:

$$a_w = \frac{\Delta H_1 - \Delta H_2}{P_O} \quad (2)$$

where P_O is calculated at T_R as was done by Labuza et al. (1976). It should be noted that either the average room temperature during the test or the final room temperature when the height of the legs is measured have been used previously.

RESULTS & DISCUSSION

EVACUATION of the sample flask leads to evaporation of some water from the sample, and the heat for which is provided by the sample. In consequence a drop in temperature of the sample is observed as seen in Figure 2. The decrease in the sample temperature is also dependent on the initial mass of the solution. For example, in the first 2 min, a saturated solution of $BaCl_2$ cools down by 4.4°C at an initial mass of 47.81g and by 12.6°C when 14.12g is used. The resultant difference between solution temperature and room temperature ensures an unsteady state heat flux towards the sample flask and thus a slow increase in sample temperature is observed. To reach temperature equilibrium between the sample and surrounding air, times as long as 120 min were necessary for some of the salts tested. In general, it can be stated that the time of temperature equilibration is dependent on the sample mass, the state and temperature of the surrounding air and the water activity of the sample. Therefore, it cannot be predicted easily. Thus a long measurement time is needed if the sample temperature is not measured.

The water activity determination accomplished through the use of the actual sample temperature only requires less than 70

Table 1—Temperatures and ΔH_1 values during a_w measurement for two satorated salt solutions

Salt	Time min	Temperature °C Room T_R	Manometer T_o	Sample T_s	ΔH_1
	40	21.5	21.8	20.8	22.2
NaCl	50	21.5	21.8	21.1	22.6
a_w	60	21.8	21.7	21.3	23.0
∿0.76	70	21.3	21.6	21.4	23.0
	80	21.6	21.7	21.5	23.0
$Mg(NO_3)_2$	30	21.4	21.5	20.5	16.0
a_w	40	21.3	21.6	20.8	16.3
∿0.32	50	20.8	21.4	21.0	16.3

min. The equilibrium time for measurement, as seen in Table 1, depends primarily on the water activity of the sample. At high water activity a longer time is needed to achieve a constant ΔH_1. However, there are other factors affecting the time at which the calculated a_w attains a constant value because there is no straight relationship between the time of measurement and the water activity of the sample as was found for four different salts.

It can also be noted from Table 1 that the apparent steady state of the manometer legs is not an indication of temperature equilibrium between sample and surrounding air. For $Mg(NO_3)_2$ a steady state in the manometer legs was observed at a temperature difference as large as 0.5°C. However, in most cases it was found that the differences in sample and room temperature amounted to only 0.1–0.2°C, but only in very few instances was it noted that temperature equilibrium coincided with the steady state of the manometer legs. The lack of temperature equilibrium at the steady state of the manometer legs is due to the inertia of the VPM apparatus. The response of the apparatus to a fluctuating room temperature is very slow. The reproducibility of the a_w value based on room temperature will be strongly affected by the amplitude of room temperature fluctuations which were variable in our laboratory. The standard deviation of water activity calculated on the basis of using the average room temperature for calculation of P_o amounts to ±0.0232 and ±0.0091 at temperature fluctuation amplitudes of 2.84 and 0.48°C respectively as seen in Table 2 for seven salt solutions. Water activity based on the final room temperature is even more unprecise, but is not significantly different from that based on the average room temperature. In the collaborative study of Labuza et al. (1976), final room temperature was used for standardizing the salt solutions' a_w values, thus the reported values are suspect.

Calculation of a_w on the basis of the actual sample temperature for P_o and accounting for the water vapor expansion due to the temperature difference between the sample and manometer space as indicated by Eq 1, yields a standard deviation of only ±0.0011. The precision of the measurement is increased by a factor of 70 compared to the values based on the average or final room temperature. In effect, the water activity can be measured to the third decimal place with a high degree of confidence.

The food materials investigated in this study were of solid nature. Heat transfer in these products proceeds entirely due to conduction, and the inertia of the system to the temperature changes is much larger than that of saturated salt solutions. Although the temperature probe can be inserted into a solid piece of material or immersed in a ground food product, the measured temperature is not exactly equal to the water

Table 2—a_w value for saturated salt solutions at two external temperatures and based on different measured temperatures for calculation of P_o (duplicate samples)

Salt	Avg T_R 22.53 ± 1.42°C Water activity calculated on the basis of Avg T_R[a]	Final T_R[a]	Avg T_R 21.65 ± 0.24°C Water activity calculated on the basis of Avg T_R[a]	Final T_R[a]	T_s[b]
LiCl	0.1065	0.1060	0.1180	0.1200	0.1190
	0.1092	0.1079	0.1180	0.1174	0.1164
CH_3COOK	0.2032	0.2038	0.2334	0.2361	0.2313
	0.1901	0.1871	0.2334	0.2334	0.2298
$MgCl_2$	0.3226	0.3180	0.3333	0.3320	0.3365
	0.3276	0.3255	0.3270	0.3272	0.3348
$Mg(NO_3)_2$	0.6756	0.6703	0.5369	0.5467	0.5457
	0.6022	0.6022	0.5383	0.5396	0.5475
$CuCl_2$	0.7218	0.7218	0.7013	0.7212	0.6740
	0.7066	0.7066	0.7200	0.7195	0.6742
NaCl	0.7540	0.7577	0.7537	0.7567	0.7633
	0.7557	0.7643	0.7603	0.7566	0.7638
Li_2SO_4	0.8083	0.7890	0.8382	0.8470	0.8545
	0.8492	0.8504	0.8733	0.8798	0.8557
Variance	$53613 \cdot 10^{-8}$	$64474 \cdot 10^{-8}$	$8344 \cdot 10^{-8}$	$11930 \cdot 10^{-8}$	$121 \cdot 10^{-8}$
Std dev	± 0.0232	± 0.0254	± 0.0091	± 0.0109	± 0.0011

[a] Eq (2)
[b] Eq (1)

Table 3—Water activities of foods based on use of the measured temperatures.

Food sample	Avg T_R 21.86 ± 0.54°C Water activity calculated on the basis of Avg T_R[a]	Final T_R[a]	T_s[b]
Rough and Ready Natural	0.9605	0.9549	0.9245
Hickory smoked beef sticks	1.0694	0.9492	0.9198
Rough and Ready Pickled	0.9692	0.9740	0.9947
Beef sausage	0.9789	0.9628	0.9841
Ken-L-Ration Burger	0.8712	0.8567	0.8454
Dog food	0.8846	0.8946	0.8604
Kellogg Corn Flakes	0.0936	0.0927	0.0895
Breakfast cereal	0.0935	0.0933	0.0912
Sun-Maid California	0.5784	0.5859	0.5516
Seedless raisins	0.5619	0.5646	0.5604
Gelatin gel 47.57% solids	0.9863	0.9908	0.9841
	0.9738	0.9712	0.9609
Distilled water	0.9803	0.9836	0.9988
	1.0099	1.0233	1.0034
Variance	$95982 \cdot 10^{-8}$	$28633 \cdot 10^{-8}$	$7137 \cdot 10^{-8}$
Std dev	± 0.0310	± 0.0169	± 0.0085

[a] Eq (2)
[b] Eq (1)

evaporation temperature in the system due to this inertia from the surface to the inside of the material. In addition, the heterogeneity of food products, the degree of grinding and the

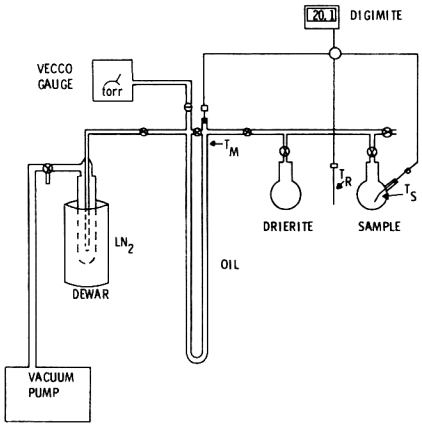

Fig. 1—Schematic diagram of VPM apparatus: T_m = manometer space temperature; T_R = room temperature; T_s = actual sample temperature.

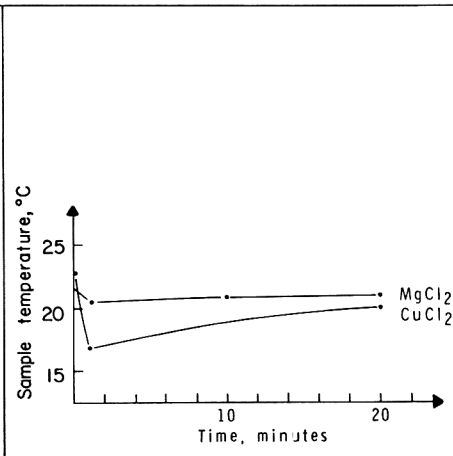

Fig. 2—Sample temperature as a function of measurement time for two saturated salt solutions during VPM measurement.

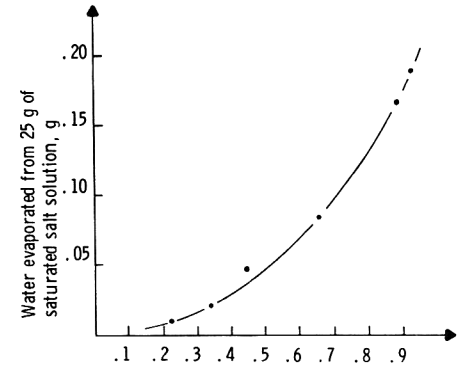

Fig. 3—Amount of water evaporated from saturated salt solutions at different a_w's during a_w measurement.

presence of volatiles all will contribute to random error in the a_w measurement.

Table 3 shows that the precision of a_w measurement in food systems is much lower than in the case of saturated salt solutions. However, the sample temperature measurement is very beneficial, and it increases the precision of the method by a factor of more than 10 compared with values calculated on the basis of average room temperature.

The amount of water which is evaporated from the sample depends primarily on the sample water activity as seen in Figure 3. At low water activities a small amount of water is evaporated, but at high water activities a substantial change in dry matter content of material may take place. The amount of water lost from the material during the a_w measurement will depend also on the sample size, surface area of material, the air-space of the VPM apparatus and final vacuum attained in the system at the end of the evacuation process. Therefore, it is recommended that water content of the material under investigation be measured after the a_w measurement and not before.

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STEVE L. TAYLOR, ELLEN R. LIEBER and MATTHEW LEATHERWOOD
Letterman Army Institute of Research, Dept. of Nutrition
Food Hygiene Div., Presidio of San Francisco, CA 94129

A SIMPLIFIED METHOD FOR HISTAMINE ANALYSIS OF FOODS[1]

ABSTRACT

The described analytical method for histamine determination in foods requires sample homogenization in methanol, heating, centrifuging or filtering, several extractions, and fluorometric detection of histamine with o-phthalaldehyde. The method eliminates potential interference by other amines through a selective extraction step. Samples of 20 foods, including seafood products (fresh, frozen and canned), comminuted meats, cheeses and sauerkraut (canned), were analyzed for histamine content. Canned sauerkraut and tuna fish had the highest average histamine content among tested foods. This method can be used to detect histamine in food samples that contain as little as 0.02 mg histamine/100g of food.

INTRODUCTION

SOME FOOD POISONING incidents resulting from the consumption of various types of fresh and processed fish have been associated with high levels of histamine in the implicated products (Kawabata et al., 1955; Merson et al., 1974; Sakabe, 1973). Histamine occurs in food primarily as the result of microbial decarboxylation of histidine. Consequently, histamine is a normal constituent of fermented foods such as sauerkraut (Mayer and Pause, 1972), cheeses (Voigt et al., 1974) and wines (Ough, 1971) and food with a relatively high histidine concentration that has been exposed to microbial degradation, such as tuna fish (Lerke and Bell, 1976). The small amounts of histamine normally occurring in foods present no appreciable hazard to consumers. The ingestion of an estimated 70–1000 mg of histamine in one meal is necessary for the onset of the clinical symptoms of intoxication (Henry, 1960). An outbreak of scombroid fish poisoning in 1973 was attributed to commercially canned tuna fish that contained 68–280 mg histamine per 100g of fish muscle (Merson et al., 1974).

The possibility of histamine-associated food poisoning outbreaks has necessitated implementation of histamine analysis as a routine quality control procedure, particularly in the tuna fish processing industry. The currently accepted method of histamine analysis in foods (AOAC, 1975) is tedious and time-consuming. This method requires column chromatographic separation in addition to homogenization, heating, filtration, and several extractions before detection of the histamine. A variety of other procedures for histamine analysis have been developed which employ variations in the chromatographic separation step (Lerke and Bell, 1976; Ough, 1971; Voigt and Eitenmiller, 1974), the homogenization and extraction conditions (Rice et al., 1975; Shore, 1971; Shore et al., 1959; Voigt et al., 1974), and the detection method (Lerke and Bell, 1976; Shore, 1971; Voigt et al., 1974). Lerke and Bell (1976) have shown that the AOAC (1975) histamine detection method is unreliable for the analysis of food samples that contain less than 5 mg histamine per 100g. A recent study has shown that the fluorometric procedure of Shore (1971) is the most sensitive and specific histamine detection method available (Taylor and Lieber, 1977). Even with the advent of more sensitive and specific histamine detection methods, the analysis of histamine in foods still requires a tedious chromatographic separation step. Due to the presence of interfering substances, simplified histamine analysis procedures similar to those used in tissue histamine analysis (Shore et al., 1959) have not proven useful. This report describes a study of various homogenization and extraction procedures which has resulted in development of a simplified method for histamine analysis in foods whereby the cumbersome chromatographic separation step has been eliminated.

MATERIALS & METHODS

Food samples

Food samples were obtained from local retail markets. Canned foods were stored at room temperature and opened immediately before analysis. Refrigerated foods, such as cheese and luncheon meats, were stored at 4°C until analysis. Raw meat samples were either stored at 4°C and analyzed within 24 hr after purchase or stored frozen at −20°C and thawed at 4°C approximately 18 hr before analysis.

Histamine analysis method

The homogenization and heating portions of the procedure were carried out with minor modifications of the AOAC (1975) procedure. Ten grams of a well-mixed food sample were homogenized with 50 ml of reagent grade methanol for 2 min in a Waring Blendor. The homogenized sample was transferred to a 100 ml volumetric flask. The blender cup was rinsed with methanol, and the rinsings were added to the volumetric flask. The flasks were stoppered and placed in a 60°C water bath for 15 min. After cooling, the contents of the flasks were adjusted to 100 ml with methanol and transferred to capped polypropylene centrifuge tubes. The samples were centrifuged at 2000 rpm for 5 min in an IEC PR-6000 centrifuge. A 2 ml portion of the supernatant was diluted 1:20 with deionized water. The extraction of histamine from these aqueous solutions was performed with conditions similar to those described by Shore et al. (1959) except that Na_2CO_3 replaced NaCl, water-saturated n-butanol was used, and heptane was eliminated. A 5 ml portion of the diluted supernatant was added to a 16 × 150 mm test tube containing 1 ml of 5N NaOH. Saturating amounts of granular Na_2CO_3 were added, and the samples were mixed thoroughly. Six ml of water-saturated n-butanol were added, and histamine was extracted into the butanol phase by vigorous mixing. Three ml of this organic phase were transferred to a second set of test tubes that contained 3 ml of 0.1N HCl. After mixing of these samples and aspiration of the upper, organic layer, a 2 ml portion of the acid phase was used for histamine detection by the fluorometric o-phthalaldehyde (OPT) procedure of Shore (1971). Fluorescence instrumentation and calibration have been described previously (Taylor and Lieber, 1977).

Histamine concentrations were calculated from comparison of the fluorescence intensities of the sample and an external histamine standard after correction for the fluorescence intensity of the blank. The external histamine standard was prepared by substituting 5 ml of a 5 μM aqueous solution (25 nmoles) of histamine dihydrochloride for the sample supernatant at the butanol extraction step. The blank was prepared by substituting 5 ml of water for the sample supernatant at the butanol extraction step. The external standard and blank were then treated identically to the other samples during both extraction and fluorescence development. Histamine recoveries were estimated from internal standards prepared by the addition of 25 nmoles of histamine to a duplicate food sample before homogenization. Histamine concentrations are expressed in mg histamine per 100g food product.

Homogenization procedures

In addition to the method described, a modification of the perchloric acid homogenization procedure (Shore, 1971), which Rice et al.

[1] Reprint requests to: Commander, Letterman Army Institute of Research, ATTN: Library, Presidio of San Francisco, CA 94129

Table 1—Effect of saturating concentrations of various salts on histamine extraction[a]

Salt	Relative fluorescence intensity		Partition coefficient
	Aqueous	Organic	
Na_2CO_3	3.2	92	29
NaCl	36	127	3.5
Na_2HPO_4	55	111	2.0
$Na_2B_4O_7$	44	105	2.4

[a] The aqueous phase was 1.0N NaOH with the various salts and 100 nmoles of histamine added. The solvent used for extractions was water-saturated n-butanol.

Table 2—Effect of Na_2CO_3 concentration on histamine extraction[a]

Molar Na_2CO_3 conc in aqueous phase	Relative fluorescence intensity		Partition coefficient
	Aqueous	Organic	
0	99	7	0.07
0.10	51	77	1.5
0.25	44	83	1.9
0.50	46	95	2.1
1.0	20	103	5.2
2.0	7	139	20
Saturated	5	127	25

[a] The aqueous phase contained 100 nmoles histamine and 1.0N NaOH. The solvent used for extraction was water-saturated n-butanol.

Table 3—Effect of NaOH concentration on histamine extraction[a]

Normality of NaOH in aqueous phase	Relative fluorescence intensity		Partition coefficient
	Aqueous	Organic	
0	10	99	10
0.05	7	117	17
0.10	7	123	18
0.50	5	114	23
1.0	5	127	25
2.5	5	126	25
5.0	5	123	25

[a] The aqueous phase contained 100 nmoles histamine and a saturating concentration of Na_2CO_3. The solvent used for extraction was water-saturated n-butanol.

Table 4—Effect of solvents on histamine extraction[a,b]

Solvent	Relative fluorescence intensity		Partition coefficient
	Aqueous	Organic	
n-Butanol	4	132	33
Benzene:n-butanol (3:2)	13	87	6.7
n-Butanol:chloroform (3:2)	114	4	0.04
Chloroform	34	2	0.06
Hexane	38	1	0.03

[a] The aqueous phase was 1.0N NaOH with a saturating concentration of Na_2CO_3 and 100 nmoles histamine.
[b] All solvents were water-saturated.

(1975) used with food products, was tried. Ten-gram tuna fish samples were homogenized in 75 ml of 0.4N perchloric acid. After homogenization, the samples were allowed to stand at room temperature for 5 min. The homogenates were centrifuged at 2000 rpm for 5 min. The extraction conditions were identical to the conditions employed in the usual histamine analysis except 5 ml of heptane were added during the acid extraction (Rice et al., 1975; Shore, 1971). The o-phthalaldehyde detection method (Shore, 1971) was used. Standards were prepared as described in the preceding section with perchloric acid substituted for methanol.

A modification of the homogenization procedure, devised by Lerke and Bell (1976), was also tried. Ten-gram tuna fish samples were homogenized in 50 ml of 10% trichloroacetic acid (TCA). Homogenates were transferred to a 100 ml volumetric flask, and the final volume was brought to 100 ml with additional 10% TCA. Centrifugation, extraction, and detection conditions were identical to the conditions employed in the usual histamine assay. Standards were prepared as described previously except that 10% TCA was substituted for methanol.

Effect of various extraction conditions

During attempts to optimize the initial solvent extraction step, numerous extraction conditions were tested including the use of various salts, the effect of Na_2CO_3 concentration, the effect of NaOH concentration, and the use of various solvents. For these experiments, 100 nmoles of histamine dihydrochloride were placed in 2 ml of the appropriate aqueous phase. Two ml of the appropriate solvent were added, and the mixtures were extracted by vigorous stirring on a Vortex mixer. After allowing several minutes for phase separation, 100 µl aliquots of the aqueous and organic phases were taken for histamine analysis. In these experiments histamine was detected with fluorescamine using the optimal reaction conditions for fluorochrome development (Taylor and Lieber, 1977). Fluorescamine was used in these experiments so that histamine could be detected directly in the organic phase. The partition coefficient was calculated as the ratio of the corrected relative fluorescence intensity of the organic phase to that of the aqueous phase.

Specificity of solvent extraction

To determine the specificity of the solvent extraction, 100 nmoles of several potentially interfering amines were placed in tubes with 2.0 ml of a 1.0N NaOH-2.0M Na_2CO_3 solution. The amines utilized were histidine, histidyl-L-leucine, histidyl-L-tyrosine, histidyl-L-serine, spermidine, cysteine, carnosine, glycylglycine, norepinephrine and glucosamine. Mixtures were extracted with 2 ml of water-saturated n-butanol. Following separation of the phases, 100 µl aliquots of each phase were taken for amine analysis. Since not all of the listed amines are easily detected with OPT, the samples were assayed with fluorescamine, a more general amine detection reagent, as described by Taylor and Lieber (1977).

RESULTS & DISCUSSION

MANIPULATION of various factors in the aqueous phase had a profound effect on histamine extraction (Tables 1, 2 and 3). The key factor in optimizing the extraction of histamine was the use of saturating concentrations of sodium carbonate. Table 1 indicates that other salts, such as NaCl, Na_2HPO_4, and $Na_2B_4O_7$, were not adequate replacements. The advantage of sodium carbonate addition over either saturating concentrations of NaCl (Rice et al., 1975; Shore, 1971; Voigt et al., 1974) or no salt addition (AOAC, 1975) is obvious from Table 1. Table 2 shows that the optimal concentration of Na_2CO_3 for the extraction of histamine is near saturation. The limiting molar concentration of Na_2CO_3 in 1.0N NaOH at room temperature is slightly over 2.0M. Table 3 shows that NaOH is also critical for the extraction of histamine. A final concentration of 1.0N NaOH in the aqueous phase was chosen for the routine procedure. Most previous procedures (AOAC, 1975; Rice et al., 1975; Shore et al, 1959) utilized similar NaOH concentrations.

The choice of solvent was also critical for a successful extraction of histamine. As shown in Table 4, the best solvent for histamine extraction was water-saturated n-butanol. The AOAC (1975) procedure used the benzene:n-butanol solvent, while most other methods (Rice et al., 1975; Shore, 1971) have used n-butanol. Shore (1971) suggested the use of n-butanol:chloroform (3:2) for samples that contained high concen-

trations of histadine. When the partition coefficient achieved with this solvent (Table 4) is considered, the use of n-butanol: chloroform should be seriously questioned.

