5 September-October 1975
Volume 40 : Number 5
Coden: JFDSAZ 40(5) 899–1107 (1975)

JOURNAL of FOOD SCIENCE

–CONTENTS CONTINUED (on the inside of the front cover) . . .

A PUBLICATION OF THE INSTITUTE OF FOOD TECHNOLOGISTS

RESEARCH NOTES

September-October 1975
Volume 40 : Number 5

JOURNAL of FOOD SCIENCE

- **MANUSCRIPTS** (3 copies) should be submitted to:

  Dr. Bernard J. Liska
  IFT Scientific Editor
  P.O. Box 3067
  Lafayette, IN 47906 USA

NO RESPONSIBILITY is assumed by the Institute of Food Technologists for statements and opinions expressed by the contributors to its publications.

MANUSCRIPTS should conform to the style used in *Journal of Food Science*. Authors should request from the Director of Publications the revised leaflet "Style Guide for Research Papers." *Journal of Food Science* reserves the privilege of editing manuscripts to make them conform with the adopted style of the journal or returning them to authors for revision. Editing changes may be reviewed by authors before publication.

PAGE CHARGES for Publications. The IFT Executive Committee has established a page charge of $50 per printed page for all papers published in *Journal of Food Science*. The page charge shall not constitute a bar to acceptance of research manuscripts because the author is unable to pay the charge.

- **SUBSCRIPTIONS:** All communications related to handling of subscriptions, including loss claims, change of address, orders for back issues, and 100 or more reprints should be sent to:

  Subscription Department
  Institute of Food Technologists–Suite 2120
  221 N. LaSalle Street
  Chicago, IL 60601 USA

Member Subscriptions–$10 per year.

Non-Member Subscriptions–Accepted only on a calendar year basis–no refunds. Rates include postage. Payment must accompany order. Domestic and Pan American Union–$30; all other destinations–$35. Reduced rates for 2- and 3-year subscriptions.

Change of address notice, with old address label, is required 8 weeks before issue date.

Claims for lost copies are allowed only if received within one month after publication (3 months for foreign subscribers).

Single copies and available back issues. $6 each; remittance to accompany order.

- **REPRINTS:** Request single reprints from AUTHORS. Price Schedule for 100 or more reprints available upon request from Subscription Department.

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

Letters *TO THE SCIENTIFIC EDITOR*

Letter to the Editor

April 20, 1975

Dear Sir:

The confusion regarding the description of Hunter Color data (A.C. Little, "Off on a tangent," J. Food Sci. 40(2): 410; F.J. Francis, "The origin of $\tan^{-1}$ a/b," J. Food Sci. 40(2): 412) stems from the manipulation of instrumental data to mathematical equations. One simple solution to this confusion is to avoid trigonometrical functions and use only rectangular and polar coordinates. The $\tan^{-1}$ b/a (Little) is the angle θ subtending with the "a" axis and the $\tan^{-1}$ a/b (Francis) is $90° - \theta$ which is the angle subtending with the "b" axis. Both these functions and $r = (a^2 + b^2)^{1/2}$ locate the sample in the Hunter a,b diagram. Working with egg yolk color which falls both in the (a,b) and (−a,b) quadrants, this author found that $\tan^{-1}$ a/b is better because it measures the deviation ($\pm\theta$) from the yellow axis (+b). Since most foods fall in the (a,b) and (−a,b) quadrants, it may be more practical and useful to use +b axis as reference rather than the conventional "a" axis. After all, the Hunter L, a, b system was an answer to the conventional and nonuniform CIE system.

THOMAS PHILIP
Dept. of Agricultural Biochemistry
The University of Arizona
Tucson, AR 85721

Letter to the Editor

May 16, 1975

Dear Sir:

In my note, "Off on a tangent," I pointed out the incompatibility of the co-existence of two opposing definitions of hue expressed as an angular function in Hunter space. In his letter, Philip suggests that a simple solution to the confusion is to avoid trigonometrical functions and use only rectangular and polar coordinates, but he fails to indicate how that can be done. In order to determine the magnitude of the hue angle (i.e., the polar coordinate θ) from measured values of a and b, one must resort to trigonometrical functions. And indeed, Philip acknowledges this by stating his preference for using the +b axis as reference, and defining θ as $\tan^{-1}$ a/b, with $\pm\theta$ measuring the deviation around the yellow axis (+b).

The angle identified as θ in the proposed scheme is, of course, the complement of the conventional hue angle θ, and the source of the confusion to which I had originally addressed my remarks. In addition, in this scheme the angle generated by clockwise rotation is designated positive, and that by counterclockwise rotation as negative, exactly opposite to conventional practice and thus potentially a further source of confusion.

I fail to understand how the proposal provides greater convenience and usefulness than the conventional one. Let us consider an example. For the case of $\tan^{-1}$ a/b, if $+\theta = 30°$ and $-\theta = 30°$, then the range covers a span of 60°, deviating ±30° around the +b axis. In the conventional method, $\theta_1 = 60°$, $\theta_2 = 120°$, covering a range of 60°, and deviating ±30° around the +b axis. However, if for example an angle of 80° is found to correspond to a measure of optimum hue, then the same data can be used to evaluate deviation around that angle. Thus, the conventional method provides both convenience and flexibility in an unambiguous manner.

The situation generated by conflicting definitions of hue angle is not analogous to that generated by the transformation of the CIE-space to Hunter space. In the former case, an angle becomes confused with its complement; in the latter, any point located in one space can be easily and directly translated to the second by well-established transformation equations.

ANGELA C. LITTLE
Dept. of Nutritional Sciences
University of California
Berkeley, CA 94720

Call For Volunteered Papers

IFT 36th ANNUAL MEETING

June 6-9, 1976 Anaheim, California

ALL IFT MEMBERS ARE CORDIALLY INVITED to submit original research papers dealing with any aspect of food science or technology for consideration by the 1976 Program Committee.

Volunteered papers are a fundamentally important part of each Annual Meeting. Have you some interesting work to share with IFT's membership? The Program Committee welcomes your contribution. To assist the Committee in its review and scheduling, please indicate on the Official Abstract Form the area of food science and technology under which your work falls. A number of areas are listed below. Indicate others on the Official Abstract Form if appropriate.

- Biochemistry of Meats
- Biochemistry of Plants
- Chemistry, Basic Food
- Chemistry, Flavor
- Environmental Aspects of Food
- Enzymes
- Food Packaging
- Food Processing
- Food Service Products & Systems
- Meat and Fish Technology
- Microbiology
- New Product Development
- Nutrition
- Physical and Chemical Analysis
- Pollution Control
- Proteins
- Quality Assurance
- Sensory Evaluation and Flavor Chemistry
- Storage Stability of Foods
- Thermal Processing

DEADLINE FOR TITLES AND ABSTRACTS IS DECEMBER 22, 1975
USE ONLY THE OFFICIAL ABSTRACT FORM

BEFORE WRITING YOUR ABSTRACT, please read the **Rules Governing Volunteered Papers Presented at IFT Annual Meetings** and **Publication Rights & Procedures**, below; and the **Instructions for Preparation of Abstracts**, on the next page.

RULES GOVERNING VOLUNTEERED PAPERS PRESENTED AT IFT ANNUAL MEETINGS

No volunteered paper shall be presented at an Annual Meeting unless at least one of the authors is a member in good standing of the Institute of Food Technologists or affiliate organizations.

No paper shall be presented at an Annual Meeting which has previously been given before other groups, or which has appeared in print prior to the Meeting.

Volunteered papers will NOT be accepted **without** abstracts suitable for publication in the Annual Meeting program booklet. These abstracts will be used by the Program Committee to determine the appropriateness of the paper based on whether it is original work, significant, contains new and useful information, and has a scientifically reasonable approach. The Program Committee reserves the right to reject the paper based on these criteria.

PUBLICATION RIGHTS & PROCEDURES

The Institute shall have first right to publish papers presented at an Annual Meeting of the Institute. However, after a paper has been accepted for the Meeting program, the author may, with supporting evidence, request its release, either before or after oral presentation. Under no circumstances shall release, if granted, permit publication of the paper before the oral presentation. Within fewer than 60 days after receiving the complete manuscript and a request for release, the Institute shall give the author a decision to release the paper or to accept it for publication subject to the necessary editorial revision. If accepted, the author or authors shall assign all rights in the paper to the Institute, and after publication the Institute shall, **upon request, reassign all rights back to the author or authors.**

Manuscripts may be submitted as soon as completed to the Institute of Food Technologists so that the papers may be reviewed and cleared for publication following the meeting.

All manuscripts not submitted prior to the meeting **MUST** be turned in during the Annual Meeting.

MANUSCRIPTS must conform to the "Style Guide for Research Papers," which is available from the Director of Publications, Institute of Food Technologists, 221 N. LaSalle St., Chicago, IL 60601 USA.

3 COMPLETE COPIES OF EACH MANUSCRIPT ARE REQUIRED. ONE IS AN ORIGINAL, TYPED COPY ON GOOD QUALITY WHITE BOND. THE SECOND AND THIRD COPIES MAY BE CLEAR AND EASILY READABLE CARBON OR PHOTOCOPIES.

INSTITUTE OF FOOD TECHNOLOGISTS—1976 Annual Meeting

ABSTRACTS MUST BE RECEIVED BY DECEMBER 22, 1975

READ BEFORE WRITING ABSTRACT

Use Official Abstract Form—see facing page

INSTRUCTIONS FOR PREPARATION OF ABSTRACTS

Procedure

Use a reasonably dark typewriter ribbon. TYPE abstracts (do NOT write) as directed on the Official Abstract Form. Use the printed form for the original and make three carbon or photocopies.

Title of Paper

Capitalize first letter of first word and of proper nouns ONLY, as:

Quick-cooking large Lima and other dry beans.

Authors and Institutions

Type SPEAKER's initials and complete surname in capital letters. For all other authors' names, capitalize only the initials and the first letters of the surnames. List company or institution names in sequence. Show complete mailing address for company or institution at which the research was conducted ONLY. Addresses usually can be abbreviated; use standard abbreviations. In case of multiple authors and multiple affiliations, use asterisks to identify, as:

J.B. KING*, P.J. Jones**, and H.W. Melton**
*Hunt Foods Co.; **Dept. of Food Science, U. of Illinois, Urbana, IL 61801

Content of Abstract

An abstract should contain a concise statement of: (1) the problem under investigation; (2) the experimental method used; (3) the essential results obtained. The text should cite quantitative data from representative experiments, or summary data; (4) what findings are new over the known art; and (5) conclusions. Do not state, "The results will be discussed."

Self-Addressed Post Cards

Enclose TWO standard sized, self-addressed and stamped postal cards for notification of the date your paper is scheduled for presentation, and for acknowledgement by the Program Committee of receipt of your abstract. **Include a separate set of cards for each abstract submitted. For each set of post cards, type paper title on back of both return postal cards.**

Submit Original and 2 Copies

Please submit completed original of Official Abstract Form and two copies, together with the self-addressed postal cards.

Mail Abstracts To:

Dr. F.J. Francis
1976 IFT Program Chairman
Dept. Food Science & Nutrition
University of Massachusetts
Amherst, MA 01002

Other communications concerning the program should be sent to the same address.

Extra Abstract Forms

Additional Official Abstract Forms, if needed, can be secured from the Institute of Food Technologists, Suite 2120, 221 N. LaSalle Street, Chicago, IL 60601.

Oral Presentation

The maximum time allowed for presentation of a paper is 15 minutes, including time for discussion. Authors should prepare the complete manuscript and be sure it can be presented easily within the allotted time, including time for presentation of slides.

To eliminate visual aid problems and to upgrade paper presentations, IFT has standardized slide projection. Remote control Kodak Carousel projectors requiring 2" X 2" slides will be used. **Therefore, all slides must be prepared on 2" X 2" (50mm X 50mm) slide mounts.**

Authors whose papers are accepted for presentation will receive, prior to the Annual Meeting, a copy of Kodak pamphlet #22, *Effective Lecture Slides*, to assist them in preparing art work for their slides.

PLEASE NOTE

At least one author of each volunteered paper must be a member in good standing of IFT.

Incomplete or improperly executed abstracts will be rejected by the Program Committee.

Failure to complete and sign the checklist on the back of the "Official Abstract Form for IFT Annual Meeting Papers" can be considered grounds for rejection of your paper.

OFFICIAL ABSTRACT FORM FOR IFT ANNUAL MEETING PAPERS

Read Instructions First (see facing page)—All Lines Must Be Double-Spaced—Type Only

YOU MUST COMPLETE AND SIGN CHECKLIST ON NEXT PAGE

TITLE: Capitalize first letter of title only (also first letter of proper nouns). **See instructions on facing page.**	
AUTHORS: Capitalize name of speaker ONLY. See instructions on facing page.	
AFFILIATIONS: Show complete mailing address ONLY for institution at which research was conducted. See instructions on facing page.	

USE THIS FORM ONLY—PUT ABSTRACT BELOW THIS LINE

Area of Food Science & Technology (see List in Call for Papers):

– CHECKLIST –

CHECK & SIGN BEFORE MAILING ABSTRACT

- ☐ I have read the "Rules Governing Volunteered Papers Presented at IFT Annual Meetings" and the "Publication Rights & Procedures," and I agree to abide by the terms of these rules.
- ☐ Abstract has been prepared according to the "Instructions for Preparation of Abstracts."
- ☐ Original and 2 copies of the abstract are enclosed.
- ☐ Two self-addressed post cards are enclosed.

(SIGNED) _____

Name of author who is a member in good standing of IFT if other than the person whose signature appears above:

_____

ABSTRACTS:
IN THIS ISSUE

DIELECTRIC PROPERTIES AT MICROWAVE FREQUENCIES OF AGAR GELS. Similarity to the Dielectric Properties of Water. B.D. ROEBUCK & S.A. GOLDBLITH. *J. Food Sci.* **40,** 899–902 (1975)–At 1.0 GHz and 3.0 GHz, the dielectric properties (κ' and κ'') of agar gels were determined at 5, 25, 45 and 65°C for concentrations as high as 3% agar. The dielectric constant κ' of the agar gels at all temperatures studied was found to be similar to the κ' of water at the corresponding temperature and frequency. The dielectric loss κ'' of the agar gels for the corresponding temperature and frequency showed maximum deviation from κ'' of water for the higher temperatures, the higher agar concentrations, and the lower frequency. Agar gels, having dielectric properties similar to water yet rigid in form, can conveniently be used for preparing models of real foods for microwave oven experiments of thawing, cooking and processing of foods.

INHIBITION OF PSYCHROTROPHIC BACTERIA BY LACTOBACILLI AND PEDIOCOCCI IN NONFERMENTED REFRIGERATED FOODS. S.E. GILLILAND & M.L. SPECK. *J. Food Sci.* **40,** 903–905 (1975)–The growth in milk at 5–7°C of *Pseudomonas fragi* and a gram negative psychrotrophic isolate was inhibited by cells of *Lactobacillus bulgaricus.* The growth of psychrotrophic bacteria in the natural flora of ground beef was also inhibited at 5°C by cells of *L. bulgaricus, L. lactis* and *Pediococcus cerevisiae,* which do not grow under these conditions. Cells of *L. bulgaricus* were inhibitory to the growth of psychrotrophic bacteria in refrigerated crab meat. Hydrogen peroxide was involved in the inhibitory action exerted by the lactobacilli. Apparently other factors were responsible for the antagonistic action produced by *P. cerevisiae.*

CHARACTERIZATION OF GLUCOSE OXIDASE AND CATALASE ON INORGANIC SUPPORTS. P.F. GREENFIELD & R.L. LAURENCE. *J. Food Sci.* **40,** 906–910 (1975)–Glucose oxidase and catalase were immobilized on a number of inorganic supports both individually and simultaneously. The effect of support type, immobilization technique, and concentration of enzyme in the immobilizing solution on the initial activity of the immobilized enzyme was measured. The effect of operating pH and temperature on the initial activity was studied as was the storage stability. The results indicate that moderate activity levels can be achieved with inexpensive clay supports using relatively crude mixtures of glucose oxidase/fungal catalase immobilized by the glutaraldehyde coupling procedure. Although direct adsorption of the enzyme is by far the most convenient method of coupling, it was found that the enzyme was readily eluted from the clay supports under flow conditions in the presence of 2M potassium chloride, and hence it is not satisfactory.

VOLATILE COMPONENTS OF ROASTED COCOA: BASIC FRACTION. O.G. VITZTHUM, P. WERKHOFF & P. HUBERT. *J. Food Sci.* **40,** 911–916 (1975)–Roasted cocoa volatiles were isolated using the following technique: extraction with supercritical CO_2 under pressure followed by atmospheric steam distillation; adsorption on Porapak Q and subsequent extraction with purified diethylether; separation of the organic phase into basic and neutral fractions. Identification of basic cocoa aroma constituents was accomplished by mass spectrometry in combination with a 200m × 0,31 mm i.d. glass capillary column. For the first time 59 compounds are reported in roasted cocoa including alkyl-, alkenyl-, acyl-, furyl- and alicyclic pyrazines, pyridines, quinoxalines, oxazoles, quinoline and methyl o-aminobenzoate. The CO_2-extraction process described is generally applicable to studies on volatile aroma constituents in food odor research.

FUSEL OIL AND METHANOL CONTENT OF LEBANESE ARAK. S.M. DAGHER & I.G. RUHAYYIM. *J. Food Sci.* **40,** 917–918 (1975)–Samples of a Lebanese alcoholic beverage were analyzed for higher alcohols and methanol. Methanol content varied from 60–750 ppm and the average content of propanol, isobutanol and amyl alcohol were 143, 211 and 523 ppm, respectively. Juice samples of local grape varieties were analyzed for their amino acid composition. Valine levels differed slightly among varieties, but the amounts of leucine and isoleucine varied considerably. Arak produced from such varieties differed markedly in their higher alcohol composition. Varietal differences and distillation techniques seemed to influence the composition of the final product.