A recent study of amines (Taylor and Lieber, 1977) showed that histidine, histidyl dipeptides, spermidine, cysteine, glycylglycine, norepinephrine, and glucosamine could potentially interfere with the Shore (1971) method for histamine detection. Other amines known to occur in foods such as tyramine and tryptamine (Voigt et al., 1974) did not interfere with histamine detection with the o-phthalaldehyde reagent (Taylor and Lieber, 1977). Therefore, any successful extraction procedure should selectively separate histamine from these interfering compounds. Table 5 suggests that the standard histamine extraction procedure described in MATERIALS & METHODS selectively concentrates histamine in the butanol phase while leaving the other interfering amines in the aqueous phase. While some spermidine, histidyl-L-leucine, and histidyl-L-tyrosine are extracted into the butanol phase, any interference would be markedly decreased by this extraction step. While the effect of various food chemical components on the extraction of histamine and interfering amines into the butanol phase was not determined, the use of this selective extraction would seem to preclude the necessity of any chromatographic separation step. Other less selective preparative methods (AOAC, 1975; Lerke and Bell, 1976; Ough, 1971; Voigt et al., 1974) have required the inclusion of a cumbersome chromatographic separation for the removal of these interfering materials.

Several different homogenization media have been used for the initial solubilization of histamine from foods or tissues including methanol (AOAC, 1975), 0.4N perchloric acid (Rice et al., 1975; Shore, 1971), and 10% trichloroacetic acid (Lerke and Bell, 1976). In a comparison of these three homogenization media in the extraction of histamine from tuna fish, the methanol homogenization resulted in a 103% histamine recovery, while 86% and 43% recoveries were obtained by the trichloroacetic acid and perchloric acid treatments, respectively. Consequently, the methanol homogenization procedure (AOAC, 1975) was adopted as the routine method for all food samples. An average histamine recovery of 106% was achieved by using the methanol homogenization procedure with 11 additional samples (Table 6).

The utility of the described method based on methanol homogenization and selective solvent extraction was tested in a variety of foods. In Table 6 the average histamine levels and recoveries in canned tuna, canned mackerel, canned sardines, lobsters, crabs, clams, oysters, canned sauerkraut, various meat items and cheddar cheese are presented. The highest average histamine level was found in canned sauerkraut with 4.07 mg/100g. Tuna, mackeral, salami, thuringer-cervelat and cheddar cheese also exhibited rather high average histamine concentrations (1.86–3.46 mg/100g). The presence of reasonably high levels of histamine in foods other than fish is not unexpected since occurrences of poisoning due to histamine have been reported with other foods such as sauerkraut (Mayer and Pause, 1972) and cheese (Doeglas et al., 1967). Considerable variability was observed among the food samples in most groups. However, more complete surveys with a larger number of samples will be required to determine if such differences are significant and to ascertain the frequency of occurrence of samples with unusually high histamine contents within each food group.

A comparison of the histamine content of foods analyzed with this simplified procedure (Table 6) with earlier estimates from more tedious procedures indicates that the simplified method is equally appropriate. In a survey of commercially canned tuna, Mietz and Karmas (1977) found histamine levels in the range of 0.1–8.0 mg/100g which compares favorably with the range of 0.7–7.4 mg/100g reported in Table 6. Vandekerckhove (1977) determined that dry fermented sausages have an average histamine content of 4.10 mg/100g while Rice et al. (1974) reported average histamine concentrations of 0.36 mg/100g for semi-dry sausage and 0.29 mg/100g for dry sausages. In tests on five types of sausage (Table 6), the histamine content ranged from 0.19–18.4 mg/100g. Voigt et al.

Table 5–Specificity of histamine extraction[a]

Amine	Relative fluorescence intensity		Partition coefficient
	Aqueous	Organic	
Histamine	5	116	23
Histidyl-L-leucine	19	44	2.3
Histidyl-L-tyrpsine	10	15	1.5
Histidyl-L-serine	10	0	0
Histidine	36	0	0
Spermidine	18	58	3.2
Cysteine	27	1	0.04
Glycylglycine	16	0	0
Norepinephrine	8	0	0
Glucosamine	6	1	0.17
Carnosine	29	0	0

[a] The aqueous phase contained 100 nmoles of amine in 1.0N NaOH–2.0M Na_2CO_3 solution. The solvent used for extraction was water-saturated n-butanol.

Table 6–Histamine content in food products

Food product	No. of samples	Avg histamine conc[a]	Range histamine conc[a]	Avg histamine recovery[b]
Light tuna, canned	11	3.46	1.60–7.41	106
Albacore tuna, canned	11	1.45	0.66–2.21	92
Mackerel, canned	18	2.25	1.20–4.50	101
Sardines, canned	10	0.79	0.31–1.38	99
Lobsters, frozen	4	0.16	0.09–0.27	96
Crabs, fresh	5	0.13	0.05–0.24	98
Clams, fresh	6	0.19	0.06–0.32	92
Oysters, fresh	7	0.18	0.04–0.64	93
Comminuted beef, fresh	24	0.58	0.40–0.79	98
Comminuted beef-soy, fresh	16	0.48	0.37–0.67	106
Comminuted turkey, frozen	10	0.15	0.00–0.26	98
Communited pork, fresh	30	0.12	0.03–0.36	101
Salami	10	2.34	0.36–18.4	102
Bologna	10	0.52	0.19–0.84	111
Thuringer	6	0.58	0.31–1.09	113
Thuringer-Cervelat	6	2.14	1.13–3.63	108
Beef summer sausage	8	0.98	0.69–1.31	110
Cheddar cheese, mild-mellow	11	1.86	0.17–7.81	101
Cheddar cheese, sharp-aged	16	2.76	0.57–11.3	99
Sauerkraut, canned	10	4.07	3.53–4.52	90

[a] mg histamine per 100g food product.
[b] Percent recovery calculated from the ratio of the corrected relative fluorescence of an internal standard to the sum of the corrected relative fluorescence of the sample and the external standard as described in MATERIALS & METHODS. Both standards were prepared with 25 nmoles of histamine.

(1974) reported histamine in cheddar cheese to range from nondetectable levels to 21 mg/100g which is somewhat higher than the range of 0.17–11.3 mg/100g reported in Table 6.

The described method is considerably less tedious than other histamine analysis methods used with foods (AOAC, 1975; Lerke and Bell, 1976; Mietz and Karmas, 1977; Ough, 1971; Voigt et al., 1974; Vandekerckhove, 1977) due to the elimination of the cumbersome chromatographic separation step. Certain elements of this simplified procedure are similar to those of previous methods including the methanol homogenization procedure (AOAC, 1975), portions of the extraction procedure (Shore et al., 1959), and the detection of histamine with OPT (Shore, 1971). However, the inclusion of saturating levels of Na_2CO_3 during the extraction procedure resulted in a more highly selective extraction of histamine which eliminated the need for any chromatographic separation step. The optimization of other analysis conditions, such as the use of NaOH in extraction (Shore et al., 1959), the choice of n-butanol (Shore, 1971) over benzene:n-butanol (AOAC, 1975), and the use of methanol in sample homogenization (AOAC, 1975) rather than perchloric acid (Shore, 1971) or TCA (Lerke and Bell, 1976), was also important in development of this selective extraction procedure. In addition the simplified procedure has proven useful for histamine analysis in a wide variety of foods.

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ANALYSIS OF FREEZING PROCESSES IN FOODSTUFFS . . . *From page 243*

where again $\underset{\sim}{N}$ is the shape function and $\underset{\sim}{H}$ is the enthalpy value at nodal points.

By definition it is:

$$\rho c = dH/dT \tag{20}$$

Therefore, evaluation of the above derivative with reference to the oriented directions of the temperature gradient yields:

$$\rho c = \frac{\partial H}{\partial s}\Big/\frac{\partial T}{\partial s} = \left(\frac{\partial H}{\partial x}\ell_{sx} + \frac{\partial H}{\partial y}\ell_{sy}\right)\Big/\frac{\partial T}{\partial s} \tag{21}$$

where ℓ_{sx}, ℓ_{sy} are the direction cosines of $\underset{\sim}{s}$.

Since we have:

$$\ell_{sx} = \frac{\partial T}{\partial x}\Big/\frac{\partial T}{\partial s}; \quad \ell_{sy} = \frac{\partial T}{\partial y}\Big/\frac{\partial T}{\partial s} \tag{22}$$

and:

$$\frac{\partial T}{\partial s} = \left[\left(\frac{\partial T}{\partial x}\right)^2 + \left(\frac{\partial T}{\partial y}\right)^2\right]^{1/2} \tag{23}$$

from Eq (20) it follows that:

$$(\rho c) = \left(\frac{\partial H}{\partial x}\frac{\partial T}{\partial x} + \frac{\partial H}{\partial y}\frac{\partial T}{\partial y}\right)\Big/\left[\left(\frac{\partial T}{\partial x}\right)^2 + \left(\frac{\partial T}{\partial y}\right)^2\right] \tag{24}$$

This averaging process always gives representative values of heat capacity and preserves a correct heat balance by avoiding the possibility of missing peak values of the quantity ρc. Obviously, in zones at constant temperature ($\partial T/\partial s \cong 0$), recourse is made to direct evaluation of heat capacity.

Similar techniques are used in the program also for the best determination of thermal conductivity values.

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LEA HYVÖNEN, RAKEL KURKELA, PEKKA KOIVISTOINEN and ANNI RATILAINEN
Dept. of Food Chemistry & Technology
University of Helsinki, 00710 Helsinki 71, Finland

FRUCTOSE-SACCHARIN AND XYLITOL-SACCHARIN SYNERGISM

ABSTRACT

Synergism in fructose-saccharin and xylitol-saccharin mixtures whose sweetness in solution corresponded to that of a 5% sucrose solution was measured at three temperatures (5°C, 23°C and 50°C). In the sensory evaluations a procedure consisting of a magnitude estimation, a paired comparison and a triangle test was applied. Synergism between fructose and saccharin and xylitol and saccharin was found to be greatest when the sweeteners were almost equal in the mixture in relation to their sweetness at the prevailing temperature. Mixtures of saccharin and fructose or xylitol without the aftertaste typical of saccharin were prepared. The sweetness of fructose-saccharin and xylitol-saccharin mixtures in coffee was enhanced compared with that in the corresponding water solutions. It is concluded that coffee, tea and juices can be prepared to conventional taste and sweetness standards using these mixtures, but with 40–70% less energy than when sucrose is used as the sweetener.

INTRODUCTION

SYNERGISM is inferred when the sweetness of a mixture is greater than the sum of the sweetnesses of its components. Synergistic effects have been found in mixtures of sweeteners at certain concentrations (Stone and Oliver, 1969; Yamaguchi et al., 1970; Moskowitz and Klarman, 1975). However, the quantitative measurement of synergism is difficult. The amount of synergism depends on the calculation of the sum of the component sweetnesses. In calculating the sweetness, its intensity vs concentration should be taken into account. Moskowitz (1973) has proposed equations which can be applied to mixtures of sweeteners. It seems that there are optimal combinations of sweeteners as to the extent of synergism. According to Weickmann et al. (1969) synergism has its maximum when the components of a mixture contribute about the same amount to the sweetness of the mixture.

The objectives of this study were to determine the ratio of the components in fructose-saccharin and xylitol-saccharin mixtures where the synergism was maximal and when the sweetness corresponded to a concentration commonly used in coffee and tea. Estimates of the sweetnesses of the components of the mixture were obtained from the sweetness value vs concentration graphs drawn on the basis of the magnitude estimation tests. It was thought that the sensory system sums up subjective intensities by summing the sweetnesses of the two components of the mixture. The sweetness of the mixture was predicted to be isosweet with a 5% sucrose solution, and synergism was inferred when the sweetness of the mixture was greater than this. The sweetnesses of the mixtures were determined by a paired comparison method and expressed as isosweet sucrose concentrations. The study was performed at three temperatures and the mixtures were applied in some foods having a low energy value.

MATERIALS & METHODS

SUCROSE, purity > 99.9%, fructose and xylitol, purity 99.5%, products of Finnish Sugar Co. Ltd. and Na-saccharin (APODAN), purity > 97%, were used.

In the sensory evaluations the following methods were applied: magnitude estimation, paired comparison and triangle test.

The taste panel consisted of 10 experienced members from the personnel of the University laboratories. Each evaluation in the magnitude estimation was repeated twice by the panel with the exception of the saccharin solutions at 23°C, which were evaluated three times. Paired comparisons and triangle tests were also repeated twice by the panel. The judges were asked not to swallow the samples and to rinse their mouths with distilled water (at 23°C) between samples. In the magnitude estimation tests four to five fructose, xylitol or saccharin solutions and four to five sucrose solutions were evaluated per session. In paired comparison tests four pairs of samples, with a randomized presentation order and order within pairs, were given to each judge at each session. The judges were asked to indicate the sweeter member of each pair. The mixture-sweetened sample was always one member of the pair and the other was a sucrose solution in a concentration which varied in an appropriate way from pair to pair (Table 1a).

The entire procedure consisted of three main phases.

1. Determination of relative sweetness of fructose, xylitol and saccharin solutions in terms of a reference sucrose solution.
2. Determination of concentrations of fructose and saccharin mixtures and those of xylitol and saccharin mixtures in solutions giving the sweetness of the reference sucrose solution.
3. Determination of synergism between fructose and saccharin and xylitol and saccharin.

First, the relative sweetnesses of fructose, xylitol and saccharin in water solutions were determined by magnitude estimation. The judges were asked to give numerical values to the sweetness of solutions of four to five concentrations of fructose, xylitol or saccharin as well as of sucrose, using one of the solutions of their own choice as the reference and an arbitrary scale. The assigned values were afterwards normalized to the standard value 10 which represented the sweetness of the 5% sucrose in each test.

Graphs were drawn in which the abscissa represents fructose, xylitol or saccharin concentrations and the ordinate the sweetness values. Relative sweetness graphs were determined at three temperatures 5°C, 23°C and 50°C (Fig. 1).

Secondly, mixtures of fructose and saccharin and those of xylitol and saccharin whose theoretical sweetness would correspond to that of the 5% sucrose solution and therefore to the sweetness value 10 on the graph, were determined in the following way: e.g., fructose concentration 3% would correspond on the fructose graph to a sweetness value 6.5, which is subtracted from 10. The difference, 3.5, indicates the sweetness value of saccharin needed in the mixture. The corresponding concentration of saccharin is obtained from the saccharin graph, in the reference case 0.0058% (Fig. 2)

Thirdly, because synergism between fructose and saccharin and xylitol and saccharin was anticipated (Weickmann et al., 1969; Yamaguchi et al., 1970), whereby the sweetness of a mixture is greater than the sum of the sweetness of its components, mixtures were prepared in which the proportions of fructose and saccharin or xylitol and saccharin were varied, but in which the calculated sweetness value was maintained as 10 (Table 1a). Solutions of these mixtures were then compared to a series of sucrose solutions of various concentrations by a paired comparison (Table 1b). The judges were asked to indicate, which of the two solutions compared in a pair was the sweeter one. A paired comparison rather than a direct scaling technique was used to find out synergism, since mixture solutions were estimated to be isosweet with a 5% sucrose solution and since it was believed that small differences in sweetness could be detected by the paired comparison method.

In the graphic evaluation of the results the percentage of judges who had regarded the sucrose solutions of the pairs as sweeter than the respective fructose-saccharin or xylitol-saccharin mixture, was plotted against sucrose concentrations (as an example, Fig. 3). From the resulting curve one can determine the sucrose concentration of the solution which was regarded by 50% of the judges as sweeter than the solution sweetened with the fructose-saccharin or xylitol-saccharin mixture. In other words the two solutions can be regarded as isosweet at that point.

Table 1a—Composition of theoretically derived solutions of fructose-saccharin and xylitol-saccharin mixtures isosweet with a 5% sucrose solution, the sweetness of which was given the value 10, and the empirical sweetness of the mixture solutions at three temoeratures.

Temp	Mixture composition	Component sweetness		Sum	Empirical
		Fructose	Saccharin	predicted	sum
5°C	1% Fructose + 0.0109% Saccharin	0.6	9.4	10	13.0
	2% Fructose + 0.0079% Saccharin	3.9	6.1	10	15.2
	3% Fructose + 0.0049% Saccharin	7.2	2.8	10	15.8
23°C	1% Fructose + 0.0132% Saccharin	1.1	8.9	10	13.4
	2% Fructose + 0.0096% Saccharin	3.7	6.3	10	14.8
	3% Fructose + 0.0058% Saccharin	6.5	3.5	10	15.0
	4% Fructose + 0.0016% Saccharin	9.5	0.5	10	12.6
50°C	1% Fructose + 0.0205% Saccharin	0	10.0	10	11.2
	2% Fructose + 0.0173% Saccharin	1.8	8.2	10	13.4
	3% Fructose + 0.0130% Saccharin	4.2	5.8	10	14.0
	4% Fructose + 0.0088% Saccharin	6.6	3.4	10	14.4
	5% Fructose + 0.0045% Saccharin	9.0	1.0	10	13.0
5°C	2% Xylitol + 0.0103% Saccharin	1.4	8.6	10	15.0
	3% Xylitol + 0.0073% Saccharin	4.5	5.5	10	15.4
	4% Xylitol + 0.0044% Saccharin	7.6	2.4	10	16.0
23°C	1% Xylitol + 0.014% Saccharin	1.0	9.0	10	11.8
	2% Xylitol + 0.011% Saccharin	3.1	6.9	10	14.4
	3% Xylitol + 0.008% Saccharin	5.2	4.8	10	13.8
	4% Xylitol + 0.005% Saccharin	7.3	2.7	10	14.2
	5% Xylitol + 0.002% Saccharin	9.4	0.6	10	13.4
50°C	3% Xylitol + 0.0172% Saccharin	1.9	8.1	10	13.4
	4% Xylitol + 0.0132% Saccharin	4.1	5.9	10	14.0
	5% Xylitol + 0.0093% Saccharin	6.3	3.7	10	15.0
	6% Xylitol + 0.0065% Saccharin	8.5	1.5	10	14.8

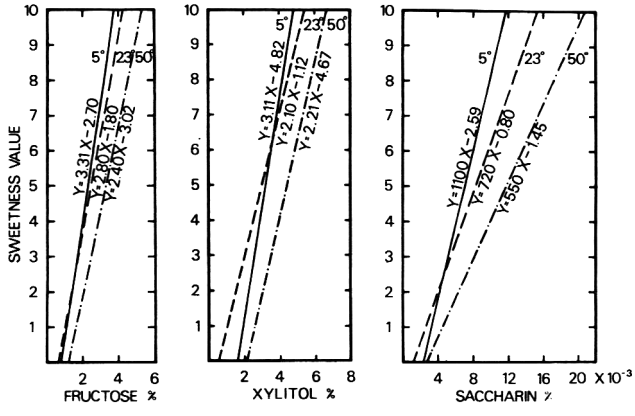

Fig. 1—Relative sweetnesses of fructose, xylitol and saccharin at 5°, 23° and 50°C. The sweetness values were obtained by the method of magnitude estimation. Regression lines were calculated using the averages from 20 judgments per sample (30 judgments for the saccharin graph at 23°C).

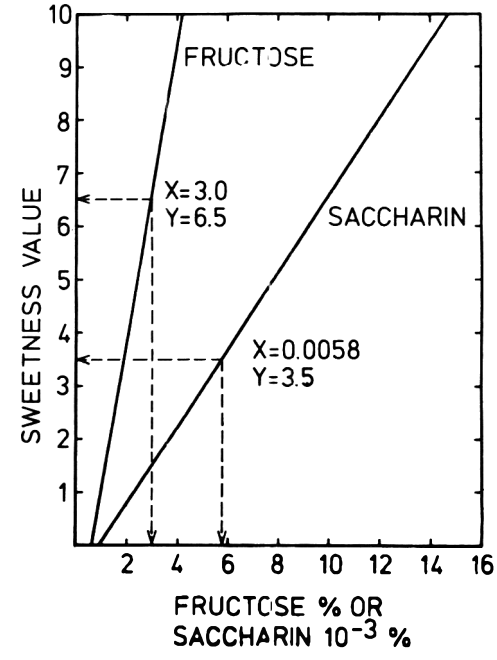

Fig. 2—Determination of a mixture of fructose and saccharin whose theoretical sweetness in solution corresponds to that of a 5% sucrose solution at 23°C and the sweetness value 10.

RESULTS

THE RELATIVE SWEETNESS of fructose, xylitol and saccharin solutions at various temperatures as functions of concentration is illustrated in Figure 1. Using these graphs, solutions of fructose and saccharin and xylitol and saccharin mixtures were prepared, the sweetness of which theoretically corresponded to the sweetness of the 5% sucrose solution, since the sensory system was thought to sum up subjective intensities by summing the sweetnesses of the components of the mixture (Table 1a). In Figure 4 the sweetnesses of solutions of the test fructose-saccharin and in Figure 5 those of xylitol-saccharin mixtures are expressed as percent concentrations of isosweet sucrose solutions. Synergism was inferred, when the sweetnesses of the mixtures were greater than the sweetness of the 5% sucrose solution. At 5°and 23°C the maxima of synergism were found in the solutions of the fruc-

Table 1b—Fructose-saccharin and xylitol-saccharin solutions compared in pairs with sucrose solutions of various concentrations

Temp	Mixture composition	Conc of sucrose soln (%)	Mixture composition	Conc of sucrose soln (%)
5°C	1% Fructose + 0.0109% Saccharin	5/6/7/8 or 6/7/8/9	2% Xylitol + 0.0103% Saccharin	5/6/7/8 or 6/7/8/9
	2% Fructose + 0.0079% Saccharin	6/7/8/9 or 7/8/9/10	3% Xylitol + 0.0073% Saccharin	5/6/7/8 or 6/7/8/9
	3% Fructose + 0.0049% Saccharin	6/7/8/9	4% Xylitol + 0.0044% Saccharin	6/7/8/9
23 °C	1% Fructose + 0.0132% Saccharin	4/5/6/7	1% Xylitol + 0.014% Saccharin	5/6/7/8
	2% Fructose + 0.0096% Saccharin	5/6/7/8	2% Xylitol + 0.011% Saccharin	5/6/7/8
	3% Fructose + 0.0058% Saccharin	7/8/9/10	3% Xylitol + 0.008% Saccharin	5/6/7/8 or 6/7/8/9
	4% Fructose + 0.0016% Saccharin	5/6/7/8	4% Xylitol + 0.005% Saccharin	5/6/7/8 or 6/7/8/9
			5% Xylitol + 0.002% Saccharin	5/6/7/8
50°C	1% Fructose + 0.0205% Saccharin	5/6/7/8	3% Xylitol + 0.0172% Saccharin	5/6/7/8
	2% Fructose + 0.0173% Saccharin	5/6/7/8	4% Xylitol + 0.0132% Saccharin	5/6/7/8
	3% Fructose + 0.0130% Saccharin	5/6/7/8 or 6/7/8/9	5% Xylitol + 0.0093% Saccharin	5/6/7/8 or 6/7/8/9
	4% Fructose + 0.0088% Saccharin	5/6/7/8 or 6/7/8/9	6% Xylitol + 0.0065% Saccharin	5/6/7/8 or 6/7/8/9
	5% Fructose + 0.0045% Saccharin	5/6/7/8		

tose-saccharin mixtures containing 2.5–3% fructose and at 50°C in a solution containing 4% fructose. Increasing temperature from 5° to 50°C decreased synergism. At 5° and 23°C the maxima of synergism were found in the solutions of the xylitol-saccharin mixtures containing 2–4% xylitol and at 50°C in a solution containing 5–6% xylitol. The synergism at 5°C was greater than that at 23° and 50°C.