CHARACTERISTICS OF RED WINES OF SIX CULTIVARS OF *Vitis rotundifolia* Michx. D.E. CARROLL, W.B. NESBITT & M.W. HOOVER. *J. Food Sci.* **40,** 919–921 (1975)–Six black-skinned cultivars of muscadine grapes (*Vitis rotundifolia* Michx.) were made into wine. The wines were characterized in terms of chemical composition, color specification and sensory quality. Over a test period of 3–5 yr, Noble and Tarheel consistently yielded wines of good overall quality while Albemarle, Hunt, Magoon and Thomas yielded wines of inferior quality due principally to poor sensory characteristics, especially poor color.

CORRELATION OF CAROTENOID VISIBLE ABSORBANCE AND NUMERICAL COLOR SCORE OF ORANGE JUICE. D.R. PETRUS, R.L. HUGGART & M.H. DOUGHERTY. *J. Food Sci.* **40,** 922–924 (1975)–Visible absorption spectra of alcoholic solutions of juices of Florida Hamlin, Pineapple and Valencia orange varieties were all found to be similar in shape although not in absorption intensities. Absorption maxima were recorded at 465, 443 and 425 nm. A slightly broad shoulder was also observed at about 395–398 nm. Results showed that the carotenoid visible absorbance increased with maturity and/or variety. The correlations of the sum of carotenoid absorbances (absorbance at 465, 443 and 425 nm) with color scores were high, regardless of extractor type or setting, °Brix, percent sinking pulp, maturity or variety. The coefficient of correlation was $r = 0.973$ or greater with a coefficient of determination of $r^2 = 0.947$ or greater.

EPICUTICULAR WAX ON THE JUICE SACS OF CITRUS FRUITS: A POSSIBLE ADHESIVE IN THE FRUIT SEGMENTS. I. SHOMER, A. FAHN & I. BEN-GERA. *J. Food Sci.* **40,** 925–930 (1975)–Transmission and scanning electron microscopy were employed to study the structure of citrus fruit segments and the nature of their compact packaging. Deposits of a waxy nature, as determined by chemical analysis, were present on the outer surfaces of the juice sacs. Removal of a waxy deposit in a number of different organic solvents or by melting it in water at a temperature higher than 50°C, resulted in disintegration of the segments and disconnection of adjoining juice sacs. Structural damage through disintegration, resulting from cryogenic freezing in liquid N and liquid Freon, is discussed.

TOTAL PEEL OIL CONTENT OF THE MAJOR FLORIDA CITRUS CULTIVARS. J.W. KESTERSON & R.J. BRADDOCK. *J. Food Sci.* **40,** 931–933 (1975)–The total quantity of cold-pressed peel oil was determined for each of 12 different citrus cultivars. Data for lime and tangelo oils were collected for 3 yr, tangerine, grapefruit and orange oils for 4 yr and lemon oils for 5 yr. The range in average peel oil content (lb

oil/ton fruit) for the major cultivars was as follows: oranges (7.8–13.5), grapefruit (5.6–6.5), tangerine (15.5), tangelo (11.3), lime (8.1) and lemon (15.1). Information regarding processing dates and percentages of the total crop being processed has been presented for each of the varieties. Based on the annual crop tonnage being processed, it was shown that the total oil content from Florida citrus was approximately 92 million lb as follows: oranges (82 million lb), grapefruit (7 million lb) and mandarins, limes, lemons (3 million lb).

GLUTAMIC OXALOACETIC TRANSAMINASE ACTIVITIES IN PEACHES DURING MATURATION. J.J. JEN & C.S. GRAHAM. *J. Food Sci.* **40**, 934–936 (1975)–Glutamic Oxaloacetic Transaminase (GOT) activities of 'Redhaven' and 'Redskin' peaches were measured at weekly intervals during the maturation period. The specific activity of GOT reached a peak 2 wk after the pit-hardened stage of maturation, then declined to a minimum until just prior to ripening when another surge in activity occurred. Peaches from trees that had received succinic acid-2,2-dimethylhydrazide treatment had higher GOT activity than fruits from untreated trees. The pH optimum of peach GOT was 7.8–8.1. A 1:1 ratio of aspartate to oxoglutarate concentration was optimum for GOT reaction.

CUCUMBER POLYGALACTURONASE. R. PRESSEY & J.K. AVANTS. *J. Food Sci.* **40**, 937–939 (1975)–A water-soluble polygalacturonase from cucumbers has been partially purified and characterized. It is an exo-splitting enzyme which removes monomer units from the nonreducing ends of the substrate molecules. The polygalacturonase has a pH optimum at pH 5.5 and is activated by Ca^{2+} ions. The maximum velocity and affinity for the substrate increase with increasing substrate chain length and are maximal for the largest substrates.

EFFECT OF WHEAT PHYTASE ON DIETARY PHYTIC ACID. G.S. RANHOTRA & R.J. LOEWE. *J. Food Sci.* **40**, 940–942 (1975)–Studies with rats showed that although wheat phytase does not account for the majority of hydrolysis of dietary phytate, it is appreciably involved in its hydrolysis in the stomach and probably to a lesser extent in the small intestine. Measured under simulated gastrointestinal conditions, activity of wheat phytase (native and isolated) was substantial in the stomach only.

EFFECT OF GAMMA RADIATION ON PHYSICO-CHEMICAL CHARACTERISTICS OF RED GRAM (Cajanus cajan) STARCH. S.P. NENE, U.K. VAKIL & A. SREENIVASAN. *J. Food Sci.* **40**, 943–947 (1975)–Total reducing and nonreducing sugars in red gram (*Cajanus cajan*) are not affected by radiation treatment (1 Mrad). Oligosaccharides, reported as flatulence factors in legumes, namely stachyose and raffinose, are slightly decreased on cooking the irradiated samples. Irradiated and cooked red gram starch is more susceptible to alpha-amylase action than the unirradiated sample. Rheological properties of red gram starch, such as gelatinization viscosity, swelling power and solubility improve on irradiation resulting in a final cooked product with better textural properties.

CHANGES IN VOLATILE FLAVOR COMPOUNDS DURING THE RETORTING OF CANNED BEEF STEW. R.J. PETERSON, H.J. IZZO, E. JUNGERMANN & S.S. CHANG. *J. Food Sci.* **40**, 948–954 (1975)–Significant qualitative and quantitative differences were found in the volatile flavor compounds of canned and fresh beef stew by gas chromatography and sniffing of the GC effluents. A total of 102 compounds was identified in the canned stew volatiles; a number of them for the first time in foods. The principle compounds identified consisted of saturated and unsaturated aliphatic hydrocarbons, saturated cyclic hydrocarbons, aromatic hydrocarbons, alcohols, aldehydes, ketones, furan compounds and nitrogen/sulfur-containing compounds. It was established that the "retort flavor" of canned stew is not due to a single compound but is probably due to a relatively complex mixture of a number of components which might include oxygenated furan derivatives, some heterocyclic compounds, such as benzothiazole and pyrroles, and some low molecular weight sulfur compounds, such as H_2S and dimethyl sulfide.

POSTMORTEM GLYCOLYSIS AND ISOMETRIC THAW TENSION DEVELOPMENT AND DECLINE IN BOVINE SKELETAL MUSCLE UNDERGOING THAW RIGOR. A.O. OKUBANJO & J.R. STOUFFER. *J. Food Sci.* **40**, 955–959 (1975)–Rapid rates of degradation of ATP, pH decline and a concomitant accumulation of inorganic phosphate were associated with the phenomenon of thaw rigor in bovine sternomandibularis muscle. These changes were almost completed in 300, 120 and 60 min at 3°, 25° and 37°C, respectively. The peak isometric thaw tension increased significantly between 3° and 25°C and only slightly at 37°C. The time to attain this peak decreased with rise in temperature. Significantly less time was required to reach peak thaw tension in oxygen than in carbon dioxide or nitrogen at each of the temperature levels. It was demonstrated that the ability of the sarcoplasmic reticulum to accumulate Ca^{2+} is destroyed so that Ca^{2+} is released in the presence of ATP and the clear demarcation between rigor mortis and ATP concentration becomes evident.

THAW RIGOR INDUCED ISOMETRIC TENSION AND SHORTENING IN BROILER-TYPE CHICKEN MUSCLES. R.C. WHITING & J.F. RICHARDS. *J. Food Sci.* **40**, 960–963 (1975)–Isometric tension and shortening of red (B. femoris) and white (P. major) muscles from broiler chickens frozen at varying times prerigor declined with increasing aging time until the muscles were in rigor whereupon relatively little response was observed. Tension in red muscle was greater than white in both normal rigor mortis and thaw rigor. Maximum thaw rigor shortening for both muscles was about 60%, but when shortening was less than maximum, red muscle consistently exhibited greater shortening than white. The degree of shortening increased as the nominal thawing temperature of prerigor-frozen muscle was increased from −1° to 5° to 21°C. Increasing the holding time at −1°C between 0 and 5 hr resulted in a reduction in shortening of muscle when it was subsequently raised to 5° or 21°C.