The results obtained in fructose-saccharin mixture solutions in particular support the assumption that synergism is greatest when the two components of a mixture contribute about the same amount to the sweetness of the solutions (Weickmann et al., 1969). As can be seen in Figure 1, 2.5–3% fructose solution at 5° and 23°C produces about half of the sweetness in the solution of the fructose-saccharin mixture isosweet with the 5% sucrose solution, whose sweetness is regarded as 10. At 50°C the sweetness of fructose is lower and therefore 4% fructose is needed to produce half of the sweetness in the mixture solution. In the solutions of the xylitol-saccharin mixtures the tendency was not so clear, but the maxima of synergism were also broader in those mixtures.

For reducing the sweetness of the solutions of those mixtures, whose synergism was greatest to the level of the 5% sucrose solution, the mixture-sweetened solutions were reconstituted taking into account the extent of the synergism while keeping the ratio of the sweeteners in the mixture unchanged. The sweetness of the solutions thus obtained were compared by a triangle test to the 5% sucrose solution. After this revision no significant difference was observed at any temperature between the sucrose solution and those sweetened with either the fructose-saccharin or xylitol-saccharin mixtures. No saccharin after taste was observed by the panel (Table 2).

The relation of the concentration to sweetness intensity of the mixture-sweetened solutions (at 23°C) was measured by magnitude estimation. In normalizing the assigned values the 5% sucrose solution with a sweetness value of 10 was used as the reference. The sweetness intensity of the mixture-sweetened solutions grew as a function of concentration such that in the equation, $S = kC^n$, n = 1.6 for the fructose-saccharin mixture and n = 1.3 for the xylitol-saccharin mixture. The mixture-sweetened solutions behaved like sugar solutions, the n-values of which in most cases are greater than 1.

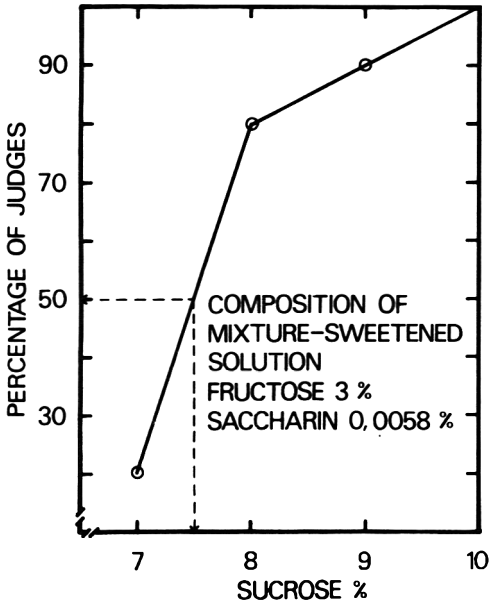

Fig. 3—Determination of the sucrose concentration of a solution which is isosweet with one sweetened with a fructose-saccharin mixture. The percentages are based on 20 judgments in two paired comparison tests.

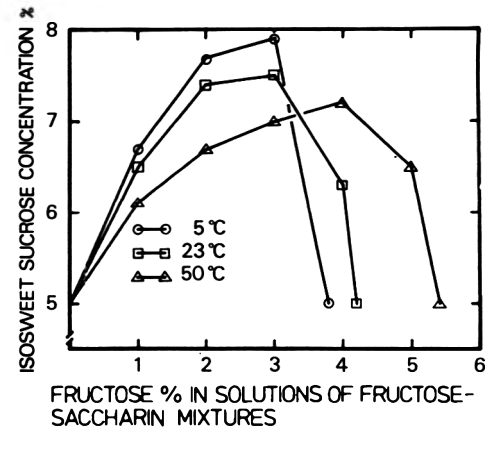

Fig. 4—Sweetness of fructose-saccharin-sweetened solutions expressed as concentration percentages of the isosweet sucrose solutions. The points plotted are the averages of 20 judgments in two paired comparison tests.

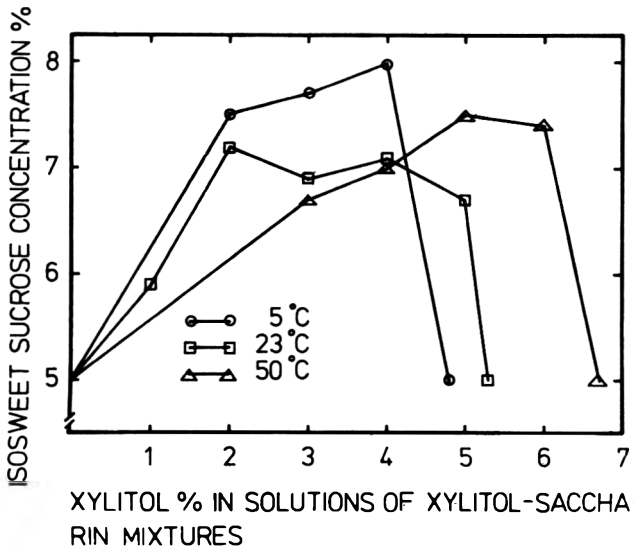

Fig. 5—Sweetness of xylitol-saccharin-sweetened solutions expressed as concentration percentages of the isosweet sucrose solutions. The points plotted are the averages of 20 judgements in two paired comparison tests.

Table 2—Comparison of a 5% sucrose solution with the mixture-sweetened solutions calculated as isosweet. Data from the triangle tests

Temperature	Revised solutions	Proportion of correct separations
5° ± 2°C	1.9% Fructose 0.0031% Saccharin	6/20
23° ± 2°C	2% Fructose 0.0039% Saccharin	9/20
50° ± 3°C	2.8% Fructose 0.0061% Saccharin	5/20
5° ± 2°C	2% Xylitol 0.0047% Saccharin	7/20
23° ± 2°C	1.4% Xylitol 0.0077% Saccharin	8/20
50° ± 3°C	2.9% Xylitol 0.0092% Saccharin	9/20

Applications

Coffee, tea and juice were sweetened with fructose-saccharin and xylitol-saccharin mixtures. Mixtures respectively containing 2.8% fructose and 0.0061% saccharin or 2.9% xylitol and 0.0092% saccharin which were isosweet with 5% sucrose in 50°C water solutions were used. The sweetness of the mixtures was significantly enhanced in coffee, the difference being significant at the 0.01% risk level. Tea and black-currant juice sweetened with the mixtures were not distinguishable by triangle test from those drinks sweetened with sucrose (Table 3).

Table 3—Comparison of mixture-sweetened coffee, tea and black-currant juice with the same drinks sweetened with 5% sucrose at 50° C in a triangle test. Test mixtures were proved isosweet with 5% sucrose in aqueous solution at 50°C (Table 2)

Proportion of sweeteners in test drinks	Test drink	Proportion of correct separations
2.8% Fructose	Coffee	15/20*
0.0061% Saccharin	Tea	6/20
	Juice	8/20
2.9% Xylitol	Coffee	18/20*
0.0092% Saccharin	Tea	10/20
	Juice	7/20

* Difference is significant at the 0.01% risk level

Table 4—Saccharin/fructose (S/F) and saccharin/xylitol (S/X) ratios in the mixtures, amounts of those mixtures of greatest synergism necessary to sweeten a drink to the common sweetness level (isosweet with 5% sucrose) at 5°, 23° and 50°C, and the corresponding energy values

Temp (°C)	S/F and S/X (%) in mix.	Amt of mix./ 150 ml drink (g)	Corresponding amt of sucrose (g)	Energy value of mix. (kJ)
5° ± 2°	0.16	2.85	7	45
23° ± 2°	0.19	3.00	7	47
50° ± 3°	0.22	4.20	7	66
5° ± 2°	0.24	3.00	7	52
23° ± 2°	0.55	2.11	7	36
50° ± 3°	0.32	4.38	7	75

In addition, the amounts of fructose-saccharin and xylitol-saccharin mixtures of the greatest sweetening power which were necessary to sweeten 150 ml of a drink to the common sweetness level (isosweet with 5% sucrose) at three temperatures, were calculated (Table 4).

Comparing the energy content of isosweet solutions, energy savings of 40–60% were achieved with the fructose-saccharin mixture, and 30–70% with the xylitol-saccharin mixture over the 7g (116 kJ) of sucrose in the sucrose sweetened solution.

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HARVEY T. CHAN JR. and RONALD A. HEU
USDA Hawaii Fruit Lab., ARS
1920 Edomndson Road, Honolulu, HI 96822
C-S. TANG,[1] ERNEST N. OKAZAKI[2] and STANLEY M. ISHIZAKI[3]
University of Hawaii, Honolulu, HI 96822

COMPOSITION OF PAPAYA SEEDS

ABSTRACT

Papaya seeds, which constitute 22% of the waste product from the papaya puree industry in Hawaii, were characterized in a preliminary study to evaluate their possible utilization. Oil was extracted from the seeds, and its fatty acid composition determined. Crude protein and crude fiber contents of the defatted meal were especially high, 40.0% and 48.9%. Benzylisothiocyanate content in the seed oil was 0.56% (w/w), and the benzylglucosinolate content in the seed meal was 1.08% (w/w).

INTRODUCTION

OVER 50% of the weight of papayas that are processed into puree appears as waste product (Brekke et al., 1977). The characterization of this waste is now in progress so that it might be utilized. We report our work on the characterization of the seed component of the waste, specifically of the seed oils and the defatted seed meal.

Held and Curl (1944) reported that papaya seeds are edible and have a spicy, pungent flavor. They also reported that seeds and peel residues have been used in poultry feed. Ettlinger and Hodgkins (1956) attributed the spicy, pungent flavor to the presence of benzylisothiocyanate (BITC), which has been shown by Tang (1973) to be formed from benzylglucosinolate by the action of thioglucosidase.

MATERIALS & METHODS

Obtaining and preparing seeds for analyses

Seeds of Solo papaya (*Carica papaya*), obtained from the fresh fruit and from the waste line of a papaya puree processing line, were soaked in water overnight to swell the membrane (sarcotesta) for easier removal. After manual removal of the sarcotesta, the seeds were air dried in a forced-draft oven at room temperature, then stored under N_2 in glass bottles at −18°C.

Moisture determination

Fresh, wet seeds with sarcotesta were weighed and air dried for 2–3 days in a forced-draft oven at 25°C. They were reweighed and ground with a mortar and pestle. A weighed portion was then dried in a vacuum oven at 60°C overnight.

Lipid extraction

Air-dried seeds were ground in a Waring Blendor and placed in Whatman cellulose extraction thimbles (80 mm × 20 mm). The powder was extracted in a Goldfisch apparatus with refluxing diethyl ether for 3 hr. The ether was evaporated from several such extracts, and the residues were then pooled and filtered through Watman #4 filter paper under dimmed light and N_2 flow. The oil was stored under N_2 in glass vials at −18°C.

Methylation

One gram of seed oil was dissolved in 10 ml benzene, and the fatty acids were transmethylated in a 40-ml, screwcapped glass centrifuge tube (Metcalfe and Schmitz, 1961) with 20 ml BF_3-methanol (14%). The tube was capped tightly and boiled for 20–30 min. The solution was then cooled, mixed well with 20 ml water and 10 ml hexane, and centrifuged at 511 × G for 5–10 min. The upper hexane layer was pipetted into another centrifuge tube, washed 3 times with water; dried over anhydrous Na_2SO_4, and concentrated with N_2 flow to ca 1 ml. The sample was stored under N_2 in a glass vial at −18°C.

Gas-liquid chromatography (GLC)

A Hewlett Packard model HP5831A Reporting Gas Chromatograph with dual flame ionization detectors was used. The carrier gas (nitrogen) flow rate was 27 ml/min and the hydrogen flow rate was 20 ml/min. The methylated fatty acids were tentatively identified by GLC on dual 6-ft × 0.093-in.-i.d. stainless steel columns packed with 10% Silar 5-CP Chromosorb W 70/80. The columns were operated isothermally at 125°C for 1 min, then programmed to 250°C at 2°C/min, then held for 1 min. The injector temperature was 220°C and detector 260°C. For the separation, identification and quantitation of the methyl esters of the fatty acids, dual 9-ft × 0.093-in.-i.d. stainless steel columns were packed with 20% DEGS on Chromosorb W 70/80. The columns were run isothermally at 190°C. The detector and injector temperatures were 225°C and 205°C, respectively.

The retention times for the methyl esters were compared to those of known esters (Applied Science). Peak areas and retentions of the methyl esters were calculated and reported by the digital processor. Gas chromatographic determination of BITC in seed oil and seed meal was based on the method of Tang (1973).

Characteristics of the seed oil

Values for the refractive index, specific gravity, percent gravity, percent unsaponifiable matter, saponification value, and iodine number were obtained by AOCS methods (1962).

Mineral analysis

Mineral analyses was done by X-ray fluorescence spectrometry with an ARS model 73,000 X-ray fluorescent Quantometer (Applied Research Laboratories.

Protein nitrogen

Nitrogen was determined with a Technicon Autoanalyzer by the principles of Kjeldahl digestion and the colorimetric determination of NH_4^+ (Schuman et al., 1973).

Crude fiber, dry matter, ash and carbohydrate

Crude fiber, dry matter, and ash were determined by AOAC (1975) methods 7.050, 7.003 and 7.010, respectively. Soluble carbohydrate was determined by difference.

RESULTS & DISCUSSION

SEEDS accounted for about 14.3% of the weight of fresh papayas. The promixate composition of papaya seeds is shown in Table 1. On a dry weight basis the oil content of papayas seeds was 32.97%, which is relatively high as compared with that of seeds from other fruit, such as figs (23.5%), pears (14.1%), apples (19–23%), grapes (12–22%) and cantaloupe (30.4%) (Jacobs, 1951). Also, on a dry weight basis the pro-

Table 1—Percent composition of papaya seeds

Moisture	71.89%
Fat	9.50
Protein	8.40
Ash	1.47
Total carbohydrate	9.44

[1] Dept. of Agricultural Biochemistry
[2] Dept. of Agronomy & Soils Science
[3] Dept. of Animal Sciences

Table 2—Characteristics of papaya seed oil

Refractive index (40°C)	1.4627
Specific gravity (25°C)	0.9130
Unsaponifiable matter	2.11%
Saponification value	193.4
Iodine no.	74.77
Free fatty acids	1.11%
Viscosity (centipoises)	339.41

Table 4—Composition of papaya seed meal

Crude protein	40.0
Crude fiber	48.9
Ash	6.86

tein content of papaya seeds, 29.16%, is comparable to that of soybeans, 35% (Jacobs, 1951), a highly touted vegetable protein source.

Ether extraction of papaya seeds yielded a slightly green oil. The results of a further analysis of this oil is shown in Table 2. The saponification number (193) indicates that the average chain length of the fatty acids in the papaya seed oil was comparable to that in oils from other sources, such as soybeans (192), cotton seed (195), sesame (192), and olive seed (192) (Jacobs, 1951). The iodine number for papaya seed oil was 74.8, and indicated that the oil was relatively low in polyunsaturated fatty acids. The indication was supported by the GLC analysis of the fatty acids (Table 3). The major fatty acids in order of decreasing abundance was oleic acid, palmitic, linoleic and stearic acid. Trace quantities of lauric, myristic, arachidic, linolenic and behenic acids were also found. The fatty acid profile and iodine number of the papaya seed oil were similar to those of teaseed, olive, cashew nut, pistachio, macadamia and almond oils (Jacobs, 1951; Hilditch and Williams, 1964; Cavaletto et al., 1966) and all these oils have high concentrations of oleic acid. The presence of high concentrations of oleic and palmitic acids in papaya agrees with the observation of Hilditch and Williams (1964) that the concentrations of these acids definitely tend to be high in plants native to subtropical and tropical regions.

BITC content

The BITC content in the seed oil was 0.56% (w/w), and the content of benzylglucosinolate in the defatted seed meal was 1.08% (w/w). While the goitrogenic effect of products containing certain isothiocyanates and their derivatives is of major concern in the cruciferous oil seed industry such as that of rape oil (Niewiadomski, 1970), the physiological effect of BITC on animals has not been clearly defined. Thus, feeding experiments are necessary for evaluation of those products as animal feed.

Proximate and mineral analyses of papaya seed meal

Table 4 shows that the defatted papaya seed meal contained high amounts of crude protein (40.0%) and crude fibers (49.9%). These values seem especially high when compared with those for pineapple bran and pineapple leaf meal, which are other by-products of Hawaiian agriculture. Ross (1966) reported that pineapple bran and pineapple leaf meal contain 3.4–4.5%, and 7.51% protein, respectively, and that they also contain 14.3–18.2, and 23.43% crude fiber, respectively.

The principal minerals of the papaya seed meal were Ca, P and Mg (Table 5).

Commercial potential availability of papaya seeds

A recent study of the processing of papaya puree (Brekke et al., 1977), showed that every ton of papayas produced

Table 3—Fatty acid composition of papaya seed oil

Fatty acid	Percent
Lauric	0.13
Myristic	0.16
Palmitic	15.13
Stearic	3.61
Oleic	71.60
Linoleic	7.68
Linolenic	0.60
Arachidic	0.87
Behenic	0.22

Table 5—Mineral analysis of papaya seed meal

Element	%	Element	%
P	0.84	Mn	0.0053
K	0.33	Fe	0.0111
Ca	1.66	Cu	0.0031
Mg	0.64		
S	0.46		

about 1280 lb of waste of which 286 lb or 22.3% was due to seeds. Hence, about 27 lb of oil might be recovered from every ton of fruit being processed.

The statewide industry projection for papaya production in Hawaii by 1980 is 40,000 tons per year (Souza, 1976). If such a goal is realized, there should be 11,000 tons of waste available for by-product utilization.

CONCLUSION

THE RESULTS of this study indicate that papaya seeds are a potential source of useable oil and seed meal. However, further studies must be conducted on the possible toxic effects of BITC before any recommendation be made on consumption or utilization of papaya seed products.

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R. J. BRADDOCK and J. W. KESTERSON
University of Florida, Institute of Food & Agricultural Sciences
Agricultural Research & Education Center, P.O. Box 1088, Lake Alfred, FL 33850

EVALUATION OF PRODUCTS MADE FROM CITRUS PROCESSING PLANT GRADING LINE REJECTS

ABSTRACT

Products were manufactured from cull oranges rejected from processing plant grading lines. Cull fruit juice yields were 20% less than from sound fruit. Frozen concentrate had a detectable stale fruit off-flavor. Total reducing sugars were 10% higher and vitamin C was 8% lower in the juice from the cull fruit, while color, cloud and viscosity were about the same as in juice from sound fruit. The aldehyde content of cold pressed oil from cull oranges was too low to meet U.S.P. specifications. Dried pulp cattle feed and molasses yields from culls were greater than from sound fruit. Crude protein content of the cull dried pulp was 6.6% and total sugars (reducing and sucrose) of cull fruit molasses was 58%; while the cull orange molasses had a viscosity of over 20,000 c.p.

INTRODUCTION

CITRUS FRUIT to be used for processing are commonly dropped from the trees to the ground by the pickers, loaded by hand into containers and transported from the grove interior to a large truck trailer for hauling to the processing plant. These trailers contain approx 20 metric tons (mt) of fruit which are unloaded in less than 15 min at the plant by dumping into a conveyor system. The unloaded fruit are immediately inspected and graded to remove injured, split and decayed fruit. The sound fruit are stored in bins and receive a final inspection and grading, after washing, prior to juice extraction (USDA, 1962). Fruit rejected by the graders are considered culls according to regulations (Fla. Dept. of Citrus, 1975) and are either destroyed or sent to a feed mill.

Present picking, hauling and handling operations result in some fruit damage; however, the future use of abscission chemicals and mechanical harvesting machinery could result in increased quantities of injured fruit delivered to the processing plants. Grierson (1968) reported two to three times more post-harvest decay (during storage for the fresh fruit market) in mechanically picked oranges than in hand picked controls. Other workers have shown that dropping fruit through the tree branches to the ground is a significant factor in damaging the fruit, allowing molds and other soil-borne organisms to enter the fruit, thus decreasing the packout of fresh fruit (Smoot and Melvin, 1975). In studies conducted at a commercial processing plant, Coppock (1977) found that mechanically harvested fruit treated before harvest with an abscission chemical showed two to three times as many culls as untreated controls. Increased quantities of cull fruit resulting from mechanical harvesting and use of abscission chemicals may be difficult to remove by hand grading, thus mechanical grading systems have been proposed (Bryan et al., 1974). Based on the authors' observations, mechanical grading results in increased amounts of rejected fruit because of the increased numbers of sound fruit included with the culls. Hand graders also tend to reject many sound fruit.

Factors such as overmaturity and freeze damage also contribute to increasing the number of culls, necessitating data concerning the quantity and quality of these fruit. This paper reports such data obtained from a cooperative study with a commercial citrus processor. Information is included to show yield and quality differences which exist between sound and cull fruit concentrated juice, cold pressed peel oil, dried pulp and molasses.

EXPERIMENTAL

Fruit

Cull fruit were taken from a commercial processing plant on three occasions, Jan. 26 (midseason oranges), Apr. 14 and May 3, 1976 (Valencia oranges). On each occasion, every fruit rejected by the graders at the fruit unloading conveyors was taken until approx 1 mt of culls were collected (2–4 hr). After collection, the fruit were brought to our pilot plant for processing. For all experiments, controls were processed by the plant and consisted of sound fruit from the same loads sent to the processing plant storage bins. Sample sizes are listed in Table 1.

Processing

The cull fruit collected at the processing plant were washed at our pilot plant with a detergent (fruit cleaner 220, FMC Corp.) in a brush washer and rinsed with a water spray. After the washing operation, the juice and peel oil emulsion were recovered using a commercial extractor (Model 391-FMC Corp.). The pulp, peel, membrane and seeds were saved for processing into dried cattle feed and molasses.

Frozen concentrate

The raw juice was passed through a finisher (screen size 0.5 mm) in preparation for concentrate manufacture and held in a refrigerated tank for feeding to the evaporator. Evaporation was performed on a pilot plant high temperature (90°C) short time (2 min) evaporator (Gulf Machinery Corp.) with approx 230 kg/hr water evaporation capacity. The cull fruit juice evaporator pump-outs (65 °Brix) were blended with 12 °Brix commercial cut-back (fresh raw juices) from sound fruit to yield 44.8 °Brix final products. A good quality cold pressed Valencia oil meeting U.S.P. specifications (USP, 1975) was added to the final products in amounts sufficient to have 0.015% oil in the reconstituted single-strength products. Commercial concentrates were made by the cooperating plant from sound fruit using the same lots of raw juices for Brix cut-back as we used for the cull fruit juices. The concentrates made by the plant from sound fruit were used as controls for all comparisons with cull fruit concentrates.