MECHANISM OF LIPID OXIDATION IN MECHANICALLY DEBONED CHICKEN MEAT. Y.B. LEE, G.L. HARGUS, J.A. KIRKPATRICK, D.L. BERNER & R.H. FORSYTHE. *J. Food Sci.* **40**, 964–967 (1975)–A series of oxidation studies were conducted to characterize the mechanism of lipid oxidation in mechanically deboned chicken meat. The catalytic function of MDCM homogenate was most active at neutral and alkaline pH. Addition of ascorbic acid, β-mercaptoethylamine and cyanide partially or completely inhibited the oxidation of linoleate, whereas EDTA had no effect. When hemoproteins were destroyed by a prior treatment of homogenates with H_2O_2, the catalytic function was decreased to less than 10% of the original activity. It was concluded that hemoproteins were the predominant catalysts of lipid oxidation in MDCM. Furthermore, the relative concentration ratio of polyunsaturated fatty acids to hemoproteins was in the range where heme catalyzed oxidation would occur at or close to maximum rate.

PERSISTENCE OF ECHOVIRUS AND POLIOVIRUS IN FERMENTED SAUSAGES. Effects of Sodium Nitrite and Processing Variables. M.A. KANTOR & N.N. POTTER. *J. Food Sci.* **40**, 968–972 (1975)–Dry and semidry varieties of fermented sausage inoculated with high titers of poliovirus and echovirus were prepared. The sausages contained sodium nitrite at added levels of 150.0, 75.0, 37.5 and 0 ppm. The commercial operations of fermentation, heating and drying were simulated, and aliquots of meat were analyzed at various intervals for virus titer, bacterial plate counts, moisture, pH and residual nitrite. Except for a loss of about 90% in cervelat after heating, both viruses persisted in high titers and were virtually unaffected by any level of nitrite and by the wide range of processing conditions employed.

EFFECT OF NITRATE AND NITRITE ON COLOR AND FLAVOR OF COUNTRY-STYLE HAMS. B.D. EAKES, T.N. BLUMER & R.J. MONROE. *J. Food Sci.* **40**, 973–976 (1975)–Country-style hams were produced with and without nitrate and nitrite by dry curing and brine pumping methods. Evaluations of chemical characteristics, color, flavor and residual nitrate and nitrite were made after 30, 60, 90 and 100 days of processing. Moisture decreased while salt and fat increased over processing time, but treatment effects were similar. Hams cured with nitrate and nitrite either alone or in combination had more acceptable color than hams cured with salt and sucrose only. Aged flavor development was not significantly affected by curing treatment. Nitrite was depleted after 30 days at 4°C and nitrate gradually decreased with days in storage.

EFFECT OF VARIOUS LEVELS OF POTASSIUM NITRATE AND SODIUM NITRITE ON COLOR AND FLAVOR OF CURED LOINS AND COUNTRY-STYLE HAMS. B.D. EAKES & T.N. BLUMER. *J. Food Sci.* 40, 977–980 (1975)–Hams and loins were dry cured with and without nitrate and nitrite at various levels. Added concentrations ranged from 0–130 ppm in the loins and 70–160 ppm in the hams. Color development of loins cured 16 days without nitrate or nitrite was unacceptable; however, nitrate and nitrite either alone or in combination at 70 ppm resulted in adequate color development. Hams processed 90 days and containing 70 ppm of nitrate and/or nitrite also had acceptable color. Aged flavor of the hams was not affected by the level of nitrate and nitrite. Nitrite was depleted at sampling and nitrate was slightly reduced.

INHIBITION OF N-NITROSAMINE FORMATION IN MODEL FOOD SYSTEMS. J.I. GRAY & L.R. DUGAN JR. *J. Food Sci.* 40, 981–984 (1975)–The effect of certain compounds, some of which are endogenous to cured meat systems and some which may be added for preservative or other purposes on the N-nitrosation reaction was investigated in both aqueous and low moisture carboxymethylcellulose systems. Essentially complete inhibition of the reaction was achieved when the ratio of ascorbic acid (or bisulfite) to nitrite was greater than 2:1. Antioxidant compounds were also effective in blocking dimethylnitrosamine formation. The inhibiting effect of amino acids, cysteine, glutathione and methionine varied with the pH of the system.

KINETICS OF THIAMINE DEGRADATION BY HEAT. A new method for studying reaction rates in model systems and food products at high temperatures. E.A. MULLEY, C.R. STUMBO & W.M. HUNTING. *J. Food Sci.* 40, 985–988 (1975)–The rate of destruction of thiamine hydrochloride in phosphate buffer and selected low-acid foods was studied over the temperature range 250–280°F using a thermoresistometer. The reaction involved in the thermal degradation of the thiamine molecule was found to be of the first order type (as evidenced by straight line destruction rate curves at constant temperature), and the decimal reduction times (D values) of the breakdown reaction were linearly related to the temperature over the entire range studied. The results showed that thiamine was destroyed more rapidly in phosphate buffer than in the food systems under study.

KINETICS OF THIAMINE DEGRADATION BY HEAT. Effect of pH and form of the vitamin on its rate of destruction. E.A. MULLEY, C.R. STUMBO & W.M. HUNTING. *J. Food Sci.* 40, 989–992 (1975)–The degradation kinetics of 0, 30, 65 and 100% thiamine hydrochloride (100, 70, 35 and 0% co-carboxylase, respectively) at 265°F in phosphate buffer between pH 4.5 and pH 6.5 were obtained using a thermoresistometer. At every pH, first order rates of reaction were observed for both thiamine hydrochloride and co-carboxylase as well as for mixtures of the two. Under identical heating conditions, co-carboxylase was destroyed more rapidly than thiamine hydrochloride. When both forms of thiamine were present together, the increased lability of co-carboxylase became apparent only when its concentration in the mixture exceeded 35%.

THIAMINE: A CHEMICAL INDEX OF THE STERILIZATION EFFICACY OF THERMAL PROCESSING. E.A. MULLEY, C.R. STUMBO & W.M. HUNTING. *J. Food Sci.* 40, 993–996 (1975)–A new procedure has been developed to test the sterilizing efficiency of a conventional thermal process. Thiamine hydrochloride was added to pea and beef purees (conduction-heating foods) and to peas-in-brine (a convection-heating food) taken in #2 cans and still-retorted at 250°F in a vertical steam retort for varying lengths of time. Predicted thiamine retentions were calculated on the basis of kinetic and heat-penetration data. In the case of pea puree and peas-in-brine, analyzed values for thiamine (obtained by the thiochrome method) were not more than about 5% below the predicted retention. This was not true for the most severely heated beef puree. Because of liquid and solid phase separation of beef puree during severe thermal processing, it would not be expected that accurate predictions for thiamine degradation under these conditions could be accomplished by mathematical procedures developed for application with uniform phase conduction-heating or convection-heating only (results obtained clearly bear this out).

EFFECTS OF HEATING METHODS ON VITAMIN RETENTION IN SIX FRESH OR FROZEN PREPARED FOOD PRODUCTS. C.Y.W. ANG, C.M. CHANG, A.E. FREY & G.E. LIVINGSTON. *J. Food Sci.* 40, 997–1003 (1975)–Six products, mashed potatoes, pot roast with gravy, peas with onions, beans with frankfurters, carrots and breaded fish in bulk packs were analyzed for vitamin content before and after treatments, simulating (1) conventional institutional handling, i.e., fresh preparation followed by holding at 180°F for 0, 1/2, 1-1/2 and 3 hr, and (2) convenience food system handling, i.e., preparation followed by freezing and reheating to 180°F using hot air convection, infrared, high pressure steam or microwave and followed by holding for 1/2 hr. Riboflavin was found to be very stable and different treatments had similar effects on retention. Significant losses of thiamine and ascorbic acid occurred in products held at 180°F after preparation. Microwave and infrared heated frozen foods retained similar or higher levels of thiamine than the freshly prepared foods held for 1-1/2 hr but retained lesser amounts of ascorbic acid as compared to other treated samples. Retentions of the heat-labile nutrients in the convection oven were either comparable to or lower than the infrared and/or microwave heating. High pressure steam heating in all instances resulted in substantially lower levels of thiamine and riboflavin than other reconstitution methods, but this method had the advantage of preserving ascorbic acid in mashed potatoes and in most instances it was better than hot-holding for 3 hr.