Cold pressed oil

Oil emulsions from the juice extractor oil recovery system were pumped to holding tanks (200L) and allowed to partially separate by standing for 4–5 hr, an oil rich emulsion floating to the top third of the tank. The dilute bottom layer was drained off, the emulsion held in a

Table 1—Amount of cull fruit and total fruit unloaded at a commercial processing plant in 3 hr

Date	Unloaded (mt)	Culls (mt)
1/26/76	184	1.1
4/14/76	375	1.2
5/3/76	138	0.5

cold room (2°C) overnight, further concentrating the oil rich emulsion layer. The concentrated oil emulsion was separated by centrifugation.

Dried pulp and molasses

The peel residues from the extractors were limed, chopped, pressed and dried in our pilot plant in the conventional manner for making dried citrus cattle feed and molasses (Kesterson and Braddock, 1976). This involves curing the peel with lime, pressing to recover soluble solids which are concentrated to 72 °Brix in a vacuum steam evaporator, and drying the press cake in a furnace-heated rotating triple-pass drum dehydrator (Vincent Proc., Inc.).

Material balances

Total fruit weights were obtained from the processing plant where the fruit were unloaded. Culls rejected by the graders were weighed (Table 1). Juice yields from the sound fruit were obtained by the processor while we determined yields from the culls. Actual weights of juice, wet peel residue, cold pressed oil, dried cattle feed, juice finisher pomace, peel frits and molasses were recorded. Peel frits are small bits (1–2 mm) of peel shredded by the juice extractor during peel oil recovery.

Analyses

Certain analytical tests, common to citrus processing, were performed on the juices and other products manufactured from both cull fruit and the sound fruit controls. Analyses on juices and concentrates included the following determinations described by Praschan (1976): soluble solids (°Brix), acid, pH, % oil, color, sinking pulp, cloud and vitamin C. Finished product plate counts on orange serum and potato dextrose agars were made by the commercial processor. Apparent viscosities were determined by Brookfield viscometer with appropriate rpm and spindle. Total reducing sugars, glucose, fructose and sucrose were measured in juices and molasses by the method of Ting (1956). Alcohol content of raw juices was determined by ebulliometer.

Cold pressed oils were compared on the basis of chemical and physical properties described in the USP (1975). Additionally, aldehyde, acid, ester and free alcohol contents were determined as described by Braddock and Kesterson (1976).

Dried pulp cattle feed and molasses were analyzed for protein, fat, fiber, ash and nitrogen-free extract. Aflatoxin analyses were made on the dried pulp and juice concentrates using the procedures of the AOAC (1975). For concentrate or dried pulp, 50g of either the 45 °Brix concentrate or pulp sample were extracted with 2 × 500 ml of dichloromethane to obtain samples for aflatoxin analysis.

Table 2—Product material balances for processed sound and cull fruit

Product	kg product/mt fruit[a]	
	Sound fruit	Cull fruit
Juice	544	422
Wet peel residue	373	478
Finisher pomace	33	40
Peel frits	50	60
Juice soluble solids	63	49
Dried pulp (10% H_2O)	35	61
Molasses (72° B)	16	22
Cold pressed oil	3	2

[a] Data are averages for three processing dates.

Table 3—Quality data of raw juice from sound and cull oranges for three processing dates

	Sound			Cull		
	1/26	4/14	5/3	1/26	4/14	5/3
Soluble solids (° B)	12.0	12.4	10.4	11.2	11.7	11.7
Acid (%)	0.78	1.01	0.71	0.80	1.04	0.82
Ratio (° B/A)	15.4	12.3	14.6	14.0	11.3	14.2
Color no.	37	37	38	38	37	39
Alcohol (% w/w)	tr	tr	tr	0.24	0.18	0.26

RESULTS & DISCUSSION

Product yields

Examination of Table 1 will show that on the occasions mentioned, from 0.3–0.6% of the fruit delivered to the processing plant were rejected during the initial grading. These culls should not be confused with fruit from second grading lines just prior to the juice extractors. If fruit from the second grading operation had been included, the percentage of culls would have increased. The plant of this study processes 400,000 mt of fruit per year; at 0.5%, the culls would amount to 2000 mt.

The data in Table 2 show the relationship of product yields between sound and cull fruit. Cull fruit juice yields were about 20% less and waste (residue + pomace + frits) 20% greater than from sound fruit. Increased quantities of dried pulp and molasses and decreased amounts of juice solids and cold pressed oil were recovered from the cull fruit. The major reason for yield differences in Table 2 was the soft condition of the fruit caused by decay and injury. This resulted in poor juice and oil recovery efficiencies in the extractors.

Product quality

Quality of products manufactured from cull oranges differed from those of sound oranges, since the culls contained some mandarins, grapefruit, rootstock lemons, sour oranges and unripe fruit, as well as partially decayed, split and dried fruit. Proportions of sound fruit were also included with the culls. A classification of cull fruit, similar to our observations, has been published by Bryan (1974).

Juice

The fermented condition of the cull fruit resulted in soured raw juice with a disagreeable aroma and flavor. However, the color was as good as that from sound fruit. Some quality data for the raw juices are listed in Table 3.

During concentration of the raw juice in the evaporator, the volatile fermentation aromas were removed, resulting in significant flavor improvement of the concentrated pump-out over the raw juice. Additional flavor improvement was made by blending the pump-out with cut-back and cold pressed oil. However, the disagreeable flavor could not be entirely eliminated by concentration in the evaporator or masked by addition of good cut-back and flavoring oil. General quality data of finished reconstituted juices are listed in Table 4. Differences in ratios (°Brix/acid) between the raw (Table 3) and processed juices (Table 4) are results of cut-back addition to the concentrates and evaporative loss of volatile acids from the raw cull fruit juices. The major difference between the reconstituted cull fruit juices and that of the sound fruit was flavor. The flavor score (Table 4) classification of 32–35 points is considered U.S. Grade B (USDA, 1968). The cull fruit product off-flavor was described by panelists as stale, old fruit characteristic, biting and bitter.

Microorganism plate counts after processing did not indicate any sanitation problems with either product concentrate (Table 5). Plate counts may be as high as 200,000 before plant operators become concerned. We attach little significance to the data in Table 5 showing plate count differences between cull and sound fruit samples on Jan. 26 and May 3. Frozen concentrated orange juice is not a sterile product, heat treatment to 90°C during evaporation is primarily for pectic enzyme inactivation.

Aflatoxin analyses were performed on all samples in our lab and additionally by a private lab, because of the considerable quantity of fluorescing substances (bioflavonoids, coumarins, etc.) present in orange juice which interfere with the test.

Table 4—Quality of reconstituted concentrate from sound and cull fruit for three processing dates

	Sound			Cull		
	1/26	4/14	5/3	1/26	4/14	5/3
Soluble solids (°B)	12.0	12.8	12.8	12.0	12.8	12.8
Acid (%)	0.78	0.93	0.89	0.76	0.99	0.99
Ratio	15.5	13.8	14.4	15.8	12.9	13.2
pH	3.96	3.80	3.70	4.10	3.80	3.91
Oil (%)	0.013	0.012	0.017	0.013	0.017	0.017
Color no.	37	37	38	38	37	39
Cloud (%T)	5	4	5	5	3	5
Serum visc. (c.p.)	2.3	2.0	2.0	1.6	15.3	1.7
Conc visc. (c.p.)	1215	940	1130	1700	1785	1040
Flavor (USDA)	36	36	37	34	32	34
Red. sugars (%)	4.4	4.2	4.0	4.7	4.3	4.9
Sucrose (%)	5.0	4.7	4.8	4.5	4.4	5.4
Glucose (%)	2.1	2.4	1.6	2.2	1.8	1.9
Fructose (%)	2.3	1.9	2.4	2.5	2.6	2.9
Vit. C (mg/100 ml)	52	58	41	48	44	47

Additionally, unidentified mold mycelia could be found upon close microscopic examination of the cull fruit processed juice. The tests proved negative for aflatoxins B_1, B_2, G_1 and G_2 in either the cull fruit or the sound fruit concentrate.

Cold-pressed oil

Cold-pressed oil yields from processing the cull oranges averaged 1 kg oil/mt fruit less than from the sound fruit (Table 2). The flavor and quality of oil recovered from cull fruit was poor, the aldehyde content being in the range 0.3–0.7%, too low to meet USP specifications. The free acid content of the cull oil was 0.24%, compared with 0.16% for the sound fruit oil; esters averaged 1.3% (culls) vs 0.9% (sound); while, the free alcohol content varied from 1.6% for cull to 1.1% for sound fruit oil. The cull fruit oil did not meet the quality criteria as described by Braddock and Kesterson (1976) and would not be suitable for marketing as commercial cold pressed oil. However, the oil emulsions could be steam stripped and the terpene hydrocarbon, (+)-limonene, recovered as a by-product, since the physical properties of refractive index, specific gravity and optical rotation were within USP specifications.

Dried pulp and molasses

Cattle feeds made from citrus processing residues are economically important by-products to the Florida industry, amounting to approx $85,000,000 yearly in sales to markets all over the world. Analytical data in Table 6 include some of the properties of dried pulp and molasses manufactured from cull oranges. These differences shown could affect the economic importance of the products. For instance, the higher reducing and total sugar content of the cull fruit molasses would be important to distillers using this product for alcohol production. The higher protein nitrogen content of the dried pulp and molasses would be an important plus for use in livestock feeding. However, the higher viscosity of cull fruit molasses would be a detriment, causing some handling difficulties.

Aflatoxin content of the dried pulp was determined by analysis to be negative. Occasionally, during bulk storage, improperly dried pulp will become moldy, thus the rationale for our aflatoxin analyses. We attributed the higher protein content to growth of yeasts in the peel prior to and after juice extraction, as well as in the press liquid. Microorganism growth in the raw press liquid also may have been responsible for the increase in reducing sugar content of the molasses through breakdown of the carbohydrate material in the peel prior to recovering and concentrating the press liquid.

Table 5—Microorganism plate counts for sound and cull frozen concentrated orange juices

		Organisms/ml[a]	
		Orange serum	Potato dextrose
Cull	1/26	5000	5000 (1 yeast)
	4/14	Neg	1000
	5/3	4000	4000
Sound	1/26	1000	1000
	4/14	1000	Neg
	5/3	23000	3000

[a] Concentrate (45° B) diluted by 1/1000 for plating

Table 6—Composition of dried citrus pulp and molasses made from cull fruit and compared with published data

	Published[a]	Cull[b]
Dried pulp		
Moisture (%)	9.9	10.4
Protein (%)	5.9	6.6
Fiber (%)	11.5	13.4
N.F.E. (%)	62.7	62.3
Fat (%)	3.1	2.4
Ash (%)	6.9	4.9
Aflatoxin	Neg	Neg
Molasses		
°Brix	72.0	72.0
Sucrose (%)	21.0	17.0
Glucose (%)	11.5	19.5
Fructose (%)	12.0	21.3
Moisture (%)	29.0	29.9
Protein (%)	4.1	5.8
N.F.E. (%)	62.0	60.8
Fat (%)	0.2	0.5
Fiber (%)	0.0	0.2
Ash (%)	4.7	4.7
Glucoside (%)	3.0	2.4
Pectin (%)	1.0	0.9
pH	5.0	5.7
viscosity at 25°C (cp)	2000	22200

[a] Data from Kesterson and Braddock, 1976
[b] Average of three processing runs on different dates

CONCLUSIONS

OUR DATA showed that from 0.3–0.6% culls were rejected by the graders; at times, this figure is probably as high as 2%. If, instead of being destroyed, the culls were manufactured into cattle feed and molasses, an economic return could be obtained. This should be considered by processors currently dumping grading line culls in a pasture or other remote site. From the 2000 mt/yr of cull fruit mentioned in this study, the dried pulp (Table 2) alone would have a market value of about $14,000 at today's prices. With the advent of abscission chemicals and mechanical harvesting, the amount of culls will in-

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P. STRUEBI,[1] F. E. ESCHER[2] and H. NEUKOM
Dept. of Food Science, Swiss Federal Institute of Technology
CH-8092 Zurich, Switzerland

USE OF A MACERATING PECTIC ENZYME IN APPLE NECTAR PROCESSING

ABSTRACT

The use of a commercial enzyme in the production of apple pulp for nectar beverages was investigated. During enzymatic treatment, apple pulp rheologically behaved initially as a pseudoplastic body with a yield value and later gradually shifted towards a pseudoplastic fluid. The pulp was separated by centrifugation into serum and insoluble residue fractions. Pectin analyses of the serum and residue showed that pectic substances were solubilized from the cell wall matrix essentially as highly esterified pectins. Viscosity measurements of the serum indicated some decrease in pectin chain length. The presence of water-soluble, highly esterified pectins is believed to be responsible for the desired cloud stability of apple nectar.

INTRODUCTION

FRUIT NECTARS are produced by blending fruit pulp with water, sugar and food acids. The fruit pulp content in the resulting viscous beverages can vary considerably, but must not be less than 40% according to Codex Alimentarius (Anon., 1971) and the U.S. authorities (Anon., 1968). Besides a fresh, original fruit flavor and a nonoxidized fruit color, fruit nectars should exhibit cloud stability and acceptable flow properties.

Most methods of fruit pulp preparation are based on the fact that many fruits soften sufficiently during ripening. With these fruits, a short blanching treatment followed by mechanical comminution and homogenization is sufficient to produce the desired cloud stability in the final beverage (Bertuzzi, 1961; Luh, 1971b; Gantner, 1972). Nectars from a wide range of temperate zone, subtropical and tropical fruits are manufactured by these processes (Kardos, 1966; Kuusi and Kiesvaara, 1968; Sulc and Ciric, 1968; Luh, 1971a, b; Seale, 1967).

These processes are not very suitable for the production of nectars from apples. This is probably due to the insufficient disintegration of the apple tissue during ripening. Because of this, a very intensive comminution and homogenization is necessary to reach the required pulp fineness which unfortunately results in a high degree of enzymatic browning. If the tissue is softened by prolonged blanching, the flavor of a cooked apple sauce rather than of a fresh apple nectar occurs. An alternative method of producing apple pulp for nectars became possible when pectic enzyme preparations for maceration or cell separation of plant tissue became commercially available (Grampp, 1969, 1972). Struebi et al. (1975) reported the successful application of macerating enzymes (Irgazyme M-10, Ciba-Geigy; Rohament P, Roehm & Haas). These nectars possessed not only the original fruit color and flavor, but also good textural qualities (flow properties) and cloud stability over a sufficient period of time. Struebi (1976) found that the nectars contained largely undamaged cells and cell aggregates.

Various reports are available on studies of the relation between composition of fruit purees or fruit juice concentrates and their flow properties (Holdworth, 1971; Mizrahi and Berk, 1971). Changes in insoluble particle size (fruit cell agglomerates), in the amount of soluble pectin and in total solid content of purees or concentrates are the main factors influencing flow properties. Also, several theories on cloud stability have been proposed especially for citrus juices (Joslyn and Pilnik, 1961; Baker and Bruemmer, 1972; Krop 1974). Sulc and Vujicic (1973) showed that certain pectic enzymes solubilize highly esterified pectins from apple pulp. Due to a high degree of esterification of these pectins a coagulation with ions such as calcium and a sedimentation of clouding particles are prevented. Joslyn and Pilnik (1961) have suggested a similar mechanism for the cloud stability in citrus juices.

Based on the findings of the studies discussed above, this paper investigates the rheological behavior and pectin compositional changes in apple pulp during the enzymatic maceration process, and discusses the influence of these changes on nectar quality.

EXPERIMENTAL

Preparation of apple pulp for nectars

The procedure described by Struebi et al. (1975) was used for preparing apple pulp. Fruits of the Glockenapfel variety were received from the Swiss Federal Research Station Waedenswil and stored at 4°C and 84% RH until ready for use. Batches of 10 kg of chilled apples were washed and milled to a mash with a fruit mill (Model Central 2, Bucher-Guyer Ltd., Niederweningen, Switz.). 30 mg/kg $NaHSO_3$ were added to the mash to prevent initial enzymatic browning.

To inactivate natural enzymes, the mash was heat-treated by pumping it with a Moineau type pump into a tubular swept heat exchanger (Thermalizer system, Luwa-SMS, Zurich, Switz.). The heat exchanger consisted of a heating part (1 Thermalizer unit, 0.1 m^2 heat exchange surface, 800 cm^3 product volume), a holding tube (25 mm I.D., 385 cm^3 product volume) and a cooling part (2 Thermalizer units). Saturated steam at 3.6 bar and water at 8°C were used for heating and cooling, respectively. The necessary holding time and the flow rate were derived from the time required to inactivate polyphenoloxidase as calculated by Dimick et al. (1951). At a flow rate of 2 kg/min, the mash was heated within 30 sec to 85°C, held at 85°C for 12 sec and cooled within 60 sec to 35°C.

The enzyme treatments were carried out on 5 kg of blanched mash in a reaction vessel at 38–40°C. A commercial enzyme preparation, Irgazyme M-10 (Ciba-Geigy Ltd., Basel, Switz.), at concentrations ranging from 63–1000 mg/kg mash was added as suspension in water. The mash was then stirred for 4 hr (2 hr at 72 rpm followed by 2 hr at 36 rpm). The enzyme-treated mash was passed through a 2.38 mm screen using a continuously working pulper finisher (Longsenkamp Ltd., Indianapolis, IN) to remove hard skin and core parts. For viscosity measurements, the mash was screened before enzyme treatment to avoid interference by skin and core parts. For the production of apple pulp which served as a nectar base, the mash was screened after the enzyme treatment, as the yield of finished pulp was higher in this case.

The enzyme-treated and screened pulp was used to prepare the final nectar beverage as described by Struebi et al. (1975).

Characterization of the macerating process

Flow property changes of apple pulp during enzyme treatment were determined with a rotational coaxial viscosimeter (Epprecht system, Rheomat-15, Contraves Ltd., Zurich, Switz.) at 20 ± 0.1°C. Shear stress and shear rate values were also used to determine the power law function constants for shear rates between 20 and 100 sec^{-1} using the graphical methods described by Charm (1960; 1963) (Eq 1).

$$\tau = b\left(-\frac{dv}{dy}\right)^s + C \qquad (1)$$

[1] Present address: Dept. Scientific & Industrial Research, Processing and Pomology Section, Plant Diseases Division, Auckland, N.Z.
[2] To whom requests for reprints should be directed

where τ = shear stress (dyne/cm^2); dv/dy = shear rate; b = proportionality or consistency factor; s = flow behavior index; and C = yield value (dyne/cm^2).

The apparent viscosity of apple pulp was calculated at shear rates 50 and 100 sec^{-1} (Eq 2), first by using the values of the flow curves, and secondly by using the constants C, b and s of Eq 1.

$$\eta_s = \frac{\tau}{-(dv/dy)} \qquad (2)$$

where η_s = apparent viscosity at a defined shear rate (dyne· cm/cm^2).

A shear rate of 50 sec^{-1} was chosen because there is a good correlation between sensory evaluation (mouthfeel) and rheological measurements (Wood, 1968). A shear rate of 100 sec^{-1} was selected to demonstrate the influence of increasing shear rates on the viscosity, and because higher shear rate values are important in the design of food processing equipment (Saravacos, 1970).

Apple pulp sampled at various stages of the enzyme treatment was separated into a serum and a water-insoluble marc fraction by centrifugation (20 min at 1500 × G). The relative viscosity of the serum, compared to that of water, was measured with an Ubbellohde capillary viscosimeter at 20 ± 0.1°C (water value 12.8 sec). The alcohol-insoluble residues were isolated from the serum and marc fraction by repeated washing with 95% ethanol and then dried in vacuo. The total pectin content of these residues and the degree of esterification of the pectins were analyzed by determining the carboxyl groups before and after saponification of the methylesters (Raunhardt and Neukom, 1965; Deuel et al., 1954).

RESULTS & DISCUSSION

Flow curves for apple pulp incubated with enzyme concentrations ranging from 63–1000 mg/kg for 4 hr at 38°C are shown in Figure 1. The power law values C, b and s are reported in Table 1. The yield value C decreased from the beginning of the treatment for all enzyme concentrations. For low and medium enzyme concentrations (63 and 250 mg/kg) the proportionality factor b first increased, and decreased only after 1 hr of the treatment, whereas at high concentrations (1000 mg/kg), b dropped from the beginning of the treatment. The flow behavior index s first dropped, and at a later stage of the incubation increased again with all enzyme concentrations. At enzyme concentrations of 250 and 1000 mg/kg, the value of s returned to that of nontreated pulp. Therefore, fresh as well as macerated apple pulp shows non-Newtonian flow properties and behaves rheologically as a pseudoplastic body with a yield value. With advancing maceration, the system changes more and more towards a true pseudoplastic fluid, as the yield value C is decreasing.

The apparent viscosities at shear rates of 50 and 100 sec^{-1} are summarized in Table 2, on one hand as calculated from the tabulated values of C, b and s, and on the other as directly measured with the rotational viscosimeter. There is good correspondence between the computed and the directly measured values for apparent viscosities; the correspondence improved with increasing incubation time.

Changes in the amount of serum separated by centrifugation of pulp and in the relative viscosity of the serum during the maceration process are reported in Figures 2 and 3, respectively. The initial changes which are important in maceration occurred too rapidly to be conveniently followed at high enzyme concentrations. Therefore, the results for treatment at low enzyme concentrations are more suitable for interpreting maceration changes. Up to 1-hr incubation at 63 mg/kg Irgazyme M-10, the amount of serum separated continued to decrease (Fig. 2), whereas its relative viscosity continued to increase (Fig. 3). In contrast, for an enzyme concentration of 1000 mg/kg, the serum separated increased and the serum viscosity decreased from the beginning of the treatment.

Figure 4 summarizes the relative changes of the water and alcohol-insoluble marc fraction and the alcohol-insoluble serum fraction. With increasing enzyme concentrations and incubation time, the yield of the insoluble marc fraction decreased, and the yield of the insoluble serum fraction increased.

Figure 5 reports the pectin content of the insoluble marc and the serum fractions. The pectin content of the marc fraction decreased with increasing enzyme concentration and incubation time. The percentage of the pectin in the serum fraction initially increased at low enzyme concentrations and, although subsequently declining, remained above 60% for all enzyme concentrations. Figure 6 shows that the degree of esterification of pectins remaining in the insoluble marc frac-

Table 1—Changes in yield stress C, flow behaviour index s and proportionality factor b of apple pulp incubated at different enzyme conentrations

Enzyme conc (mg/kg)	Incubation time (min)	Yield value C (dyne/cm^2)	Flow behavior index s	Proportionality factor b
0	0	240	0.47	105
63	240	81	0.34	120
125	240	64	0.44	50
250	240	36	0.46	31
500	240	31	0.41	22
1000	240	28	0.44	14
63	15	210	0.39	126
	30	170	0.30	184
	60	120	0.28	205
	120	100	0.31	165
	240	81	0.34	120
250	15	180	0.34	176
	30	144	0.31	174
	60	90	0.28	168
	120	50	0.35	90
	240	36	0.46	31
1000	15	80	0.37	95
	30	71	0.38	49
	60	54	0.48	18
	120	34	0.48	14
	240	28	0.44	14

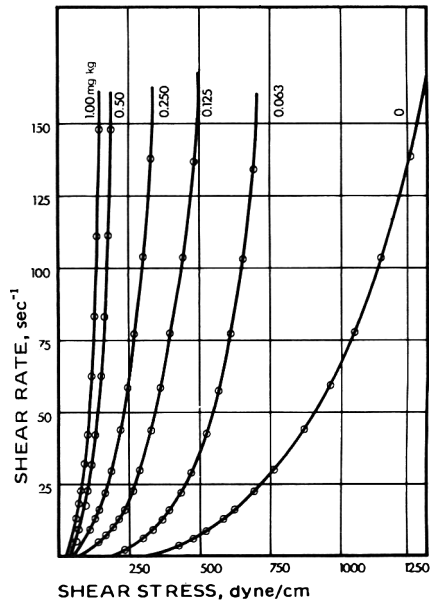

Fig. 1—Flow curve of apple pulp incubated with different enzyme concentrations for 4 hr at 38°C.

tion dropped continuously, whereas the degree of esterification of all serum pectins always remained above 50%.

It is important to correlate the compositional changes of apple pulp during maceration (Fig. 2–6) with the changes of the power law values (Table 1). In the initial phase of the enzyme treatment, increases in the amount of soluble pectin and in relative serum viscosity coincided with increases in the proportionally factor b. At a later stage the serum viscosity and the proportionality factor b declined. This decrease of b seems to be due to a decrease in chain length of the pectins. A decrease in pectin chain length is believed to have occurred because the dried alcohol-insoluble residues were fibrous at low enzyme concentration and short treatment, but became grainy and sandy at higher enzyme concentrations and longer treatment. The yield value C is also influenced to some extent by the amount of soluble pectin present in the product. As Struebi (1976) showed, the decline of the yield stress C also coincided with the continuing separation of larger cell agglomerates. No conclusive explanation for the changes in the flow behavior index s during the maceration was found. Changes in solid matter, which are suggested by Mizrahi and Berk (1971) to be the reason for changes of s, did not occur during the whole incubation period.

Table 2—Apparent viscosity at shear rates of 50 and 100 sec^{-1} of apple pulp incubated at different enzyme concentrations

Enzyme conc (mg/kg)	Incubation time (min)	Apparent viscosity η_s (dyne·sec/cm^2) D = 50 sec^{-1} a	D = 50 sec^{-1} b	D = 100 sec^{-1} a	D = 100 sec^{-1} b
0	0	18.20	17.74	11.40	11.34
63	240	10.60	10.52	6.50	6.42
125	240	6.88	6.76	4.35	4.34
250	240	4.50	4.46	3.00	2.94
500	240	2.82	2.76	1.72	1.73
1000	240	2.26	2.25	1.34	1.44
63	15	16.14	15.56	9.55	9.52
	30	15.70	15.30	9.10	9.03
	60	14.44	14.42	8.52	8.47
	120	13.10	12.88	7.84	7.72
	240	10.60	10.52	6.50	6.42
	420	8.60	8.48	5.32	5.32
1000	15	10.04	9.68	5.96	6.02
	30	5.70	5.66	3.45	3.42
	60	3.60	3.44	2.20	2.18
	120	2.62	2.52	1.60	1.62
	240	2.26	2.25	1.34	1.44

[a] Measured directly
[b] Calculated from values of C, b and s in Table 1

The initial increase in serum viscosity and decrease in percentage of serum separated at low enzyme concentration indicate swelling of the fruit tissue as well as rapid solubilization of long chain pectin molecules. After 1 hr enzyme treatment, the amount of serum increased whereas little change occurred in the marc and in the alcohol-insoluble serum fraction (Fig. 2 and 4). Since the apparent viscosity of the pulp continued to drop during this period (Table 2), it appears that solubilized pectin fractions which contribute to the viscosity were degraded. This would also explain the changes in appearance of the dried alcohol precipitates. At high enzyme concentrations, the solubilization and degradation process most probably occurred simultaneously resulting in the immediate drop in serum viscosity.

The results summarized in Figure 6 indicate, that highly esterified pectins were solubilized specifically, and that low-esterified pectins remained either in the cell wall matrix or were completely degraded. This supports the findings of Sulc and Vujicic (1973) mentioned in the introductory part. These authors were also able to isolate soluble highly esterified pectins from apple pulp after enzymatic treatment.

The rheological data and the analytical results of the maceration experiments permit some conclusions as to what changes in the apple mash lead to the desired textural qualities and to the necessary cloud stability of apple nectars (Struebi et al., 1975). The smoothness of the product is obtained by the initial enzymatic disintegration of apple into small groups of cells, which permits the final breakdown of large particles by mechanical screening and homogenization. Because the cell walls generally remain intact and strongly hydrated, the cells remain suspended in a serum containing a large proportion of water-soluble long-chain pectins. The cell hydration and the high degree of polymerization of pectins contribute to the apparent viscosity of the nectar base and to the characteristic consistency of the nectar beverage. Also, the presence of high esterified pectins seems important for cloud stability as was already outlined in the introduction. Therefore, it is essential to inactivate pectinesterases naturally present in fruits during

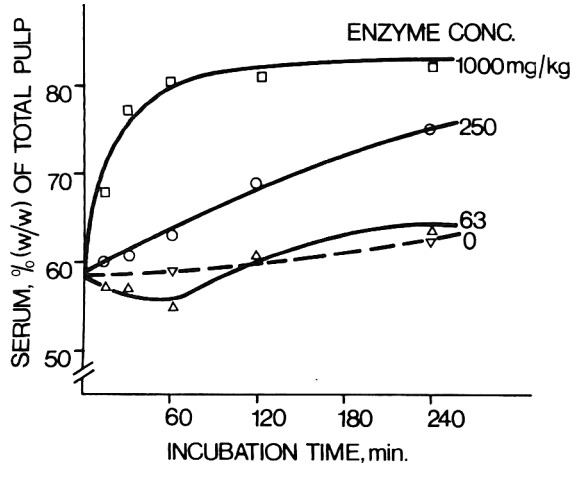

Fig. 2—Amount of serum separated from apple pulp treated with different concentrations of Irgazyme M-10 at different lengths of time.

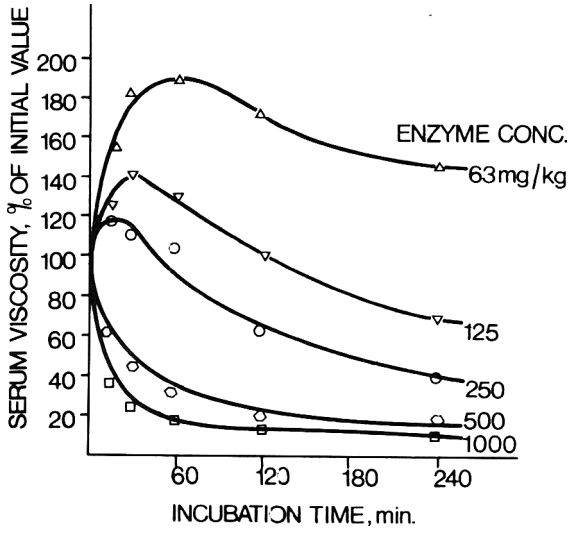

Fig. 3—Change of relative viscosity of serum during incubation with Irgazyme M-10. Initial viscosity = 16.25 × water value.

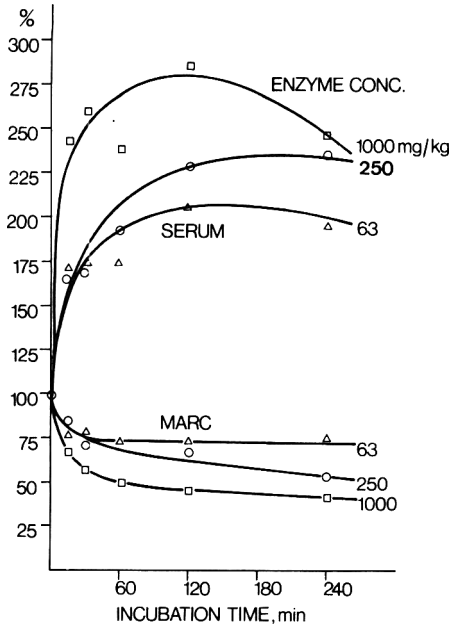

Fig. 4—Relative changes in weight of water and alcohol-insoluble marc fraction and of alcohol insoluble serum fraction during incubation of apple pulp with Irgazyme M-10. Marc: 100% = 1.50g dried water and alcohol-insoluble marc per 100g moist pulp; Serum: 100% = 0.32g dried alcohol-insoluble solids per 100g moist pulp.

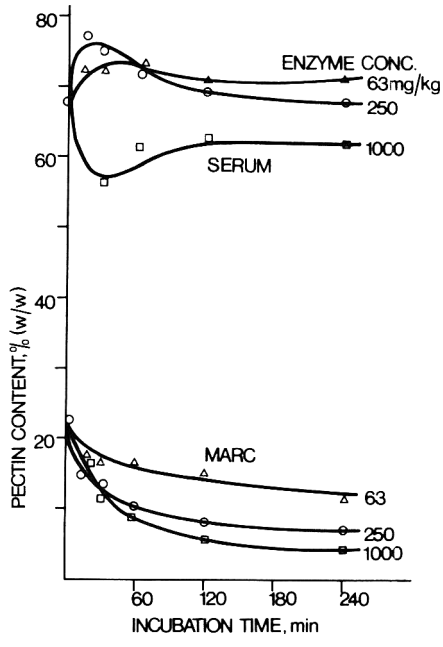

Fig. 5—Changes in concentration of pectic substances in the water and alcohol-insoluble marc fraction and in the alcohol-insoluble serum fraction during incubation of apple pulp with Irgazyme M-10.

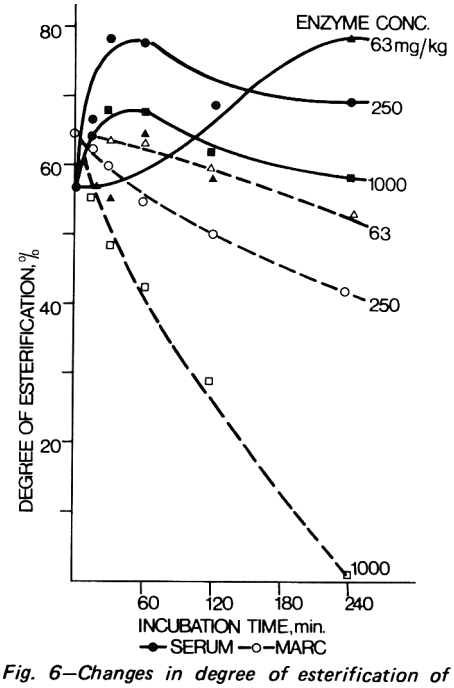

Fig. 6—Changes in degree of esterification of pectic substances in the water and alcohol-insoluble marc fraction and in the alcohol-insoluble serum fraction during incubation of apple pulp with Irgazyme M-10.

the blanching operation. The amount of esterified pectin in the serum is sufficient to maintain the cloud stability even after the nectar base has been diluted with sugar-acid solution.

No analyses were made of the non-pectin fraction (i.e. cellulose and hemicelluloses) in the water-insoluble marc. Their degradation might contribute to the cell separation and maceration of apple tissue. In their work on total liquefaction of fruits and vegetables, Pilnik et al. (1975) mention the importance of cellulolytic as well as pectolytic enzymes. However, it is not clear what the relationship between maceration and liquefaction is. Further research is required to elucidate the role and mechanism of these enzymes in producing nectars and liquefied fruit products.

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R. W. BUESCHER and R. J. FURMANSKI
Dept. of Horticultural Food Science
University of Arkansas, Fayetteville, AR 72701

ROLE OF PECTINESTERASE AND POLYGALACTURONASE IN THE FORMATION OF WOOLLINESS IN PEACHES

ABSTRACT

Chilling injury symptoms do not appear in nonripe peaches (*Prunus persica* L.) while stored at low temperatures, but a dry-mealy texture (woolliness) appears in chill-injured fruits after ripening. Reduced pectinesterase and polygalacturonase activities were associated with reduced juiciness, reduced levels of water soluble pectins, poor texture and enhanced levels of insoluble pectins in peaches which were ripened after storage at 1°C for more than 3 wk. Pectinesterase and polygalacturonase activities and levels of pectic substances were unaltered in nonripened fruits held at 1°C. Fruits transferred to 20°C for 24 or 48 hr after 1 and 3 or 2 and 4 wk of storage at 1°C did not exhibit woolliness after 5.5 wk of storage. Warming for 12 hr was inadequate to prevent the development of woolliness. The effect of intermittent warming on providing a desirable texture in subsequently ripened fruits was related to enhanced pectinesterase and polygalacturonase activities. Low temperatures appeared to induce this physiological disorder by reducing the capacity to provide adequate levels of pectin-esterase and polygalacturonase during subsequent ripening at nonchilling temperatures. Intermittent warming appeared to prevent injury by protecting the capacity to produce adequate levels of pectolytic enzymes during ripening.

INTRODUCTION

FRESH MARKET PEACHES are usually harvested prior to being ripe and stored at low temperatures (0–8°C) during marketing. Low temperatures are necessary for retarding ripening and maintaining salable fruits; however, nonripe peaches are subject to chilling injury when exposed to temperatures below 10°C (Harding and Haller, 1932, 1934; Lutz and Hardenburg, 1968). Chilling injury in peaches results in a dry-mealy (woolly) texture although injured fruits appear normal. Since the presence of woolliness is not obvious until consumption, it is detrimental to consumer acceptance and demand for peaches (Davies et al., 1937; Boyes, 1955).

Woolliness becomes evident in peaches ripened after storage for 1–4 wk at 0–10°C depending on the cultivar (Furmanski and Buescher, 1976; de Haan, 1957; Haller and Harding, 1939). Fruits pre-ripened prior to storage are resistant to injury (de Haan, 1957; O'Reilly, 1947; Scott et al., 1969; Ben-Arie and Lavee, 1971) while all nonripened stages of maturity are equally susceptible (Buescher and Griffith, 1976). Intermittent warming of peaches stored at chilling temperatures has been shown to reduce the development of wooliness and allows for extended storage (Anderson and Penney, 1975; Ben-Arie et al., 1970; Scott et al., 1969).

Formation of woolliness has been associated with impaired solubilization of pectic substances (Ben-Arie and Lavee, 1971; de Haan, 1957). Ben-Arie and Lavee (1971) have indicated that pectinesterase (PE) demethylates pectins during low temperature storage which results in an insoluble low methoxyl pectin of high molecular weight that holds water in a gel. Thus, juiciness is reduced and the symptom of woolliness is expessed.

Polygalacturonase (PG) has been associated with softening and solubilization of pectins in peaches during normal ripening (Pressey et al., 1971). Anomalous pectolytic enzyme action induced by chilling could account for impaired depolymerization of pectins and concomitant expression of woolliness.

The objectives of the study reported herein were to determine if pectolytic enzyme (PG and PE) activities were associated with the formation of woolliness in peaches. Solubility of pectic substances, taste panels, and expressible juice were used for characterizing woolliness in peaches allowed to ripen after storage at 1°C. Intermittent warming, which prevented woolliness, was also used to substantiate the hypothesis that depressed pectolytic enzyme activities were associated with the formation of this disorder.

EXPERIMENTAL

Preparation and treatment

Firm (USDA, 1969) peach fruits (cvs. Red Haven and Jefferson) of uniform size were obtained from the University of Arkansas Fruit Substation and placed in storage at 1°C and approximately 90% relative humidity. Two samples (each sample consisted of 16 fruits separated into four replicates of four fruits each) were taked after 0, 1, 2, 3, 4, and 5 wk of storage. One sample was assayed immediately after storage for pectic substances, PE, and PG activities while fruits in the other sample were allowed to ripen for 3 days at 20°C in humidified air. After ripening, fruits were assayed for PE and PG activities, pectic substances, expressible juice, taste and texture.

Effects of intermittent warming were determined by exposing fruits to 20°C for 0, 12, 24 or 48 hr after 1 and 3 wk or after 2 and 4 wk at 1°C. Each treatment consisted of 12 fruits which were separated into three replicates of four fruit each. After 5.5 wk in storage all fruits were transferred to 20°C, allowed to ripen for 3 days, and then assayed for flavor, texture, PE and PG activities

PE and PG assays

Pectolytic enzymes were extracted by homogenizing peach flesh in an aqueous solution containing 0.5M NaCl and 1% polyvinylpyrrolidone. The homogenates were centrifuged at 11,300 × G for 10 min, filtered and used for the enzyme assays.

PE activity (microequivalents/g fresh wt-min) was determined from the amount of 0.05N NaOH required to maintain a 1% citrus pectin solution containing 0.01% chloramphenicol in 0.1M sodium acetate buffer (pH 5.0) and the enzyme maintained at 30°C (Kertesz, 1951). Distilled water at 30°C was used to standardize the viscometers.

Pectic substances

Pectic substances in peach flesh were extracted by sequentially extracting the material insoluble in 80% ethanol with water, 0.5% sodium hexametaphosphate (SHMP), and 0.05N NaOH. Three extractions were made with each solvent. The uronide content in each fraction was determined by the colorimetric procedure described by McComb and McCready (1952).

Expressible juice

Juiciness was estimated by determining weight loss from tissue disks (18 mm diam, 3 mm thickness) after centrifuging for 10 min at 1700 × G. Tissue disks were supported over a wad of absorbant paper by a polyethylene net during centrifugation. From preliminary studies, results from this method correlated very well (r = 0.88) with sensory scores for juiciness.

Sensory evaluation

A sensory panel with six members was used for evaluating flavor and texture of ripened peaches. Samples were rated on a scale from 1 (poor) to 10 (excellent).

RESULTS

PE ACTIVITY declined in peaches during storage at 1°C (Fig. 1A). The greatest decline occurred in fruits stored for 1 wk and then after the third week, PE activity continuted to de-

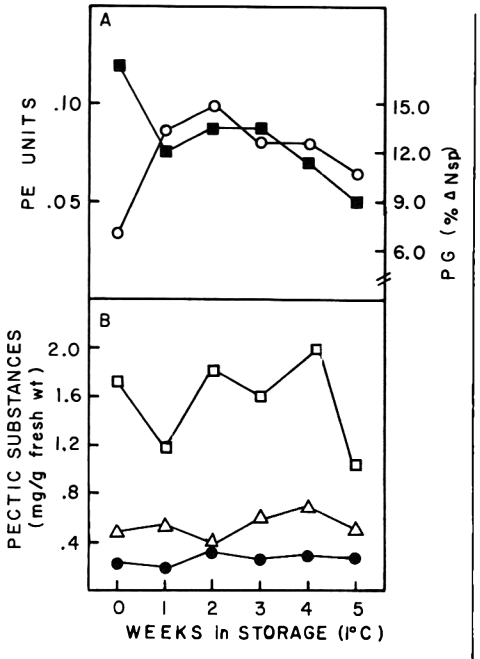

Fig. 1—PE, PG activities, and levels of pectic substances in 'Red Haven' peaches stored at 1°C. (A) PE activity (■—■), PG activity (○—○); (B) 0.05N NaOH soluble pectins (□—□), SHMP soluble pectins (●—●) and water soluble pectins (Δ—Δ).

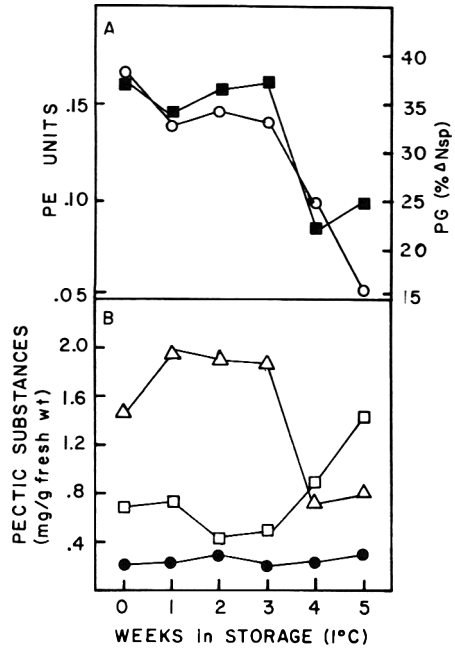

Fig. 2—PE, PG activities, and levels of pectic substances in 'Red Haven' peaches ripened for 3 days after cold storage. (A) PE activity (■—■), PG activity (○—○); (B) 0.05N NaOH soluble pectin (□—□), SHMP soluble pectin (●—●) and water soluble pectin (Δ—Δ)

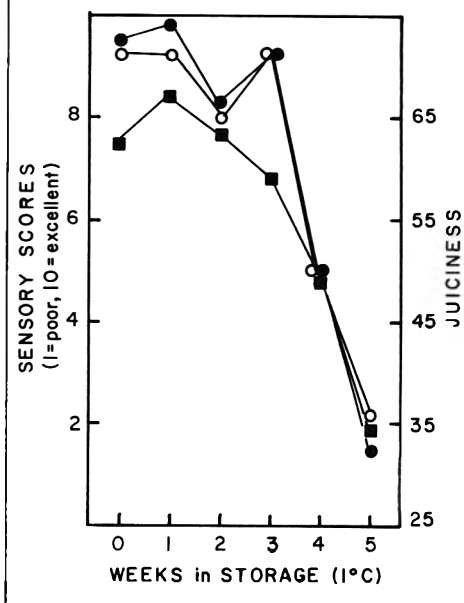

Fig. 3—Texture (●—●), flavor (○—○) and juiciness (% expressible juice, ■—■) of 'Red Haven' peaches ripened after storage at 1°C.

cline. While PG activity is low in peach fruits (Pressey et al., 1971) it was measurable after allowing the enzyme extract to react with the substrate for 24 hr. PG activity increased during the first 2 wk of storage at 1°C and then tended to decline.

Changes in pectolytic enzyme activities during 1°C storage were not reflected in altered solubility of the pectic substances (Fig. 1B). This would be expected, since PE and PG activities in fruits stored at 1°C would be much lower than those measured at 30°C.

In fruits ripened for 3 days at 20°C following storage at 1°C for 0, 1, 2, and 3 wk, PE and PG activities remained fairly constant (Fig. 2A). After 4 and 5 wk of storage, PE and PG activities were substantially reduced in the ripened fruit. Reduced pectolytic enzyme activities were associated with enhanced levels of 0.05N NaOH soluble pectins (protopectin) and reduced levels of water-soluble pectins (Fig. 2B). Levels of SHMP soluble pectins were not affected by storage. Juiciness and sensory scores for flavor and texture were markedly reduced in peaches which had been ripened after storage for 4 and 5 wk at 1°C (Fig. 3). Sensory panel members judged fruits stored for 4 and 5 wk to be dry and mealy with poor flavor, while fruits stored for 1, 2, or 3 wk had normal flavor and texture. Juiciness declined in fruits which had been stored for 3 wk, however, this was not detected by the sensory panel. It appears that a critical time of storage at chilling temperature is necessary for woolliness to become apparent after ripening. The appearance of woolliness is indicated to be closely associated with reduced PE and PG activities which results in reduced solubilization of pectic substances, loss of juiciness, and a dry, mealy texture. In this study, storage for more than 3 wk at 1°C was necessary to induce woolliness.