ASCORBIC ACID. MINERAL AND QUALITY RETENTION IN FROZEN BROCCOLI BLANCHED IN WATER, STEAM AND AMMONIA-STEAM. D. ODLAND & M.S. EHEART. *J. Food Sci.* 40, 1004–1007 (1975)–Broccoli was blanched in water, steam and NH_3-steam. Blanched samples were analyzed for total solids, ash, P, K, Na, Mn, Ca, Mg, Cu, ascorbic acid, color (Gardner), pH and titratable acidity; blanch effluents for solids and minerals. In addition to pH, acidity and ascorbic acid analyses, cooked samples were evaluated for flavor, texture, color and overall acceptability by a trained panel. Effluent from the water blanch contained 9–16 times the mineral content as condensates from steam blanches. Water-blanched broccoli was lower in solids, ash, P, K and ascorbic acid than both steam-blanched samples. Steam blanching gave the poorest color of the three methods and the addition of NH_4HCO_3 to the steamer greatly improved color. The panel rated NH_3-steam-blanched broccoli superior in color and overall acceptability to broccoli blanched by the two conventional methods.

NUTRIENTS IN SEEDS AND SPROUTS OF ALFALFA, LENTILS, MUNG BEANS AND SOYBEANS. A.M. KYLEN & R.M. McCREADY. *J. Food Sci.* 40, 1008–1009 (1975)–Dry seeds and sprouts of alfalfa, lentils, mung and soybeans were analyzed for selected nutrients. Sprouts compared on a solids basis contained calcium, iron and zinc at levels about the same as the seeds while protein was higher and fat content lower in sprouts. Total Vitamin C, thiamine, niacin and riboflavin content was higher in sprouts than seeds, vitamin C showing the greatest increase. Cooking slightly lowered heat-labile nutrients in sprouts. Galactose-containing sugars of mung beans disappeared upon sprouting. Based upon chemical analyses, nutrient levels of sprouts compare favorably with other fresh vegetables.

FACTORS INFLUENCING THE EXTRACTABILITY OF SAFFLOWER PROTEIN (*Carthamus tinctorius* L.) A.A. BETSCHART. *J. Food Sci.* 40, 1010–1013 (1975)–Classical fractionation of safflower meal protein yielded 8, 31 and 28% of the meal nitrogen as water, salt and alkali soluble, respectively. The water soluble fraction contained equivalent or larger quantities of the essential amino acids than did the meal. Conditions for extraction of total safflower meal protein included extracting a 5% (w/v) aqueous solution for 60 min at pH 9 and 25°C. Protein extraction was impaired when the meal was heated to temperatures of 107°C or higher. At pH 9 some 68, 80 and 83% of the meal nitrogen was extracted from a commercially desolventized meal, an expeller press cake meal and an unheated control meal, respectively.

COCONUT BREAD AS A MEANS OF IMPROVING PROTEIN NUTRITION. M.F. CHASTAIN, S.J. SHEEN, T.J. COOPER & D.R. STRENGTH. *J. Food Sci.* 40, 1014–1017 (1975)–An acceptable bread product using coconut flour (CF) as a protein supplement was developed. Bread containing CF up to 18% was found by taste panel scores to be as

palatable as 100% wheat flour (WF) bread. Nutritive value of the bread was evaluated by incorporating it into basal diets of male weanling albino rats at the 10% protein level, and determining protein efficiency ratios (PER) and carcass nitrogen (N) retentions. Increasing levels of CF in diets significantly increased PER and also increased carcass N retention when compared to control animals on 100% WF bread diets.

EFFECTS OF ETHYLENE ON METABOLIC AND QUALITY ATTRIBUTES IN SWEET POTATO ROOTS. R.W. BUESCHER, W.A. SISTRUNK & P.L. BRADY. *J. Food Sci.* **40,** 1018–1020 (1975)–The influence of ethylene on levels of respiration, phenolic content, peroxidase, catecholase and beta-amylase activities were investigated in fresh sweet potato roots while curing and after curing. Also associated changes in flavor, discoloration and firmness in baked roots were examined. Ethylene treatments markedly enhanced respiration, phenolic content, peroxidase and catecholase activities but decreased beta-amylase activities. When ethylene was removed, respiration declined to rates equal to that of the control although phenolic levels, peroxidase and catecholase activities remained significantly higher. While the quality attributes of flavor and firmness declined in baked roots, discoloration increased with increasing levels of phenolics, peroxidase and catecholase activities in the fresh roots.

EVALUATION OF METHODS FOR MEASURING ASPARAGUS TEXTURE. S.C. SHARMA, R.R. WOLFE & N.F. HAARD. *J. Food Sci.* **40,** 1021–1024 (1975)–The principal physicochemical methods for measuring toughness in asparagus, including mechanical fiber separation, histochemical evaluation and the shear press, were investigated and compared with organoleptic ratings. All of the methods were found to be highly correlated ($R > 0.95$). Mathematical expressions relating the various methods were established and it was determined that these relationships are independent of spear dimensions and storage conditions. Based on an understanding of spear texture as a response to lignification of fibrovascular tissue, a conceptual explanation of these methods and their interrelationships was proposed which is consistent with the experimental findings. Finally, a relationship was established between the shear press method and the asparagus fibrometer currently being used by processors.

TEXTURE OF BROCCOLI AND CARROTS COOKED BY MICROWAVE ENERGY. E. SCHRUMPF & H. CHARLEY. *J. Food Sci.* **40,** 1025–1029 (1975)–This study verified and attempted to account for the effects of microwave cooking on the texture of vegetables. Cooking by microwaves vs boiling produced a less tender, spongy outer cylinder and a more fibrous core in carrots. In broccoli, the outer layer was tough while the central portion was more tender than that cooked conventionally. Alteration of the pectic substances appears inadequate to account for the textural differences. Greater water loss, more shrunken contour of the vegetables, and more pronounced collapse of cells in tissues cooked by microwaves suggest that dehydration of the cell wall, possibly accompanied by increased crystallinity of the polysaccharide gels, may account for the greater toughness observed.

ESTIMATION OF THE COMPONENTS OF A PENETRATION FORCE OF SOME TROPICAL FRUITS. M. PELEG & L. GÓMEZ BRITO. *J. Food Sci.* 40, 1030–1032 (1975)–Penetration tests with sets of cylindrical plungers having curved contact area were performed on mangos, papayas, pineapples, plantains and agar gels. It was found that the relationship between the penetration force (F) and the plungers diameter (D) could be represented by the equation: $F/D = K'_C D + K'_S$, at least in a certain range of plunger diameters which included the two standard plungers of the Fruit Pressure Tester. The latter enables a numerical calculation of the compressive and shear components of the penetration forces from experimental results obtained by these two standard plungers only. Though considerable variations existed between the individual fruits it could be demonstrated that in penetration tests performed with the standard 5/16 in. plunger of the Fruit Pressure Tester the shear contribution was in the order of 6% for mangos, 20% for papayas, 32% for pineapples and 14% for plantains.

COMPUTER ANALYSIS OF THE VARIABLES AFFECTING RESPIRATION AND QUALITY OF PRODUCE PACKAGED IN POLYMERIC FILMS. Y.S. HENIG & S.G. GILBERT. *J. Food Sci.* **40,** 1033–1035 (1975)–Packaged produce is a dynamic system in which two main processes are taking place, respiration and permeation. Shortly after hermetic packaging, the rate of produce respiration will be equal to the rate of O_2 permeation into the package and CO_2 out of the package, and the concentrations of these gases as well as that of water vapor will be maintained at a constant level. The objective of our study was to predict the steady state concentrations of these gases taking into account all the variables affecting the system. A new method was devised to obtain respiration data under different O_2 and CO_2 concentrations in the range of 21%–2%, 0.03%–21%, respectively. These data were combined with permeability characteristics of different packaging films to predict the internal atmospheres of packaged tomatoes with varying weight of produce, area of packaging film and initial free volume. The prediction technique involves a numerical solution of the two differential equations which represent the packaging system. An iteration technique for the solution of these equations was devised using a computer, and the results of the actual storage experiments agreed with the predictions.

CHARACTERISTICS OF MECHANICALLY HARVESTED RAISINS PRODUCED BY DEHYDRATION AND BY FIELD DRYING. H.R. BOLIN, V. PETRUCCI & G. FULLER. *J. Food Sci.* **40,** 1036–1038 (1975)–Different fatty acids and their esters were used to accelerate drying of grapes to raisins, in conjunction with mechanical harvesting. A 2% suspension of either methyl or ethyl oleate provided optimum treatment to accelerate grape drying without adverse flavor problems. The oleate treatment reduced sulfur dioxide absorption by grapes and enzymatic browning in the vine-dried fruit. Raisins from oleate-treated grapes both absorbed and desorbed moisture faster than untreated raisins. The oleate treatment also softened the raisin skin.