Our results substantiate that intermittent warming prevents the development of woolliness in peaches stored at chilling temperatures (Anderson and Penney, 1975; Ben-Arie et al., 1970; Scott et al., 1969). Woolliness was prevented in peaches exposed to 20°C for 24 or 48 hr after 1 and 3 wk or 2 and 4 wk in storage at 1°C (Fig. 4A). Texture and flavor scores were higher in peaches ripened at 20°C for 12 hr than in those continuously chilled, which indicated that the severity of woolliness was reduced; however, additional time of intermittent warming was necessary to eliminate the appearance of this disorder.

PE and PG activities were greater in fruits which did not exhibit woolliness than in fruits with woolliness (Fig. 4B). Fruits exposed to intermittent warming after 1 and 3 wk in storage at 1°C contained higher activities of PE and PG than did fruits warmed after 2 and 4 wk in storage, regardless of the time of exposure to 20°C. The effects of intermittent warming on texture and flavor qualities of ripened fruit appears to be directly related to protecting against depressed pectolytic enzyme activity.

DISCUSSION

CHANGES in pectic substances and pectolytic enzyme activities during chilling temperature storage does not appear to be responsible for the formation of woolliness. Instead, the ability to complete normal ripening is affected during chilling and injury becomes apparent in chill-injured fruits when transferred to temperatures suitable for ripening. We find that woolliness is associated with reduced levels of soluble pectins and enhanced levels of insoluble pectins in ripened fruits which substantiate the finding of other investigators (Ben-Arie and Lavee, 1971; de Haan, 1957). During normal ripening, PE (Ben-Arie and Lavee, 1971) and PG (Pressey et al., 1971) activities increase and pectic substances become soluble. In chill injured peaches, we find that PE and PG activities increase during ripening but to a lower level than in noninjured fruit. Thus, reduced PE and PG activities appear to account for reduced depolymerization and reduced solubilization of pectic substances. Ben-Arie and Lavee (1971) have indicated that insoluble low methoxyl pectins are high in woolly fruit due to

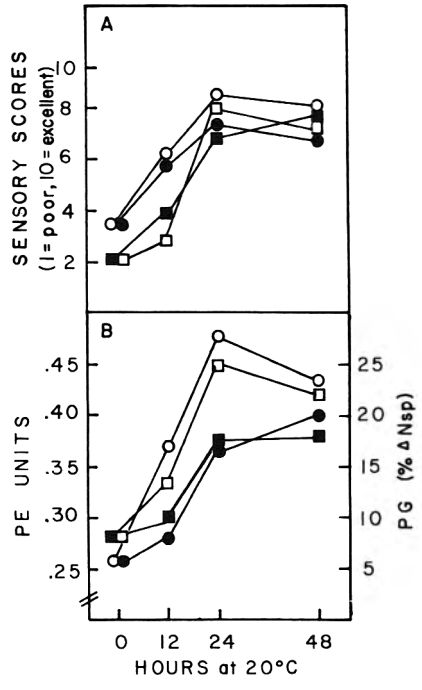

Fig. 4—Effects of intermittent warming of 'Jefferson' peaches stored at 1°C on texture, flavor, PE and PG activities after ripening. Intermittent warming after 1 and 3 wk (open points) or 2 and 4 wk (solid points). (A) flavor (○—○) and texture (□—□); (B) PG activity (○—○) and PE activity (□—□).

continuous demethylation by PE. This suggests that the action of PE is not limiting but that the level of PG activity is the limiting factor.

Recently, Strand et al. (1976) reported that PG is responsible for releasing cell wall bound enzymes. Thus, pectolytic enzymes may be involved in regulating other reactions necessary for normal ripening in addition to degradation of pectic substances. Abnormal ripening of tomatoes has been associated with little or no PG activity (Hobson, 1964; Buescher and Tigchelaar, 1975) which emphasizes the importance of PG in providing normal ripening.

Very low or no detectable PG activity in immature green fruits with large increases in activity during ripening indicate that synthesis of PG accounts for its increased activity (Pressey et al., 1971). Li and Hansen (1964) reported that the failure of pears to ripen after extensive cold storage was related to supressed capacity for synthesizing proteins. Reduced capacity to synthesize proteins elicited by low temperature storage might also account for reduced levels of pectolytic enzyme activities in chill-injured peaches during ripening.

We confirm the results of other workers that intermittent warming during storage of nonripened fruits prevents the appearance of woolliness after ripening. (Ben-Arie et al., 1970; Scott et a., 1969; Anderson and Penney, 1975). In addition, we show that 24 hr of warming after every 2 wk of storage prevented woolliness as well as warming for 48 hr. Preventing woolliness by intermittent warming appears to be associated with the development of adequate PE and PG activities during ripening. Exposing fruit to intermittent warming after 1 and 3 wk in storage resulted in higher PE and PG activities than after 2 and 4 wk in storage which indicates that the time in storage prior to intermittent warming is related to subsequent PE and PG activities during ripening. The effect of intermittent warming on protection against woolliness and preventing reduced PE and PG activities provides additional support for the hypothesis that woolliness is caused by reduced pectolytic enzyme activity during ripening.

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CHARLES J. WAGNER JR. and PHILIP E. SHAW
USDA Citrus and Subtropical Products Laboratory
Southern Region, ARS, Winter Haven, FL 33880

SENSORY EVALUATIONS ON TANGERINE-GRAPEFRUIT JUICE BLENDS

ABSTRACT

Tests were conducted on blends of tangerine and grapefruit juices reconstituted from concentrates. Flavor thresholds, preferences and influences of °Brix and °Brix/acid ratio on tangerine-grapefruit juice blends were established from evaluations by panelists who were familiar with citrus juices. Flavor thresholds of tangerine in grapefruit juice were 14–15%. Blends at concentrations of 15–35% differed significantly from and were preferred over unblended grapefruit juice. Increasing °Brix of the blends caused a significant difference among blends, but the influence of °Brix/acid ratio was negligible.

INTRODUCTION

THE VALUE OF GRAPEFRUIT for canned juice products has decreased in recent years (Weekly Citrus Statistical Report 1973–1976) and harshness, acidity and bitterness of the juices apparently were partially responsible. Over the years the industry has developed new products from citrus, such as blends of juices, to reduce surpluses, or to establish new markets. The first such product was a blend of orange and grapefruit juices. Consumers' taste reactions to three experimental blends were described (U.S. Bureau of Agricultural Economics, 1949). Sweetened grapefruit juices (sugar added) also were developed. Consumer preferences for these canned grapefruit juices were reported (Bell, 1955) and widening markets for these products have helped increase the value of grapefruit. Other grapefruit products with wide consumer appeal are needed to provide new markets.

The consumer's familiarity with the delightful flavors of tangelos has been limited to the fresh fruit. These citrus are hybrids of tangerine and grapefruit. Processed juices from many of these hybrids have been unacceptable. In addition, small crop sizes, seasonal changes in fruit quality, and processing problems such as low juice yields and excessive peel oil limit the potential for processing these hybrids as juice products. However, another possible approach for obtaining the pleasant flavors of these hybrids would be the blending of tangerine and grapefruit concentrates. The blend of tangerine with grapefruit might be mutually beneficial.

Results for this report encompass sensory evaluations on tangerine-grapefruit juice blends evaluated by taste panelists who were familiar with citrus juices. Thresholds were determined to establish the minimum concentration of tangerine needed for blends, and comparisons were made between blends and grapefruit juice. Also the influences of °Brix and °Brix/acid ratios on preferences for tangerine-grapefruit blends were examined.

EXPERIMENTAL

Materials

Blended and unblended juices were prepared from retail 6-oz cans of grapefruit concentrate and sweetened concentrated tangerine juice. In addition, grapefruit and tangerine evaporator pumpouts (concentrate devoid of flavor fractions usually added to retail concentrates) were used for the blended juices. Water at 7–8°C was used for reconstituting these concentrates. Sucrose or crystalline citric acid was used to adjust juice °Brix/acid ratios and cold-pressed grapefruit or tangerine oil from commercial sources was used to adjust oil concentration.

Methods

Analyses of juices. °Brix (total soluble solids) values were measured with a Zeiss refractometer with a sucrose scale and were corrected for temperature and acid. Citric acid levels in juice were determined on 10-g samples titrated with standardized sodium hydroxide solution to pH 8.2. Recoverable oil was measured by the bromate titration method (Scott and Veldhuis, 1966).

Calculations for juices. Amounts of chilled water for diluting concentrates were calculated from the corrected total soluble solids of the concentrates. °Brix/acid ratios were calculated by dividing corrected total soluble solids by the citric acid content, and were used to determine amounts of sucrose or crystalline citric acid that would be required to adjust °Brix/acid ratios.

Preparation of juices. Juice samples were compared with a Zeiss refractometer and a thermometer to be within 0.1 °Brix and 0.3°C before sampling. Samples (25 ml each) were then dispensed into dark red glasses that were then randomized on trays. These trays were distributed to tasters in individual booths illuminated by red lights to eliminate possible color bias. There were four booths within an air conditioned room to eliminate extraneous odors.

Tests on juices

Our objective in these tests were to determine: (1) minimum detectable concentration of each juice in the other; (2) whether blends were preferred over grapefruit juice; (3) whether °Brix and °Brix/acid ratios influence preferences for these blends; and (4) whether there is a preferred optimum concentration of tangerine in grapefruit juice. Thresholds on juices were determined using paired comparison taste tests. The definition of threshold used by Guilford (1936) which was the basis for these tests, states that threshold is the concentration of the test component for which there are 50% more correct identifications than would be expected by chance. All thresholds were obtained with a series of four to five tests representing the same number of concentrations compared each time to an unblended sample. A high concentration was used for the first test in the series to familiarize tasters with the flavor for which the threshold was sought. In the next test in the series the concentration was low to bracket the threshold, and concentrations for subsequent tests were refined gradations within the extremes. All tests in a series were given on consecutive days, and in all tests the 12 judges were asked to identify the blended sample.

Preferences for juices were determined by use of ranking tests. The same 12 tasters who had evaluated juices in the threshold tests were asked to rank four samples at a time from 1 to 4; 1 indicated the sample most liked, and 4 indicated the sample least liked.

These preference tests were used to investigate the influence of °Brix, °Brix/acid ratio and concentration on blends. In addition triangular taste tests (Boggs and Hanson, 1949) were used to determine whether oil concentrations affected thresholds. In these tests the three blends at their threshold concentrations were each tested with and without an increase in oil content equivalent to the maximum difference experienced with blended and unblended juices.

Analyses of tests

Threshold concentrations were interpolated from the linear regression of the log of concentration versus probability for each series of threshold tests; and correlation coefficients were calculated for the linear regressions Significance of contrasts from the statistical analysis of the rank means for samples in the preference testing was tested by Scheffe's (1953) procedure. Significance of triangular taste tests was determined from standard tables (Roessler et al., 1948).

RESULTS & DISCUSSION

SENSORY EVALUATIONS on blends of tangerine and grapefruit juices were conducted to determine whether the blends were preferred over grapefruit juice alone. These blends if pre-

Table 1—Analyses of concentrates used for tangerine-grapefruit juice blends

Concentrates	°Brix	% Acid	°Brix/acid ratio	% Oil
Commercial tangerine	42.6	2.42	17.6	0.012
Tangerine pumpout	58.6	4.31	13.6	0.002
Tangerine pumpout	59.4	4.50	13.2	0.005
Commercial grapefruit	37.6	3.65	10.3	0.011
Commercial grapefruit	38.0	4.49	8.47	0.009
Commercial grapefruit	38.9	4.16	9.36	0.011
Grapefruit pumpout	59.6	6.68	8.92	0.002

Table 2—Thresholds for tangerine-grapefruit juice blends

Blends	°Brix/acid ratio	Tests	Concentration[a]	Correlation coefficient R^2
Grapefruit in tangerine	17.6	5	16.1 ± 2.1	0.73*
Tangerine in grapefruit	8.47	4	15.3 ± 0.2	0.93**
Tangerine in grapefruit	10.3	5	13.7 ± 0.1	0.96**

[a] Threshold concentration is obtained from the linear regression of probability versus log of concentration and is expressed as the interval at the 95% confidence limits (C.L.). Concentration represents the percentage of total soluble solids contributed by the first named juice in these blends.
* 90% C.L.
** 95% C.L.

Table 3—Average preference rankings of tangerine-grapefruit blends with and without ratio adjustment[a]

°Brix/acid ratio	% Tangerine			
	0	15	20	25
8.47	3.4***	2.4	2.0	2.2
8.47–9.35	3.4***	2.5	2.2	1.8
9.36	3.1***	2.4	2.4	2.1
9.36–10.1	3.6***	2.3	2.3	1.7
10.3	3.0**	2.6	2.1	2.2
10.3–11.0[b]	3.4***	2.6**	2.3	1.7**

[a] Scale of 1 to 4 used for ranking samples, where 1 is the most liked and 4 is the least liked.
[b] In addition to difference between 0% tangerine and the other blends, a difference between the 15% and 25% blends was found.
** 95% Confidence limits
*** 99% Confidence limits

Table 4—Influence of °Brix on average preference rankings of tangerine-grapefruit juice blends[a]

°Brix	% Tangerine			
	20	25	30	35
10.5	2.4	2.8	2.3	2.4
12.0	3.0**	2.7	2.3	1.9

[a] Scale of 1 to 4 used for ranking samples, where 1 is the most liked and 4 is the least liked.
** 95% Confidence level

ferred could indicate the possibilities for new markets for grapefruit juice.

The inventory and analyses of concentrates used to prepare juice blends are shown in Table 1. The lack of flavor fractions in "pumpout" is indicated by the low concentrations of oil. Because the commercial tangerine concentrate was a sugar-added product, its °Brix/acid ratio was higher than the ratios for the two tangerine "pumpouts." The three commercial grapefruit concentrates represent two USDA grades (USDA, 1968). The concentrate with an 8.47 °Brix/acid ratio is grade B, while the other two with °Brix/acid ratios above 9 are grade A.

Thresholds determined for reconstituted juices from grapefruit concentrate in a sweetened concentrated tangerine juice, and for tangerine concentrate in both grade A and B grapefruit concentrates are listed in Table 2. All juices for each threshold were tasted at the ratio shown. °Brix of the juices used for the grapefruit in tangerine was 12.0, while °Brix of juices for tangerine in grapefruit was 10.0. Results indicate that thresholds for tangerine in grapefruit are in the range of 14–15% on a total soluble solids basis. The correlation coefficient for the linear regression used to obtain the threshold for grapefruit in tangerine is 0.73, this larger unexplained error in comparison to the other two might be attributed to using a sweetened tangerine product. Natural tangerine concentrate has not been available to the consumer in recent years.

Although the usual taste panel variables (such as temperature, light, etc.) as well as °Brix and °Brix/acid ratio of the juices were controlled in these threshold tests, differences in oil content between blended and unblended juices varied from 0–0.003%. For determination of the influence of oil content, blends at their threshold concentrations with and without an increase of 0.003% in oil content were evaluated by triangular taste tests, and the difference was not detected by the panel.

We compared the threshold concentration (15%) for tangerine in grapefruit and two additional concentrations (20 and 25%), to the unblended grapefruit juice. Table 3 presents average preference rankings for these comparisons. In all tests the unblended grapefruit juice differed significantly from the three blends and was the least liked sample. The tendency for the 25% blends to have good (most liked) average rankings is evident between those samples with and without ratio adjustment; and with the high °Brix/acid ratio grapefruit a significant difference was found between the 15% and 25% blends. This suggested that tasters might prefer concentrations of tangerine above 25%. In other tests the 15% blend was replaced with a 35% blend.

Before preference for the 35% blends was tested, a three-member taste panel screened the influence of °Brix on these blends. Blends of 35% tangerine in grapefruit were tasted at 9.0, 10.5 and 12.0 °Brix. While 10.5 °Brix (normal for reconstituted grapefruit concentrate) had been used for the previous juice blends, 1.5 °Brix increments on either side of this would include a 12.0 °Brix (normal for reconstituted tangerine concentrate) blend and a 9.0 °Brix juice blend. Results showed that the 9.0 °Brix juice blend was too bland for further testing.

Juice blends at both 10.5 and 12.0 °Brix were evaluated by full taste panels and results are shown in Table 4. The juice blends at 10.5 °Brix showed no significant differences among the 20, 25, 30 and 35% tangerine-grapefruit juice blends. This finding confirmed results from the USDA (1949) on consumer responses to orange-grapefruit juice blends. In those tests, consumers showed no significant preference among blends containing 40, 50 and 60% orange. When our tangerine-grapefruit juice blends were tasted at 12.0 °Brix the 20% samples were ranked significantly lower than the others at a confidence level of 95%. This difference may reflect a relationship between the amounts of grapefruit solids in these juices and flavor. Bell's (1955) work on preferences for canned grapefruit juice

showed significant differences between sweetened and natural juices at the same °Brix/acid ratio. In that work the percentage of grapefruit solids contributing to flavor of the sweetened juice was reduced by the added sugar.

In a test to further determine the influence of changes in °Brix/acid ratios, grapefruit blends were prepared with added sugar. Results of this test showed taste panelists could easily discern differences in ratios. The °Brix/acid ratios for these sweetened juice blends prepared with the 9.36 °Brix/acid ratio grapefruit were the same as the tangerine-grapefruit blends listed in Table 3 with a range of 9.36–10.1. Results indicated that the juice by itself differed significantly from the sweetened blends and that the sweetened blend with a °Brix/acid ratio of 10.1 differed significantly from the other two blends with °Brix/acid ratios of 9.78 and 9.94. (Both of those differences were at the 99% confidence level). The finding that panelists could not distinguish among tangerine blends at the same °Brix/acid ratios but could distinguish among blends with sugar added supports panelists' comments on tangerine blends. These comments indicated that tangerine masked the undesirable bitterness of grapefruit juice without the addition of unnecessary sweetness.

Preference tests, by panelists familiar with citrus juices, on tangerine-grapefruit blends showed the potential for a new grapefruit juice product. Threshold concentrations of 14–15% tangerine were established for these blends and all blends of 15–35% tangerine in grapefruit juice differed significantly from and were preferred over unblended grapefruit juice. Tests on blends with and without °Brix/acid ratio adjustment showed that °Brix/acid ratio did not influence those differences. However, increasing the °Brix of the 20, 25, 30 and 35% blends made a significant difference among them. As with other citrus blends in previous studies no preferred optimum concentration was determined. Results from these studies could be incorporated into a sensory testing program to determine consumer taste responses to tangerine-grapefruit blends.

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PROCESSING/CULL ORANGES . . .From page 259

crease, especially if the fruit is not handled rapidly. Thus, it will be necessary to remove larger numbers of fruit to maintain high quality in the industry's major product, frozen orange juice.

From the chemical analyses, the frozen concentrate made from cull fruit was comparable to the sound fruit product, but sanitation and flavor requirements would preclude direct human consumption of such a product. However, this would not limit its usefulness as a chemical or fermentation substrate for manufacture of certain food or chemical products *viz* alcohol or single-cell protein production by fermentation, or chemical purification to manufacture sugars. The peel oil was of inferior quality for use as a flavoring, yet could be manufactured from the oil emulsion and sold as (+)-limonene, a chemical used in the manufacture of chemicals, plastics and adhesives. In some plants, which send culls to the feed mill, (+)-limonene is recovered from the press liquor during molasses manufacture.

We have shown that processing cull oranges in the same manner as sound fruit is feasible. From the volume of culls expected, a citrus plant might be justified in setting up a special processing line to handle these fruit, rather than destroying or diverting them to a feed mill. Production of economical by-products from culls might also stimulate processing plants to do more efficient grading of fruit streams, segregating greater proportions of these fruit from the sound fruit. The net result would be quality improvement of frozen concentrated orange juice.

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J. O. REAGAN, J. A. CARPENTER and C. P. BLANCHARD
Dept. of Food Science, University of Georgia, Athens, GA 30602

A Research Note

EFFECTS OF PROCESSING TREATMENTS ON CHEMICAL AND ORGANOLEPTIC PROPERTIES OF CORNED BEEF

ABSTRACT

Eighteen beef briskets obtained from USDA Choice carcasses were boned and randomly assigned to either a 4°C or −10°C storage treatment for 72 hr. The frozen briskets were then placed in a 4°C cooler and held until attaining an internal temperature of 3–4°C. After thawing, all briskets were pumped to 0, 10 or 20% of their green weight (3 briskets/group/pumping treatment) and held for 2 days in a cover pickle of the same salinity. Weight losses were determined after a 2-day immersion period and chemical and organoleptic samples were obtained 2, 7 and 14 days after pumping. These data indicate that processing yields were not significantly affected by degree of pump, storage at −10°C prior to processing or specific gravity values. Residual nitrite values were not significantly affected by freezing treatments prior to processing or specific gravity values. Nitrite levels were similar at 2 and 14 days after pumping at all pumping levels. After 7 days of storage, residual nitrite levels were less than 50 ppm in all briskets. Sensory ratings for tenderness and juiciness were observed to increase as time after pumping increased.

INTRODUCTION

CORNED BEEF is a cured meat product which is generally produced by pumping beef briskets or rounds with a curing solution composed of salt, sugar, sodium nitrite and corned beef seasoning. It is a common commercial practice to pump the individual cuts prior to packaging in a plastic bag containing additional curing solution. The product is shipped immediately to the retail stores, thus allowing the product to cure during transit. Currently, there is little information available concerning the effects of brisket quality or methods of processing on the characteristics of the finished product. The present study was designed to investigate the effects of freezing, fat content of the brisket, percentage of brine injected and length of storage on the quality of the final product as measured by processing shrinkage, residual nitrite levels and organoleptic characteristics.

MATERIALS & METHODS

EIGHTEEN BEEF BRISKETS (0.79–1.79 kg) from USDA Choice cattle were purchased from a commercial packing plant and transported to the University of Georgia Meat Science Laboratory in a refrigerated truck. Nine randomly selected briskets (Group I) were placed in a −10°C freezer for a 2-day period while the remaining nine briskets (Group II) were placed in a 4°C holding cooler. After 2 days, the frozen briskets (Group I) were transferred to the 4°C cooler and held until an internal temperature of 3–4°C was attained (approximately 2½ days). The briskets (Groups I and II) were then stitch pumped to 0, 10 or 20% of their green weight (3 briskets/group/pumping treatment) and held for 2 days in a cover pickle of the same salinity. The pumping and cover pickles were composed of water (94.2 kg), salt (22.7 kg), sugar (0.45 kg), ascorbic acid (0.45 kg) and nitrite (0.14 kg). The level of corned beef seasoning was reduced as the level of pumping was increased for each treatment to compensate for differences in flavor intensity; however, no adjustments were made in the amount of nitrite added to each brine solution. Upon completion of the immersion period, individual briskets were air dried (15 min), weighed for weight loss determinations, then equally subdivided into 3 equal segments so that samples for nitrite determination could be obtained 2, 7 and 14 days after pumping. The segments remaining after nitrite samples were obtained were doubled wrapped in freezer paper and held in a −10°C freezer for organoleptic determinations.

The following individual brisket weights were obtained: (1) green weight, (2) weight in water, (3) after pumping, (4) after immersion, (5) after storage, (6) prior to cooking, and (7) after cooking. The fat content of each brisket was estimated by the specific gravity procedure of Kraybill et al., 1952. Nitrite analyses were conducted according to AOAC procedures (1970).

Six randomly selected samples were individually cooked in boiling water to an internal temperature of 72°C, sliced and served hot to panel members at each sensory panel session. A 12-member trained panel evaluated corned beef slices (0.3 cm thick) using 8-point scales for tenderness (1 = extremely tough, 8 = extremely tender), flavor intensity (1 = extremely undesirable, 8 = extremely desirable) and juiciness (1 = extremely dry, 8 = extremely juicy), and a 4-point scale for saltiness (1 = lacking salt, 4 = extremely salty).

All data were subjected to analysis of variance according to the methods of Steel and Torrie (1960). Simple correlations were made to determine the relationship between treatments and quality attributes of the finished product (Snedecor and Cochran, 1967).

RESULTS & DISCUSSION

MEAN VALUES and standard deviations for processing yields and nitrite levels for briskets pumped three different levels of brine are reported in Table 1. Processing yields did not differ significantly between the three pumping levels although the

Table 1—Mean values and standard deviations for processing yields and nitrite levels of beef briskets pumped with three different levels of brine[a]

Pumping levels (%)	Processing yields (%)		Nitrite levels (ppm)[b]					
			2 days		7 days		14 days	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	101.77a	9.86	76.68a	25.3	22.71a	5.5	20.68a	4.4
10	107.8a	3.67	113.14a	24.2	44.71b	28.8	29.12a	41.0
20	110.46a	6.10	78.40a	30.5	29.71a	26.9	28.51a	23.9

[a] Mean values in the same columns bearing different letters differ significantly ($p < 0.05$).
[b] Samples obtained at 2, 7 and 14-day intervals after pumping

higher numerical difference observed for those briskets pumped 10 and 20% of their green weight when compared with those pumped 0% may be of economical significance in actual commercial production. The small differences in processing yields of briskets pumped to 10 and 20% levels indicate that at the higher pumping levels (20%) an excessive amount of the injected brine may be lost. While values for nitrite levels (ppm) were similar for all pumping treatments after 2 and 14 days, nitrite levels were significantly higher after 7 days for the briskets pumped to 10% of their green weight. Because of the large standard deviations among values for nitrite, this significant value in itself is not as important as the general decrease in concentration of nitrite in all treatments 2, 7 and 14 days after pumping. It is important to note that nitrite levels (ppm) were within the APHIS allowances (200 ppm) only 2 days after pumping and all briskets contained less than 50 ppm 7 days after pumping.

Analysis of variance procedures were performed to determine the effects of using fresh or frozen briskets in the manufacturing of corned beef on processing yields and nitrite levels of the final product. These data indicate that the freezing of briskets prior to processing does not alter the processing yields (fresh, $\bar{x}$ = 107%; frozen, $\bar{x}$ = 106%) or significantly affect the level of residual nitrite in the product. Residual nitrite levels for briskets frozen prior to processing were always within 5 ppm of those values determined for corned beef produced from fresh briskets. Similarly, values for specific gravity determined by the Kraybill et al. (1952) method did not significantly affect processing yields or nitrite levels in the final product. These data indicate that if the specific gravity determinations measured were accurate, levels of fatness in briskets are not important in determining processing yields or nitrite levels. There is a possibility that the specific gravity method employed is not a good indicator of fatness in beef briskets.

Mean values for nitrite levels, tenderness and juiciness ratings at 2, 7 and 14-day intervals after pumping are reported in Table 2. Although values for residual nitrite were reduced significantly ($p < 0.05$) between 2 and 7 days, there appears to be no advantage in holding corned beef beyond 2 days after pumping as residual nitrite levels were well within the limits specified by APHIS. Since corned beef would be 2–5 days in the sales chain before reaching the consumer, only minimal traces of residual nitrite would be present at the time of consumption. Tenderness ratings were higher ($p < 0.05$) 14 days after pumping than those ratings obtained for the 2-day samples. The increased ratings for tenderness could have been due to the natural tenderization which occurs with beef held for 2 wk at refrigerated temperatures. Similarly, juiciness ratings also increased as the time after pumping increased; however, juiciness scores did not increase significantly after 7 days.

A further analysis of these data indicated that the level of pump (0, 10 or 20% of green weight) did not significantly affect any of the sensory panel ratings with the exception of juiciness scores. Juiciness ratings observed 2 days after pumping were significantly higher for those briskets pumped to 10 and 20% of their green weights; however, these differences dissipated after 7 and 14 days. Values for percent cooking loss were also not affected by the level of pump used which would be expected since the processing yields for the three pumping levels did not vary significantly. Juiciness ratings observed for the 14-day samples were significantly higher for the fatter briskets as estimated by specific gravity methods. Values for specific gravity did not significantly affect any sensory panel traits or percent cooking loss. No differences were noted by panel members for flavor desirability or flavor intensity.

Table 2—Overall mean values and standard deviations for nitrite levels, tenderness and juiciness ratings for corned beef samples taken at 2, 7 and 14-day intervals after pumping[a]

	Sampling intervals after pumping (days)					
	2		7		14	
Traits	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Nitrite (ppm)	89.4a	39.0	32.4a	13.5	26.1a	9.6
Tenderness[b]	4.2a	0.76	4.6ab	0.57	4.8b	0.76
Juiciness[b]	4.2a	0.50	4.5b	0.65	5.1b	0.60

[a] Mean values in the same rows bearing unlike letters differ significantly ($p < 0.05$).
[b] Means based on 8-point scales where: 8 = very tender or juicy; 1 = very tough or dry; 12 judgments per sample mean.

Several significant correlations between quality and processing factors and organoleptic responses were observed in the present study. Green weights (brisket weight prior to pumping) were significantly correlated with specific gravity values (r = 0.48) and with percent cooking loss at 2, 7 and 14-day intervals after pumping (r = 0.49, 0.65 and 0.48, respectively). These findings indicate that the heavier briskets were fatter and sustained a higher percentage of cook loss than the lighter weight briskets. Heavier briskets were also associated with higher juiciness and flavor intensity ratings for samples obtained 14 days after pumping (r = 0.53 and 0.60, respectively). Weight after pumping was significantly ($p < 0.05$) correlated with percent overall shrink (r = −0.74) and with percent cook loss (r = 0.56) and flavor intensity (r = 0.50) after 14 days. Levels of brine injection were associated with only tenderness (r = 0.47) and juiciness (r = 0.56) at 2 days after pumping. Ratings for flavor intensity were positively correlated with overall shrinkage at 2, 7 and 14-day intervals (r = 0.74, 0.55 and 0.58, respectively). Higher levels of shrinkage should tend to concentrate the spices remaining in the product.

In conclusion, these data indicate that the levels of pump utilized in the present study did not significantly affect processing yields, residual nitrite levels or sensory panel traits with the exception of juiciness ratings for the 2-day samples. Similar processing yields and residual nitrite levels were observed for corned beef produced from fresh and frozen briskets.

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J. E. BREKKE and A. L. MYERS
USDA, ARS, 1920 Edmondson Road, Honolulu, HI 96822
and C. R. N. DE AQUINO
Centro de Pesquisas e Desenvolvimento—CEPED
Calixa Postal, 40,000 Salvador, Bahia, Brazil

A Research Note

VISCOMETRIC BEHAVIOR OF GUAVA PUREES AND CONCENTRATES

ABSTRACT

Guava purees and concentrates had flow characteristics of pseudoplastic fluids as determined with a tube viscometer. Puree treated with a pectinolytic enzyme could be concentrated significantly more than untreated puree.

INTRODUCTION

PUREES of tropical fruits are used in nectars, breakfast drinks, jams and preserves, and other fruit products. A high quality concentrate would be a desirable form in which to transport purees from the tropics to markets in the temperate zone. An important characteristic of a concentrate is its consistency or thickness, which is limiting in the performance of some evaporators, and can be a problem during pumping or filling of the product into containers. To investigate this characteristic further, we determined the rheological properties of guava purees and puree concentrates with a tube viscometer.

Other purees and concentrates have been shown to be non-Newtonian fluids (Harper, 1960; Saravacos, 1968; Rao and Palomino, 1974) with pseudoplastic flow characteristics. The flow of these fluids follow the power-law equation, $\tau = K\gamma^n$, relating shear stress (τ) and shear rate (γ) (Metzner, 1956). K (fluid consistency coefficient) characterizes the thickness of the fluid, and n (flow behavior index) denotes the extent of deviation from Newtonian flow behavior. Depending on the magnitude of n, the fluid can be classified: n = 1 Newtonian; n $<$ 1 pseudoplastic; and n $>$ 1 dilatant.

EXPERIMENTAL

Tube viscometer

The tube viscometer used was similar to the one used by Saravacos (1968) and consisted of a 9.5L stainless steel reservoir connected with a 4-mm diam stainless steel tube 92.2-cm long through large diameter fittings and a ball valve. Compressed air was admitted to the reservoir through a pressure regulator, and air pressure was measured by a gauge.

Guava puree

Guavas of the Beaumont variety and its seedlings were harvested from University of Hawaii experimental plots. The fruits were stored overnight at 7.2°C then sorted, washed, and comminuted in a Fitzmill Model D6. The seeds were removed by a paddle-type pulper fitted with a 0.033-in. (0.838-mm) screen. Stone cells were then removed by a paddle-type pulper fitted with a 0.020-in. (0.508-mm) screen. The finished puree was packed in 30-lb tins lined with 3-mil polyethylene bags and stored at −18°C for 6 months to 3 yr before viscometric measurements were made.

Preparation of concentrate

Frozen puree was brought to room temperature and thoroughly mixed with a power stirrer. It was concentrated at low pressure (62–72 mm Hg) in a Centritherm CT-1B. Some samples were treated with Pectinol 10-M (0.15–0.20% of the weight of the puree), which reduced their viscosity or consistency. Those samples were held at room temperature for 1 hr and concentrated to the desired extent by recycling through the evaporator.

Viscometric measurements

Puree or concentrate was filled into the viscometer reservoir, the lid was securely fastened, and compressed air was admitted through a pressure regulator. At the desired pressure, the system was allowed to equilibrate for 15 sec. The ball valve was opened fully, and the material was allowed to flow for a few seconds until flow rate was steady. Then the fluid outflow was collected for a measured period of time (10–180 sec); 100g or more was collected. The pressure was varied from 5–60 psig, and the temperature was 24° ± 1°C.

RESULTS & DISCUSSION

THE NOTATIONS and mathematical analysis used were substantially those of Saravacos (1968). For each measurement, the air pressure in the reservoir was noted, and the rate of flow of puree from the tube was measured. Using data for puree not treated with pectinolytic enzyme and for concentrates of different solids content prepared from this puree after enzyme treatment, we found that plots of the logarithms of shear stress vs shear rate were straight lines (Fig. 1). The slopes represent n, the flow behavior index. Changes in consistency were indicated by changes in the slopes of the lines. Treatment with pectinolytic enzyme was necessary to facilitate concentration to more than threefold. The concentrates had lower flow rates than the puree and their n $<$ 1 values indicated that they were pseudoplastic fluids.

Table 1 lists data for six different lots of purees, two of which were not treated with pectinolytic enzyme (A and B). Lots A, B and F were pseudoplastic fluids. Like dilatant fluids, the enzyme-treated purees, C, D, E, had n values well above 1; for these, K values were less than 1 and were not recorded. Concentrates of puree not treated with pectin-degrading enzyme were much thicker than those of enzyme-treated purees. K values increased with degree of concentration more rapidly

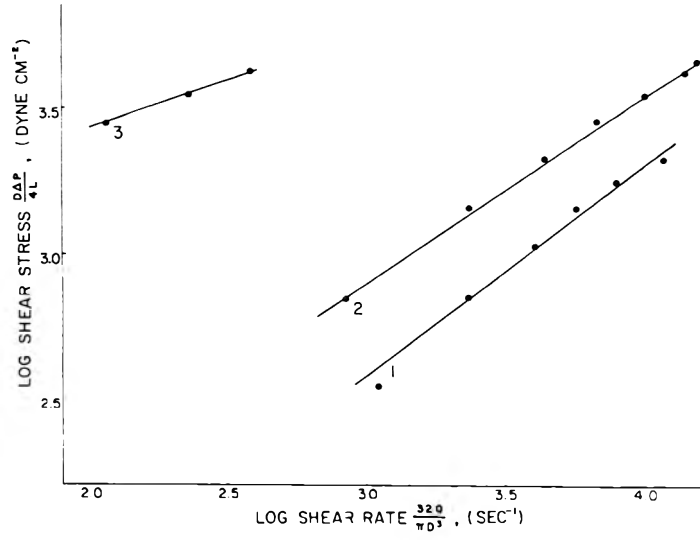

Fig. 1—Flow data of (1) guava puree 8.9° Brix, (2) 2-fold concentrate 17.6° Brix and (3) 4.6-fold concentrate 40.8° Brix.

for untreated purees than for enzyme-treated ones. Concentrates with a K value of 400–500, such as B-3, E-5 and F-3, were so thick that they were difficult to pump or pour; moreover, they could not practically be concentrated further by the evaporator. A 3.9-fold concentrate (E-4) had a K value of 226 and was fluid enough to be concentrated further in the evaporator without difficulty.

Table 1—Viscometric values for guava purees and concentrates

	Sample	° Brix	K	n
A.	1. Puree	7.2	2.6	0.68
	2. Concentrate 2.2-fold	15.9	50	0.47
B.	1. Puree	7.4	2.8	0.68
	2. Concentrate 1.7-fold	12.3	16	0.55
	3. Concentrate 3.1-fold	22.7	410	0.32
C.	1. Puree + 0.15% enzyme	8.8	<1	1.65
	2. Concentrate 1.7-fold	15.1	3	0.71
	3. Concentrate 2.7-fold	24.3	16	0.62
D.	1. Puree + 0.15% enzyme	8.9	<1	1.78
	2. Concentrate 3.5-fold	31.4	23	0.59
E.	1. Puree + 0.20% enzyme	8.6	<1	1.75
	2. Concentrate 2.1-fold	18.5	6.7	0.67
	3. Concentrate 3.2-fold	27.5	35	0.58
	4. Concentrate 3.9-fold	34.0	226	0.42
	5. Concentrate 4.5-fold	39.5	458	0.37
F.	1. Puree (before enzyme preatment)	8.9	1.6	0.77
	2. Concentrate (after 0.15% enzyme) 2-fold	17.6	8.9	0.64
	3. Concentrate (after 0.15% enzyme) 4.6-fold	40.8	495	0.34

SUMMARY

GUAVA PUREES and concentrates were pseudoplastic fluids as determined by viscometric measurements with a tube viscometer. Pectinolytic enzymes reduced the consistency of purees substantially, and this effect was reflected by the lowered consistency of its concentrates. A 3.9-fold concentrate of de-pectinized puree was easily pumped, and flowed readily in an evaporator, a 3.1-fold concentrate of untreated puree was too thick to handle in this manner.

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H. FARSAM and N. AHMADI
Dept. of Pharmaceutical Chemistry, College of Pharmacy
University of Teheran, Teheran, Iran

A Research Note

FLUORINE CONTENT OF TEAS CONSUMED IN IRAN

ABSTRACT

The objective of this investigation was to find out the fluorine content of teas consumed in Iran and to evaluate the potentiality of tea as a contributor of fluorine. The fluorine content of eight samples of tea were determined by two methods in dry and infused forms. The results obtained showed a low variation. The variation of fluorine in dry tea was 10.25–15.25 mg/100g. The fluorine content of the infusions showed a range of 0.89–1.15 ppm. The amounts of fluorine extracted into infusions varied from 68.85–86.83%. The daily ration of 3.8 g/day contributes from 0.34–0.44 mg fluorine. No specific relation was found between the fluorine content of tea and percent of its extraction. Drinking tea and feeding infants with diluted infused tea in between breast feedings in Iran may account for some dental benefit in this country.

INTRODUCTION

THE MAJORITY OF foods found in the average diet contain 0.2–0.3 ppm or less fluorine in the food as consumed. Tea and sea foods are notable exceptions (McClure, 1949). Different values are reported for fluorine content of various teas by different investigators (Wang et al., 1949; Fabre and de Campos, 1950; de Campos, 1950; Zimmerman et al., 1957; Quentin et al., 1960; Stankoviansky and Biely, 1965; Okada and Furuya, 1969; Cook, 1970; Venkateswarlu and Sita, 1971). The fluorine content of tea depends on the origin of the plant, type of soil and fertilizer, age of the leaves and the time of harvesting (Wang et al., 1949; Zimmerman et al., 1957; Garber, 1962; Okada and Furuya, 1969).

Tea is normally consumed in the infused form. The fluorine content of the infused tea depends on the percent of extraction of fluorine, which in turn is a function of type, strength and duration of infusion, but not proportionately (Harrison, 1949). Boiling increases the fluorine extraction but affects the flavor of the tea (Zimmerman et al., 1957). Five minutes of infusion produces the best flavor with least extraction of tannin (Harrison, 1949). The value of the infused tea as a source of fluorine has been evaluated by some of investigators. Ham and Smith (1960) found that one cup of tea increases the fluorine content of the diet by 0.2 mg. Quentin et al. (1960) stated that a person drinking seven cups of tea/day consumes about 1 mg of fluorine. Cook (1970) found that the fluorine intake from tea in British children was 1.26 mg as compared to 2.35 mg in adults.

Tea is an important item in the Iranian diet and drunk mostly by laborers and peasants; furthermore, diluted infused tea is used as a supplement in between breast feedings of infants. The annual consumption of tea in Iran is about 48000 tons (Iran Tea Organization). Approximately half of this amount is produced inside the country and the other half imported from other countries. The objective of this investigation was to find out the fluorine content of teas consumed in Iran and to evaluate the potentiality of tea as a contributor of fluorine.

MATERIALS & METHODS

ACCORDING TO information from Iran Tea Organization, almost all types of tea consumed in Iran are a blend of 60% Iranian product with 40% imported Ceylon and Indian teas.

In this investigation three brands of pure Iranian tea (Ghoncheh, Pouneh, Noush) and three brands of pure imported teas (Assam, Darjeeling, Ceylon) and two blended teas (Irano-India, Irano-Ceylon) were analyzed. The fluorine content of the samples was estimated by two colorimetric and potentiometric methods. In the former, the samples were ground and ashed by the method of Harrison, (1949). The fluorine was estimated by the distillation and colorimetric method of AOAC (1970) with some modification. In the later, the method of Baker, (1972) was used. In this method the fluorine was measured after a NaOH fusion, using the specific ion electrode.

The infusions were prepared in a strength of 1 g/100 ml with distilled water and infused for 5 min. Since it was aimed to estimate the fluorine content of infused tea without considering the fluorine content of the water supplies, the infusions were prepared with distilled water. The infusions were filtered, made up to volume with fluorine-free distilled water and the fluorine content was measured using methods described for dry tea.

RESULTS & DISCUSSION

THE VALUES OBTAINED are given in Tables 1 and 2. Comparison of the results obtained by two different methods showed a low variation. Therefore, both methods could be used with the same advantages.

The variation of fluorine content of various teas obtained in this investigation was from 10.25–15.25 mg/100g. Values given for fluorine content of various teas by different investigators do not agree with each other. The fluorine contents obtained in these analyses for Indian and Ceylon teas were consistent with those given by Harrison (1949) for teas with the same origin. The fluorine content of the Iranian teas was within the ranges reported by Zimmerman et al. (1957) for Chinese tea; Iranian teas are mostly from that origin.

The fluorine content of tea infusions ranged from 0.89–1.15 ppm. The amounts of fluorine extracted into infusions varied from 68.85–86.83%. No significant relation was found between the fluorine content of dry tea and percent of its extraction. However, a tendency was observed towards a decrease of fluorine extraction with an increase of the fluorine content of the teas.

The values for the daily intake of fluorine were calculated considering the percentage of fluorine extraction of the daily

Table 1–Fluorine and water content of tea[a]

Variety of tea		Water (%)	Fluorine (mg/100g) AOAC method	Fusion method	Mean
Ghoncheh	Iran	5.9	14.8	15.3	15.05
Noush	Iran	5.3	15.5	15.0	15.25
Pouneh	Iran	5.9	12.0	13.5	12.75
Assam	India	6.2	10.0	10.5	10.25
Darjeeling	India	4.6	10.8	11.8	11.30
Ceylon	Ceylon	6.7	13.5	14.0	13.75
Irano-India	Iran-India	4.9	11.8	11.0	11.40
Irano-Ceylon	Iran-Ceylon	5.0	14.0	15.5	14.75

[a] Average of five analyses

ration of 3.8g tea. The daily ration was obtained by dividing the total consumption of tea by the population without considering the variation of consumption among different groups of the people. The daily ration of tea provides quantities of fluorine from 0.34–0.44 mg (Table 2).

Considering the optimal intake of fluorine (1 mg/day) suggested for protection from dental caries, it may be said that drinking tea in Iran provides half of this amount without considering the fluorine content of water and other sources. Furthermore, the habit of tea drinking and feeding infants with diluted tea may account for some dental benefit in Iran.

Table 2–Fluorine content of tea infusions[a] and daily fluorine intake provided by a ration of 3.8g tea

Variety of tea	AOAC method (ppm)	Fusion method (ppm)	Mean	Extr of fluorine (%)	Fluorine provided (mg)
Ghoncheh	1.10	1.20	1.15	76.41	0.44
Noush	1.10	1.00	1.05	68.85	0.40
Pouneh	1.20	0.92	1.06	83.13	0.40
Assam	0.88	0.90	0.89	86.82	0.34
Darjeeling	0.98	0.96	0.97	85.84	0.37
Ceylon	0.98	0.94	0.96	69.81	0.37
Irano-India	0.95	0.93	0.94	82.45	0.36
Irano-Ceylon	1.10	1.00	1.05	71.18	0.40

[a] Strength 1 g/100 ml in distilled water, infusion duration 5 min

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A. E. WASSERMAN, J. W. PENSABENE and E. G. PIOTROWSKI
USDA Eastern Regional Research Center
ARS, Philadelphia, PA 19118

A Research Note
NITROSAMINE FORMATION IN HOME-COOKED BACON

ABSTRACT

Randomized slices of bacon were cooked at home by 25 consumers by their usual method of preparation and the edible portions were analyzed for nitrosamines. N-nitrosodimethylamine (DMNA) and N-nitrosopyrrolidine (NPyr) were found in all samples. Of the 25 preparations, five contained DMNA and five contained NPyr in concentrations greater than 10 μg/kg; only one sample contained both nitrosamines in concentrations greater than 10 μg/kg. Although the association between nitrosamine concentration, time and amount of heat is not strong, it appears that frying bacon at low or medium heat for less than 10 min can result in less than 10 μg/kg DMNA or NPyr.