REVERSE OSMOSIS RECOVERY OF FLAVOR COMPONENTS FROM APPLE JUICE WATERS. T. MATSUURA, A.G. BAXTER & S. SOURIRAJAN. *J. Food Sci.* **40,** 1039–1046 (1975)–This paper illustrates the application of a fundamental physicochemical criteria approach for predicting quantitatively reverse osmosis separations of some typical apple flavor components in dilute aqueous solutions using aromatic polyamide membranes. It is shown both by analysis and by experiment that recovery of flavor components present in apple juice waters is relatively much higher with an aromatic polyamide membrane than with a comparable cellulose acetate membrane. Reverse osmosis treatment of apple juice waters further shows that aroma recovery of flavor components increases with a decrease in operating temperature from 25°C to 7.5°C, an increase in operating pressure in the range 250–1000 psig, and a decrease in the concentration of flavor components in the feed.

STABILITY OF GRAPE ANTHOCYANIN IN A CARBONATED BEVERAGE. N. PALAMIDIS & P. MARKAKIS. *J. Food Sci.* **40,** 1047–1049 (1975)–The anthocyanin pigment of fermented grape skins was extracted with either hot water or 500 ppm aqueous SO_2 solution. The extracts were freeze dried and 0.7g of dried preparation was added as a colorant to 100 ml of a beverage containing 13.0g sucrose, 0.1g citric acid, 0.2g grape flavor, 0.05g Na benzoate and 1.7 volumes of CO_2. Ten ml samples of the beverage were stored at temperatures ranging from 3.5–38°C, in darkness, diffuse day light and continuous fluorescent light. Increasing the storage temperature resulted in faster pigment degradation, almost doubling the destruction for a 10°C rise. Light also accelerated the degradation of the pigment. In diffuse day light at 20°C the half-life of the anthocyanin extracted with SO_2 solution was 197 days. The degradation of the pigment followed first order reaction kinetics, generally. The SO_2-extracted pigment was more stable in the beverage than that extracted with hot water.

TEXTURAL PARAMETERS OF CANDY LICORICE. J. OLKKU & C.K. RHA. *J. Food Sci.* 40, 1050–1054 (1975)–Commercial candy licorice was subjected to texture profile and penetration test with an Instron Universal Testing Machine Model TM-M. Deformation speed (VCH) vs initial resistance to deformation, hardness, cohesiveness, gumminess,

chewiness, springiness, energy required for first predetermined deformation, force required for surface puncture, energy required for surface penetration, force required for the given penetration deformation, energy required for the given penetration deformation, and ratio of energy required for surface penetraton to predetermined penetration deformation were studied over a range of deformation speeds. In the deformation speed region from VCH 0.5–1.0 cm/min there were several changes in the slopes of the curves. There also were close correlations between some of the texture parameters. The textural parameters studied had no definite relationships with moisture content, N_2 content, suggesting that the processing parameters of the manufacturing condition give rise to the different textural behavior of the candy licorice.

EFFECT OF VARIETY, GROWING LOCATION AND THEIR INTERACTION ON THE FATTY ACID COMPOSITION OF PEANUTS. D.F. BROWN, C.M. CATER, K.F. MATTIL & J.G. DARROCH. *J. Food Sci.* **40,** 1055–1060 (1975)–Fatty acid compositions of oils expressed from 10 peanut varieties grown at 7 U.S. locations were determined by GLC. Higher linoleate (L) and lower palmitate (P) and oleate (O) contents were recorded in mature nuts from northern growing locations. Statistically significant latitude-related differences also were found in stearate, eicosenoate and behenate. Spanish types were less sensitive to location than the Virginia type varieties, Florunner, Va. 72R and Florigiant. Respective fatty acid percentages from the Spanhoma variety grown in southern Texas and central Oklahoma were: (L) 34.7, 38.7; (P) 11.8, 11.0; and (O) 45.5, 38.7. In Florunner peanuts they were: (L) 25.8, 36.5; (P) 9.6, 9.0; and (O) 59.1, 48.0. Differences within varieties at different locations are probably due to differences in soil temperatures, month of maturation and possibly to temperature-induced differences in metabolic rates.

RELATIONSHIP BETWEEN CHOPPING TEMPERATURES AND FAT AND WATER BINDING IN COMMINUTED MEAT BATTERS. D.D. BROWN & R.T. TOLEDO. *J. Food Sci.* **40,** 1061–1064 (1975)–Comminuted meat batters have been called "emulsions" by previous investigators and the comminution of batters to maximize fat and water binding properties has been considered as an emulsification process. In the present study, it has been observed that the bound water content of the batters as measured with a wide line NMR increased as the batters became more stable, and decreased as the batters lose their stability. In meat batters where the proportion of boneless beef proteins to fat was approximately 0.137–1, maximal binding of fat and water occurred when a temperature of 15–22°C was first reached during comminution. However, when the batters were chopped beyond the region of maximum stability, cooled and rechopped, there was a progressive decrease in binding with each temperature cycling. When the batters became unstable, they appeared to lose the ability to bind water earlier than fat, and the rate of change of fat release occurred faster than that for water. When chopping was prolonged at a constant temperature of 15°C, changes in the fat and water-holding capacities of the batter can still be observed indicating that temperature and stability are not always directly interrelated. In a batter containing a boneless beef protein to fat ratio of 0.374 to1, it appears that fat and water binding interfere with each other increasing the binding capacity of the batter for fat when there is a decreased binding capacity for water and vice versa.

BEEF PATTIES: THE EFFECT OF TEXTURED SOY PROTEIN AND FAT LEVELS ON QUALITY AND ACCEPTABILITY. S.R. DRAKE, L.C. HINNERGARDT, R.A. KLUTER & P.A. PRELL. *J. Food Sci.* **40,** 1065–1067 (1975)–Ground beef patties were formulated to contain 0, 15, 20 and 25% added textured soy protein at each of four fat levels (15, 20, 25, 30%). Analysis of the raw patties for fat found the Hobart Fat Percent Indicator and the Soxhlet extraction procedures reporting essentially the same fat values. Total cooking losses were found to be less with the addition of soy protein. Fat loss upon cooking was dependent on the amount of fat in the patty and not on the soy protein level. Moisture loss during cooking was highly dependent on the level of soy protein incorporated in the beef patties. Both trained and consumer sensory panels differentiated among patties with various levels of soy protein on the basis of flavor. No distinction among patties was made due to fat levels.

DIELDRIN, FAT AND MOISTURE LOSS DURING THE COOKING OF BEEF LOAVES CONTAINING TEXTURIZED SOY PROTEIN. M.A.M. SHAFER & M.E. ZABIK. *J. Food Sci.* **40,** 1068–1071 (1975)–Texturized soy protein ranging from 0–50% was substituted for beef in a meat-loaf system to determine the effects of soy on fat, moisture and pesticide reduction during cooking. The beef used in the study was environmentally contaminated with dieldrin. The addition of texturized soy to the meat-loaf system reduced fat losses through the drip but did not reduce moisture losses during cooking. With all levels of soy substitution, the dieldrin content of the cooked meat loaves was less than that of the corresponding raw meat loaves. The reduction in dieldrin content was dependent on both drip losses and codistillation. The amount of dieldrin found in the drip decreased as the level of soy substitution in the meat loaves increased. Significant decreases in dieldrin content, however, were accompanied by codistillation and volatile losses.

USE OF COAGULATED LACTALBUMIN FROM CHEESE WHEY IN GROUND MEATS. P. JELEN. *J. Food Sci.* **40,** 1072–1074 (1975)–Heat-acid coagulated lactalbumin curd (LC) from cottage cheese whey was used as a meat extender in ground beef. Meat balls containing different levels of LC were deep fried at 175°C for specified times. Moisture content, fat content, cooking loss, texture and organoleptic quality were determined. Increasing levels of LC decreased the cooking loss and moisture content after frying, but caused increased absorption of fat from the oil bath. The curd had a softening effect as determined by the Lee-Kramer shear press. Taste panel acceptance of the fried meat balls decreased with increasing LC addition.

EFFECT OF BONING BEEF CARCASSES PRIOR TO CHILLING ON MEAT TENDERNESS. S.N. FALK, R.L. HENRICKSON & R.D. MORRISON. *J. Food Sci.* **40,** 1075–1079 (1975)–30 Angus steers were utilized to evaluate the feasibility of removing muscles and muscle systems [longissimus (LD), semimembranosus (SM) and semitendinosus (ST)] from the unchilled carcass (hot boning) following conditioning for 3, 5 or 7 hr postmortem at 16°C, and after being refrigerated for 48 hr at 1°C (cold boned). Differences in shear force values between hot- vs cold-boned muscle were small, averaging less than 2 lb. Sarcomere length, measured only in LD was slightly, but not significantly ($P > 0.05$), smaller for the hot process. Significant differences ($P < 0.05$) in fiber diameter between the two processes occurred in all three muscles at the 3-hr holding period. Fiber diameters were greater for hot-processed muscle in the LD and ST, but the opposite occurred in the SM. Kinkiness scores were statistically different ($P < 0.05$) at all three holding periods in the LD; however, values were in the range of wavy to wavy-plus indicating that only minimal shortening had occurred. Sensory evaluation via the duo-trio test revealed that judges could distinguish between ($P < 0.05$) the tenderness of the two processes only in the 7-hr LD. Both preference and acceptability analyses showed that panelists had a slight tendency ($P > 0.05$) to prefer the 7-hr hot-boned LD to the contralateral process. The pH curves at the 3-hr holding period show the rate of pH decline was somewhat greater from 1–3 hr postmortem as compared with the 5- or 7-hr conditioning period. However, from 3–48 hr postmortem, only minor variations in pH among conditioning periods occurred. Mean pH for all hot sides at 3 hr postmortem was 5.76 ± 0.07. Additionally, the pH data together with temperature measurement provided evidence that the conditioned muscle had progressed sufficiently into rigor mortis by 3 hr postmortem to allow hot boning to proceed. Results demonstrate that bovine muscle may be boned as early as 3 hr postmortem with only minor changes in tenderness.