INTRODUCTION

N-NITROSOPYRROLIDINE (NPyr) forms in bacon on the application of heat during preparation for consumption. Although the precursor(s) of the nitrosamine and the mechanism of formation are still unknown, it has been observed that greater concentrations of the NPyr form in the adipose tissue than in the lean (Fiddler et al., 1974) and that nitrosoproline (NPro) is present in raw bacon (Kushnir et al., 1975; Ivey, 1974). The conversion of NPro to NPyr occurs through decarboxylation at elevated temperatures (Pensabene et al., 1974; Huxel et al., 1974). Several studies have demonstrated the formation of NPyr in bacon prepared by frying, broiling, or baking, with the lowest concentration in the product prepared in the microwave oven at, presumably, a lower temperature than the other methods (Herring, 1973; Pensabene et al., 1974). Pensabene et al. (1974) investigated the effects of time and temperature on NPyr formation and suggested that lower temperatures reduced the concentration of NPyr produced.

Practically all studies in the United States on the formation of NPyr in bacon have been carried out by frying for 3 min on each side in preheated electric fry-pans set at 172°, which is approximately the temperature recommended on most commercial packages of bacon. For research purposes such a standardized time and temperature program is essential, but it does not take into consideration the variations occurring during the preparation in the home. The formation of nitrosamines in bacon prepared by consumers was investigated and the results are reported here.

MATERIALS & METHODS

Bacon

For the first experiment a slab of bacon was obtained from a processor, sliced, and randomized into packages containing 10 slices each. The packages were given to 12 members of the Eastern Regional Research Center with instructions to prepare the bacon as they normally would cook it for family use. The participants completed the form shown in Figure 1 and returned it with the fried bacon strips. One package of bacon (control) was fried in the laboratory at 175°C in a preheated, calibrated electric fry-pan. This bacon was fried for 4 min on each side instead of the standardized 3 min in order to obtain a more edible-appearing product. The degree of doneness of the bacon samples was subjectively evaluated by the member of the staff responsible for preparing and evaluating bacon in the laboratory for the last 3 yr.

In the second experiment, carried out several months later, 1-lb packages of a nationally distributed bacon were purchased at a retail market, the slices were randomized as described above and distributed

Please cook this bacon in the way you normally prepare it for your family. Keep a record of the length of time of cooking. Wrap cooked bacon strips in aluminum foil and return.

Please fill in questionnaire below.

1) Type of heat:

Gas Electric

2) Method:

Fried Broiled Baked Other

3) Pre-heat cooking utensil:

Yes No

4) Heat setting (gas flame or electric):

Low Medium High Broil Temp.

5) Time of heating (from time bacon is placed in the heat):

Fig. 1—Form used to collect information on home cooking conditions.

to 13 members of the Center staff who had not participated in the previous study. The instructions and evaluations of degree of doneness, carried out by the same evaluator, were as described above.

Nitrosamine analysis

Each bacon sample was ground and thoroughly mixed. Aliquots of each sample were analyzed for nitrosamines by a modification of a previously described procedure (Pensabene et al., 1974) in which direct extraction was used to remove nitrosamine from the sample instead of the methanolic-KOH digestion. The gas chromatographic detector used for the first part of the study was the alkali-flame ionization detector in the Perkin-Elmer Model 3920 GC. The second set of samples was analyzed with a Thermal Energy Analyzer (Fine et al., 1975) interfaced with the Varian-Aerograph Model 1720 GC. Confirmation of positive nitrosamines was carried out with the DuPont Model 492 GC-mass spectrometer (Pensabene et al., 1974) operated with a resolution of 1 in 12,000 in the peak matching mode.

RESULTS & DISCUSSION

THE DATA on the formation of nitrosamines during frying of bacon in consumers' homes are shown in Table 1. Electric heat was used by 10 of the 25 participants (40%) and gas by the remainder. One bacon sample was baked for 47 min and two were broiled for 5 and 9 min; the remainder were fried in the conventional manner for preparing bacon. Approximately 50% of the participants frying with gas (8 of 15) estimated low heat was used and the frying time ranged from 10–28 min. The five participants using medium heat fried the bacon from 4–9 min; one sample was fried at a high gas heat for 6 min. Electrically, one sample was fried at low heat for 14 min and four were prepared at a medium setting for times ranging from 5 min to 15 min. Two bacon samples were fried for 10 min at a medium-high heat. Only three samples (#2, 14 and 16) were prepared in preheated fry-pans. Five samples were judged to be well-done and 17 were either medium or medium-well done;

three samples were medium-rare. The degree of doneness corresponded roughly with the amount of heat used and the time of heating.

Although the study was established to evaluate NPyr formation in the bacon samples, n-nitrosodimethylamine (DMNA) was also found. However, the formation of these nitroso compounds does not appear to follow a discernible pattern. In the first experiment, 5 of the 12 samples contained more than 10 μg/kg NPyr; only one of all the samples had more than 5 μg/kg DMNA. The most undesirable bacon, containing 31 μg/kg DMNA and 13 μg/kg NPyr, was fried electrically at a medium-high heat for 10 min, whereas the other four samples with more than 10 μg/kg NPyr were fried on low gas heat from 14–25 min. In the second experiment, however, there were no samples with 10 or more μg/kg NPyr. Two samples with high (39 and 28 μg/kg) DMNA concentrations were fried electrically at medium-high and high settings for 11 and 8 min, respectively. Two gas-fried bacon samples with 15 and 24 μg/kg DMNA were cooked at low heat for 28 and 10 min, respectively.

The formation of nitrosamines in random samples of bacon fried under home conditions emphasizes our lack of knowledge about the mechanism of formation of these compounds. While it is true that the amount of heat applied was estimated by those cooking the bacon, there is no obvious correlation between nitrosamine formation and the time and estimated heat applied. It appears, however, that frying at low to medium heat settings for less than 10 min could result in lower nitrosamine concentrations. The variation in nitrosamine content was probably not caused by the variation in composition of the bacon used since the strips were randomized, and greater randomization was achieved in the second experiment in which commercial bacon, presumably from different bellies, was used.

Nitrosamines were determined only in the edible product in this study. However, since larger concentrations of these compounds have been reported to occur in the rendered fat than in the lean tissue, the method of draining or blotting the fat from the edible portion could affect the residual nitrosamines determined.

No information is available on the possible health significance in the human diet of trace quantities of nitrosamines in the concentrations reported in this study.

Table 1—Formation of nitrosamines in bacon fried by consumers

Sample no.	Heat applied	Time (min)	Degree of doneness	DMNA[a] μg/kg	NPyr[b] μg/kg
		Experiment No. 1			
Electric heat					
Control	175°C	8	Well	3	7
1	Bake (175°C)	47	Well	3	3
2	Low	14	Medium	3	5
3	Medium	5	Medium	2	6
4	Medium	15	Med-rare	3	5
5	Med-high	10	Well	31	13
Gas heat					
6	Low	14	Med-well	–	14
7	Low	15	Med-well	3	12
8	Low	20	Well	5	14
9	Low	25	Well	4	12
10	Medium	7	Med-well	4	7
11	Medium	9	Med-well	3	9
12	High	6	Well	4	7
		Experiment No. 2			
Electric heat					
Control	175°C	8	Well	5	5
13	Medium	10	Med-rare	3	4
14	Medium	10–15	Medium	6	3
15	Med-high	11	Med-well	28	7
16	High	8	Medium	39	8
17	Broil	5	Med-well	4	4
Gas heat					
18	Low	10	Medium	24	5
19	Low	12	Medium	5	5
20	Low	20	Medium	3	8
21	Low	28	Med-well	15	8
22	Medium	4	Med-rare	4	1
23	Medium	5	Medium	3	2
24	Medium	6	Med-well	9	8
25	Broil	9	Med-well	5	7

[a] DMNA, N-nitrosodimethylamine
[b] NPyr, N-nitrosopyrrolidine

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JASJIT SINGH, R. K. KUILA and B. RANGANATHAN
National Dairy Research Institute, Karnal 132001, India

A Research Note

STUDY ON CASEIN DEGRADATION BY A GAMMA-RADIATION INDUCED MUTANT OF LACTOBACILLUS BULGARICUS

ABSTRACT

Results of starch gel electrophoresis of degraded whole casein by a gamma-radiation induced mutant of *L. bulgaricus* showed that the mutant degraded whole casein much faster than the parent culture.

INTRODUCTION

IN AN EARLIER STUDY (Singh and Ranganathan, 1974a), it was shown that gamma-radiation induced mutants of *Lactobacillus bulgaricus* exhibited 50–70% higher proteolytic activity in milk than the unirradiated parent culture. The present paper reports the caseinolytic activity of one such mutant on whole casein and its purified fractions.

EXPERIMENTAL

MUTANT AND PARENT cultures of *Lactobacillus bulgaricus* were selected for the present study. The cultures were propagated in sterilized (120°C/20 min) reconstituted nonfat milk and stored in the refrigerator until use. The test inoculum was prepared by transferring the cultures from milk into enriched medium devised by varying the composition of ingredients in tomato juice broth (Singh and Ranganathan, 1974b). The cells were harvested by centrifuging at 8000 X G for 30 min in an International Refrigerated Centrifuge (Model PR-6), washed twice with phosphate buffer (0.05M, pH 7.0) and centrifuged at 14,600 X G for 15 min to collect cells following each wash. They were then suspended in the same buffer and adjusted to 0.50–0.55 optical density at 525 nm in a photoelectric colorimeter. The inoculum used for studying degradation of casein and its fractions consisted of 2% of cell suspension.

Acid casein was prepared from fresh milk according to the precipitation method at isoelectric point, pH 4.7; α_s-, β- and κ-caseins were prepared according to the procedures of Zittle et al. (1959) and Zittle and Custer (1963). The purification of individual casein fractions was monitored by using DEAE cellulose column chromatography at pH 8.6 in the presence of 4.0M urea. Solutions of acid casein and its purified fractions were adjusted to pH 7.0 with 0.1N NaOH heated to 80°C for 5 min to destroy any protein-associated proteolytic activity as described by Moreno and Kosikowski (1973) and cooled rapidly to 25°C. Then these solutions were seitz filtered and adjusted to final concentration of 1.0% with 0.1M phosphate buffer. A 10-ml aliquot of each substrate was individually inoculated with the test inoculum. Sodium chloride (3%) and merthiolate (0.025 mg/ml) was added to the inoculated solutions according to Rymaszewski et al. (1972) and the mixtures were incubated at 37°C for 10 days. Cells were centrifuged in each case and the supernatants were analyzed for proteolytic activity according to the method of Hull (1947). In case of degraded casein samples, pH of the supernatants was adjusted to 4.6 and the insoluble fraction containing approximately 100 mg was suspended in 0.5 ml vernol buffer (pH 8.6) containing 20% urea for starch gel electrophoresis (Ganguli and Majumdar, 1967).

RESULTS & DISCUSSION

RESULTS of starch gel electrophoresis of degraded whole casein by a gamma-radiation induced mutant of *L. bulgaricus* have shown that the mutant degraded whole casein much faster than the parent culture (Fig. 1). These findings are comparable to similar observations on lactic acid bacteria by Sato and Ohmiya (1966). The present studies have also shown that the mutant culture released significantly higher amounts of tyrosine in casein substrates namely, α_s-, β- and κ-caseins as compared to *L. bulgaricus* (Table 1). There appears to be some divergence of opinion in regard to the preferential breakdown of casein fractions by lactic cultures. The results reported in the present study indicate that *L. bulgaricus* and its mutant degrade κ-casein more readily than α_s- or β-fraction, while Poznanski et al. (1965), Ohmiya and Sato (1968) observed that lactic acid bacteria degraded the α_s- or κ-casein fractions more readily than the β-fraction. On the other hand, Shidlovskaya and Dyachenko (1968) and Rymaszewski et al. (1972) found that β- and κ-casein fractions were easily hydrolyzed as compared to α_s-fraction. In the light of above reports, it would,

—Continued on page 280

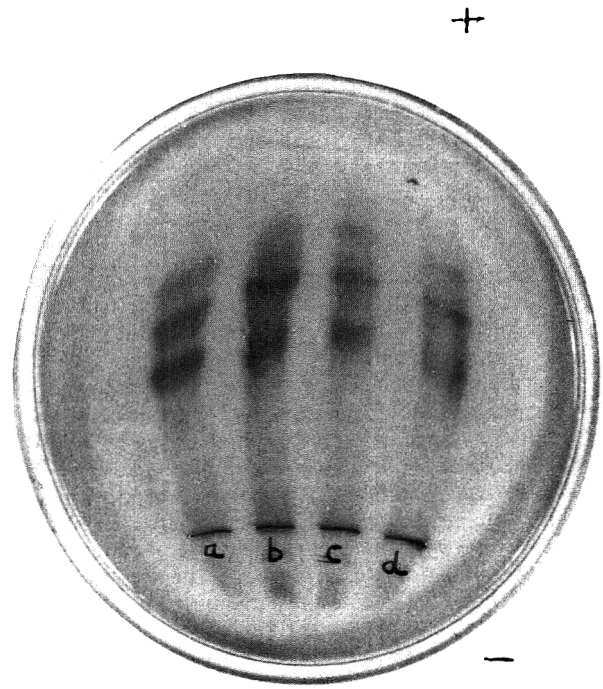

Fig. 1—Starch gel electrophoretic pattern of whole casein after incubation for 10 days with washed cell suspensions of L. bulgaricus and its mutant: (a) L. bulgaricus, 0 day; (b) Mutant, 0 day; (c) L. bulgaricus, 10 days; and (d) Mutant, 10 days.

Table 1—Proteolytic breakdown of α_s-, β- and κ-caseins by L. bulgaricus and its gamma-radiation induced mutant[a]

	Increase in free tyrosine content (mg/ml) of medium	
	L. bulgaricus	
	Parent	Mutant
α_s-casein	0.343	0.560
β-casein	0.183	0.243
κ-casein	0.575	0.840

[a] Cultures were examined after 10 days of incubation at 37°C. Results represent an average of three trials.

J. M. REGENSTEIN, L. P. GRUNDEN[1] and R. C. BAKER
Dept. of Poultry Science, Cornell University, Ithaca, NY 14853

A Research Note

PROTEOLYTIC ENZYMES AND THE FUNCTIONALITY OF CHICKEN EGG ALBUMEN

ABSTRACT

Egg white was subjected to proteolysis by ficin, bromelin, papain, trypsin and protease; changes in functional properties were monitored. Many of the changes observed were due to the addition of enzyme rather than actual proteolysis (e.g., foam volume increase, foam stability decrease, and angel cake volume increase). The angel cake volume showed a further increase with proteolysis; however, off-flavors were also noted. In experiments with ficin and bromelin, proteolysis by both enzymes decreased the coagulum strength while only ficin decreased the amount of noncoagulable protein.

INTRODUCTION

THE WORK OF GRUNDEN et al. (1974) using proteolytic enzymes as a method of improving egg albumen functionality has been continued, including the use of an additional control: a zero time sample with enzyme added. In many cases, the changes observed in the original work can be accounted for by the addition of the enzyme preparation rather than by the effect of further proteolysis.

METHODS & MATERIALS

THE METHODS of Grunden et al. (1974) were used with the following exceptions and additions:

0.001%, 0.05%, 0.1% and 0.3%, Sigma Type II ficin, bromelin, papain, trypsin and protease were used. Samples were incubated for 12 hr at 34°C unless otherwise noted. The Biuret method of Gornall et al. (1949) was used to determine protein.

Emulsification capacity was measured by the modified method of Swift et al. (1961). Forty grams of a 1% (w/v) albumen sample were mixed with 10 ml soybean oil for 15 sec in a Model 541 Osterizer (John Oster Mfg. Co., Milwaukee, WI) at 15,000 rpm. Oil was then added at 1 ml/sec until the emulsion broke. Emulsifying capacity is expressed as ml of oil added/mg protein.

Noncoagulable protein was measured by diluting 2g egg albumen with 10 ml water, cooking 10 min at 90 °C in a water bath, cooling 5 min in an ice-water bath, and spinning for 20 min at 5000 rpm, then measuring the protein content of the supernatant.

Coagulum strength was measured by weighing a 100g aliquot of albumen, into a 250 ml Griffin beaker, covering with a petri dish cover, cooking for 30 min at 90°C in a water bath, chilling for 10 min in an ice-water bath, then measuring on an Instron Universal Testing Machine (Instron Corp., Canton, MA) equipped with a CCT 381 Compression Load Cell. The head from a Curd Meter (Submarine Signal Co., Boston, MA) was attached to the load cell. Measurements were made at a crosshead speed of 39.4 cm/min. Coagulum strength was measured as the maximum pounds of force needed to penetrate 1.7 cm into the sample.

RESULTS & DISCUSSION

THE INTENSITY of albumen off-color and off-odor development after 12 hr increased as the enzyme concentrations increased. Egg albumen treated with 0.05% or more bromelin, ficin or protease developed a brownish color. Trypsin and papain treated albumen developed this color at 0.1% and higher. With the exceptions of ficin, a "fermenting fruit" off-odor was detected in albumen treated with 0.1% or higher levels of enzymes. This odor was detected in the ficin-treated albumen only at the 0.3% level.

Nonprotein nitrogen (NPN) released by the enzyme proteolysis was estimated by the Lowry method (Lowry et al., 1951) and expressed as tyrosine equivalents (Table 1). Both peptide bonds and tyrosine and tryptophan were measured. Thus, NPN could show a peak since hydrolysis of soluble peptides can cause a decrease in color. Trypsin released very little NPN, papain and ficin released intermediate quantities, and bromelin and protease released the greatest quantities of NPN.

Changes in pH from the zero time control with enzyme are also shown in Table 1. The most marked changes were observed with bromelin and protease, the two enzymes which also showed the greatest changes in NPN.

Foam volume was influenced by the addition of the enzyme (638 ml/100g albumen for the no-enzyme control up to 767 ml/100g albumen for 0.05% ficin). In general, increasingly greater volumes of foam were obtained as enzyme preparation concentration increased. However, the zero time control with enzyme and the 12 hr treated samples had about the same foam volume, suggesting that the results were not due to proteolysis. It remains to be shown what component of the crude enzyme preparations is responsible for this result. Upon addition of the enzymes at concentrations of 0.05% or higher except for trypsin, foam stability was decreased from 25–50 ml drainage/100g control up to 70–71 ml/100g of 0.01% protease treated albumen. Foam stability of no-enzyme albumen treated with 0.05% trypsin was still comparable to albumen without enzyme treatment. Addition of the enzyme rather than proteolysis was found to cause foam stability losses.

Volumes of cakes made from albumen treated with the various enzyme preparations at various concentrations is presented in Table 1. Only at the highest level of added enzyme

Table 1—Effect of Enzyme Preparation on pH, Net NPN and Cake Volume of Enzyme-treated Egg Albumen (0.3% enzyme at 34° C for 12 hrs)

Enzyme (0.3%)	Net NPN[a] (μg Tyr/g Albumen)	pH[b]		Cake volume (ml)[c]	
		Zero time with enzyme	Incubated	Zero time with enzyme	Incubated
No enzyme	–	8.6	8.6	426	–
Papain	580	8.4	8.3	422	474
Ficin	560	8.2	7.8	526	484
Trypsin	110	8.2	8.2	514	454
Bromelin	920	8.2	6.8	410	482
Protease	900	8.3	7.4	516	509

[a] Net NPN = NPN after incubation – NPN of zero time control. Each value is a mean of three replications; each replicate consists of two observations.
[b] Each value is a mean of three replications; each replicate consists of one observation.
[c] Each value is a mean of three replications; each replicate consists of three observations.

[1] Present address: General Foods Corp., Tarrytown, NY 10591

Table 2—Noncoagulable protein and coagulum strength of bromelin- and ficin-treated egg albumen (0.05%, 1.5 hr at 25°C)[a,b]

Treatment	Noncoagulable protein (mg protein/g)	Coagulum strength (lb of force)
No-enzyme control	11.6	3.3
Bromelin control	11.4	3.0
Ficin control	11.3	3.2
Bromelin	11.4	1.9
Ficin	6.2	1.3

[a] Each value is a mean of two replications; each replicate consists of three observations.
[b] Means connected by the same vertical line are not significantly different ($p < 0.01$).

(0.3%) did the cake volumes show a significant change from that of the zero time control with enzyme, except for protease which did not change. Except for bromelin and papain, the final cake volumes after proteolysis were higher than the control without enzyme. Zero time control with enzyme treatment volumes in all cases were higher than the no-enzyme control. Ficin and trypsin, however, showed further improvement with proteolysis. It was also observed that the zero time volume peaked at 0.05% with the addition of papain, bromelin or trypsin; while with ficin it peaked between 0.05% and 0.1%. This suggests that for these enzymes the volume maximizing effect of material addition has an optimum.

It was also observed that above 0.05% enzyme preparation, the angel cakes from all enzyme treatments (zero time and incubated) had off-flavors which were described as being bitter and sour. These cakes also had a darker appearance and coarser, gummier texture that angel cakes made from untreated egg albumen.

Emulsification capacity, noncoagulable protein, and coagulum strength

These studies were undertaken with ficin and bromelin only at a treatment level of 0.05% for 1.5 hr at 25°C. Both enzymes produced relatively high NPN values. Ficin caused a pH change during hydrolysis while bromelin did not.

Emulsification capacity which ranged between 57.0 and 62.0 ml oil/mg protein was not significantly affected by enzyme addition (zero time control with enzyme) or by proteolysis.

The noncoagulable protein of the incubated ficin-treated sample decreased significantly while the coagulum strength of both of the incubated ficin and bromelin-treated samples was decreased (Table 2). The incubated enzyme-treated albumen did not form a continuous proteineous network when heated, but rather a coagulum of independent, irregular pieces.

It is evident from the foregoing data and earlier work (Grunden et al., 1974) that proteolytic enzymes do have some activity on egg albumen. However, most of the changes in the foaming ability (i.e., foam volume and stability and angel cake performance) reflected the addition of enzyme preparation and not proteolysis. Although at high enzyme concentrations, effects due to proteolysis become apparent in angel cake volumes. Proteolytic activity did affect the heat coagulating properties of egg albumen.

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CASEIN DEGRADATION BY L. bulgaricus MUTANT . . .From page 277

however, be interesting to use such mutant cultures to study the nature and extent of degradation of casein and its component fractions during cheese ripening with a view to achieving a shorter ripening period.

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