PREDICTION OF TEMPERATURE OF ICED FISH. P. CHATTOPADHYAY, B.C. RAYCHAUDHURI & A.N. BOSE. *J. Food Sci.* **40,** 1080–1084 (1975)–An analytical method using an error function solution of inverse Laplace transformation is proposed for predicting the time required for melting of a rectangular column of ice used for lining during transportation of fresh fish. After complete melting of the ice, the rise in temperature of the fish is computed by solving the heat conduction equation by the finite difference method. The derived formulae are used to predict the time required for ice melting with varied ratios of ice and

fish and the temperature of fish after complete melting of ice (ice to fish ratio 1:1 by weight) using three different fish containers. The predicted values are found to be in close agreement with the experimental results.

AFLATOXIN PRODUCTION ON SOME FEEDS AND FOODS. M.W. BENSON, R.H. KURTZMAN JR., W.U. HALBROOK & R.M. Mc CREADY. *J. Food Sci.* **40,** 1085–1086 (1975)–Samples of foods and feeds were sterilized, hydrated, inoculated with an aflatoxin-producing strain of *Aspergillus flavus*, and after growth occurred, an extraction for aflatoxins was made and assayed by a thin-layer chromatographic method. Growth of *A. flavus* occurred and aflatoxins were detected in all except castor and alfalfa meal. Most foods are rarely stored under conditions where the growth of *A. flavus* would occur. However, foods moistened, sterilized and inoculated with *A. flavus* supported growth and aflatoxin was produced. Selected livestock feeds, castor and alfalfa meal, supported growth of *A. flavus* but aflatoxin was not produced.

A SIMPLIFIED METHOD FOR THE QUANTITATIVE DETERMINATION OF SUCROSE, RAFFINOSE AND STACHYOSE IN LEGUME SEEDS. M. TANAKA, D. THANANUNKUL, T-C. LEE & C.O. CHICHESTER. *J. Food Sci.* **40,** 1087–1088 (1975)–A procedure using thin-layer chromatography and the thiobarbituric acid reaction was established for the quantitative determination of oligosaccharides in legume seeds. Neither the removal of lipids and proteins is needed for the sample preparation nor is expensive analytical equipment required by this method. The overall good recovery of added sugars indicates that this method is excellent for the determination of sucrose, raffinose, and stachyose simultaneously. The compositions of oligosaccharides in soybeans, mung beans, adzuki beans and white beans were analyzed and the results were compared with previously reported values.

NATURE OF LUTEIN ACYLATION IN MARIGOLD (Tagetes erecta) FLOWERS. T. PHILIP & J.W. BERRY. *J. Food Sci.* **40,** 1089–1090 (1975)–The nature of lutein acylation in marigold (*Tagetes erecta*) flowers was examined. Lutein accounts for 60% of total carotenoids in marigold flower extracts and occurs acylated with palmitic and myristic acids. Lutein esters are soluble in vegetable oil to the extent of twenty five percent (w/w).

DONENESS OF COMMERCIALLY COOKED BROILER THIGHS AS INDICATED BY AN OBJECTIVE COLOR METHOD. C.E. LYON & B.G. LYON. *J. Food Sci.* **40,** 1091–1092 (1975)–Commercially cooked broiler thighs were evaluated for doneness in thawed and reheated states, using the Hunter Color and Color Difference Meter according to Lyon et al. (JFS 1975, 40: 133) who established the relation between Hunter a_L values and subjective estimates of doneness. Six of the seven products ranged from done to moderately underdone in the thawed state by a_L values and showed no significant change in a_L value on reheating. The one product that was moderately to very underdone in the thawed state was rendered done to slightly underdone according to a_L value by the commercially recommended reheating procedure. Need for further research in the area of subjective and objective responses to doneness is emphasized.

MUSCLE FRAGMENTATION INDICES FOR PREDICTING COOKED BEEF TENDERNESS. J.O. REAGAN, T.R. DUTSON, Z.L. CARPENTER & G.C. SMITH. *J. Food Sci.* **40,** 1093–1094 (1975)–Samples were obtained from the raw semimembranosus muscle of 20 beef carcasses for the purpose of developing a technique for predicting relative tenderness among carcasses. It was determined that homogenizing 10g samples for 60 sec provided the highest correlation coefficient between muscle fragment weights (after filtration through cheesecloth and subsequent centrifugation) and shear force values. Significant differences in muscle fragment weights were obtained when the samples were stratified into "tough" and "tender" groups. Although the fragmentation index values were not significantly associated with sensory panel evaluations of tenderness, there was a significant correlation between fragmentation index and shear force values ($r = -0.79$). These data provide presumptive evidence that myofibrils from more tender muscle as measured by the Warner-Bratzler shear machine, are more easily fragmented. This variability in fragmentation can possibly be measured and related to ultimate tenderness of beef.

APPARATUS FOR MAINTAINING HOLDING TEMPERATURE WHILE SERVING CAFETERIA ROUNDS OF BEEF. M.R. BERRY JR. & R.W. DICKERSON JR. *J. Food Sci.* **40,** 1095–1096 (1975)–Rounds of beef are often served on consumer demand and may remain on the slicing table for long periods at low holding temperatures. Since these conditions favor microbial growth, techniques were developed for maintaining proper temperatures during serving. A deep-well pan enclosed a major portion of the roast but exposed the top surface to facilitate slicing. The pan had an adjustable rack that was raised as the roast was sliced. Adequate temperatures were maintained in boneless rounds with one infrared lamp directed on the cutting surface and a 206°F steam table temperature. Effectiveness of the system also depends on proper handling of the beef. Roasts not cooked to the proper degree of doneness or not placed directly onto the serving line resulted in inadequate holding temperatures.

THE EFFECT OF TEXTURED SOY FLOUR PARTICLES ON THE MICROSCOPIC MORPHOLOGY OF FRANKFURTERS. R.G. CASSENS, R.N. TERRELL & C. COUCH. *J. Food Sci.* **40,** 1097–1098 (1975)–The addition of coarsely and finely divided textured soy flour to a frankfurter batter caused some of the lipid globules to assume an irregular or angular shape. Lipid was not incorporated into the interstices of the texturized soy flour.

EFFECT OF LINEAR APPROXIMATION OF ENTHALPY-TEMPERATURE CURVE IN SIMULATING HEAT TRANSFER DURING FREEZING. L.C. TAO. *J. Food Sci.* **40,** 1099–1100 (1975)–Simulation of heat transfer during a freezing operation was recently made by integrating numerically a set of differential equations on a digital computer. This note presents the freezing curves obtained by the simulation program of Joshi and Tao (JFS 39: 623) for the nonlinear as well as the two linearized enthalpy functions.

ANTHOCYANINS OF GARLIC (*Allium sativum* L.). C.T. DU & F.J. FRANCIS. *J. Food Sci.* **40,** 1101–1102 (1975)–This study reports isolation and partial identification of the major pigments in garlic. Prolonged chromatography of garlic pigment extract showed the presence of seven pigment bands, three major (G1, G2, G3) and four minor. Results show that acylated anthocyanins might occur more often than generally recognized, especially those of aliphatic acyl groups which might escape detection due to their lability in mineral acids and lack of absorption in the cinnamoyl region of UV spectra.

EFFECTS OF DIFFERENT CONCENTRATIONS OF SUCCINIC ACID-2,2-DIMETHYLHYDRAZIDE ON THE FLAVOR OF PUREE FROM FRESH AND CANNED FREESTONE PEACHES. S.D. SENTER, B.G. LYON & B.D. HORTON. *J. Food Sci.* **40,** 1103–1104 (1975)–Flavor was evaluated on puree from fresh and canned freestone peaches (*Prunis persica* (L.) Batch, cv. Dixiland) that had been sprayed at the beginning of pit-hardening with 1000, 1500, 2000 and 2500 ppm succinic acid-2,2-dimethylhydrazide. These comparisons indicated that the flavor of treated and nontreated peaches differed significantly in the fresh and the canned product. In fresh fruit, laboratory panels preferred the flavor of peaches treated with 2500 ppm. In canned fruit, a panel of discriminating judges preferred the flavor of fruits treated at all concentrations over nontreated.

THE RED COMPONENT OF THE EXTERNAL COLOR AS A MATURITY INDEX OF PAPAYA FRUITS. M. PELEG & L. GÓMEZ BRITO. *J. Food Sci.* **40,** 1105–1106 (1975)–The initial external coloration of fresh papaya fruits was evaluated by a Hunterlab Color Difference Meter at sites 20 mm in diameter. It was demonstrated that the yellow coloration of the skin was accompanied by the introduction of a red color component and that the latter's intensity in terms of Hunter "a" values could provide an index for safe harvest of the fruits. It was found that the great majority of the fruits having an initial positive Hunter "a" value ripened normally and that fruits showing higher Hunter "a" values had the tendency to ripen in a shorter time. The latter values, however, due to the distribution of the storage time-ripening relationship, could only indicate a trend but could not provide a satisfactory single index for the ripening time prediction.

CYANIDE CONTENT OF APRICOT KERNELS. G.S. STOEWSAND, J.L. ANDERSON & R.C. LAMB. *J. Food Sci.* **40,** 1107 (1975)–The cyanide content of kernels from five cultivars of apricots ranged from 12 to 177 mg/100g. The lowest level of cyanide was observed in the old English cultivar, Moorpark, produced from unknown parentage. This was the only kernel that tasted sweet. The relatively high level of the cyanogenetic glycoside amygdalin, indirectly determined by hydrolyzed cyanide, created an extremely bitter taste of the kernels from the other four cultivars.

REPRINTS of Articles from "Journal of Food Science"

☐ SINGLE *reprints are NOT available from* JOURNAL OF FOOD SCIENCE. *Send requests directly to the senior author (address appears near title).*

QUANTITY *reprints (minimum of 100 copies) can be ordered from IFT Subscription Department, Suite 2120, 221 N. LaSalle St., Chicago, IL 60601. Price schedule available upon request.*

NOTICE: Page Charges for Publication of Research Papers

☐ *AT ITS March 1969 meeting, the Executive Committee of IFT voted to increase the page charge for research articles published in the* JOURNAL OF FOOD SCIENCE. *The page charge of $50 per printed page is effective for all research manuscripts received AFTER April 1, 1969. The page charge shall not constitute a bar to acceptance of research manuscripts because the author is unable to pay the charge.*

JFS BASIC SCIENCE

B. D. ROEBUCK[1] and S. A. GOLDBLITH
Dept. of Nutrition & Food Science, Massachusetts Institute of Technology
Cambridge, MA 02139

DIELECTRIC PROPERTIES AT MICROWAVE FREQUENCIES OF AGAR GELS.
Similarity to the Dielectric Properties of Water

INTRODUCTION

IN STUDYING the thermal properties of foods heated in microwave ovens, water is a convenient standard because its dielectric properties are well known (Von Hippel, 1954). Water often approximates or serves as the limiting case for the physical and dielectric properties of foods. Agar gels, in many instances, have dielectric properties similar to water, without possessing the fluidity of water. Gels can be molded into various shapes, filled with soluble or insoluble materials, and used in microwave ovens and tunnels without sample containers which would attenuate microwave energy. The high viscosity of agar gels eliminates the convection currents resulting in the heating of liquids; this greatly simplifies microwave heating studies. Agar gels are transparent, odorless and tasteless.

deLoor and Meijboom (1966), Van Zante and Nakayama (1956) and Copson (1956, 1962) have used agar gels in studies of the application of microwave energy to food processing. However, the dielectric properties (dielectric constant κ', dielectric loss factor κ'') have not been extensively studied: in this paper we investigate several factors. First, the purity of the agar used is important, because the ionic conductivity of salts or ionic moieties of the agar polymer increases the dielectric loss of the gel especially at the lower frequency. Second, both κ' and κ'' are functions of temperature; the temperature dependence will be shown for agar gels. A similar dependence occurs for water and heterogeneous mixtures such as foods. Third, the concentration of the agar in the gel is important. The gel melting temperature and gel strength increase as the agar concentration of the gel increases. These physical properties of agar gels are adequately reviewed by Selby and Selby (1959). As the agar concentration increases, the dielectric properties show increasing deviation from those of pure water.

It is desirable to use as little agar as is needed to retain the shape of the water-agar gel at the necessary firmness.

MATERIALS & METHODS

DIELECTRIC MEASUREMENTS at 1.0 and 3.0 GHz were made with a Model 4 Microwave Dielectrometer (Central Research Laboratory, Red Wing, Minn.) (Anonymous, 1951). The details of measuring techniques, sample holders, and sample preparation are discussed in detail by Pace (1967) and Roebuck (1970). The computation procedures of the dielectric properties κ' and κ'' are contained in Program I of Westphal and Iglesias (1970). Repeated measurements of the dielectric constant varied less than 1% of the mean and for the dielectric loss the variation was less than 3% of the mean.

The agarose and Difco agar gels were prepared with Agarose (Grade B, Calbiochem, Los Angeles, Calif.) and Bacto-Agar (Difco Certified, Difco Laboratories, Detroit, Mich.), respectively. Glass-distilled water was used in the formulation of all gels. Moisture analysis was performed by drying the samples at 105°C under vacuum until constant weight was obtained.

RESULTS & DISCUSSION

DIFCO AGAR refers to Difco certified Bacto-Agar and Agarose refers to Calbiochem Grade B Agarose; collectively, both gels will be referred to as agar gels.

The dielectric properties (κ', κ'', and tan δ) for water and four concentrations of Difco agar at 1.0 GHz and 3.0 GHz and at four temperatures (5, 25, 45 and 65°C) are presented in Table 1. Table 2 lists similar data for the dielectric properties of Agarose. The rigidity and melting points of these agar gels depend upon the agar concentration. Agar gels of less than 0.75% agar are very soft and often do not support their own weight. Gels of a higher percentage of agar are firm. Gels of less than 1% agar liquefy at 45°C while gels of higher agar concentration become viscous liquids above 65°C. Details of the physical properties of agar gels are discussed by Selby and Selby (1959).

deLoor and Meijboom (1966) measured the dielectric properties of agar gels only at 20°C and found that for the frequencies available for commercial microwave processing, namely 0.915 GHz and 2.45 GHz, the dielectric properties are similar to the dielectric properties of water. The data in Tables 1 and 2 confirm their measurements and also show the concentration and temperature dependence of the dielectric properties.

Figures 1, 2, 3 and 4 were drawn from the data of Tables 1 and 2. For agar concentrations of less than 3% agar by weight, the κ' and κ'' values appear to be linear functions of agar concentration. This is also true for the temperature range from 5–65°C. With agar concentrations increasing to approximately 3%, the κ' values at both frequencies decrease for 5, 25 and 45°C (Fig. 1 and 2). At 65°C, κ' values at 1.0 GHz increase slightly with increasing agar concentrations. The Difco agar at 3.0 GHz and at 65°C also shows an increase in κ' as the con-

[1] Present address: Dept. of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH 44106

Table 1—Dielectric properties[a] of Difco agar gels

Concentration (wt %)		Temp	Frequency					
			1.0 GHz			3.0 GHz		
Solids	Water	(°C)	κ'	tan δ	κ''	κ'	tan δ	κ'
0.0	100	5	85.1	0.090	7.70	80.2	0.275	22.06
		25	77.1	0.052	3.99	76.7	0.157	12.04
		45	71.4	0.031	2.20	70.7	0.106	7.49
		65	65.0	0.022	1.40	64.0	0.076	4.90
0.48	99.52	5	85.0	0.094	7.98	79.6	0.280	22.3
		25	76.6	0.059	4.50	76.3	0.160	12.2
		45	71.1	0.039	2.80	70.4	0.109	7.7
		65	65.4	0.032	2.10	64.0	0.081	5.2
0.89	99.11	5	84.5	0.097	8.20	79.3	0.288	22.8
		25	76.8	0.064	4.90	76.0	0.163	12.4
		45	71.2	0.048	3.42	70.3	0.111	7.8
		65	65.1	0.046	3.00	64.4	0.090	5.8
2.30	97.70	5	84.1	0.102	8.56	77.4	0.302	23.4
		25	75.5	0.080	6.06	74.8	0.171	12.8
		45	70.4	0.075	5.31	70.1	0.123	8.6
		65	66.7	0.076	5.10	64.1	0.098	6.3
2.68	97.32	5	84.4	0.112	9.50	77.1	0.307	23.7
		25	75.2	0.088	6.58	74.5	0.169	12.6
		45	70.6	0.082	5.82	69.8	0.126	8.8
		65	66.5	0.087	5.78	64.5	0.102	6.6

[a] The dielectric constant (κ') and the dielectric loss factor (κ'') are related by the loss tangent (tan δ) where κ' tan $\delta = \kappa''$.

Table 2—Dielectric properties[a] of Calbiochem Agarose gels

Concentration (wt. %)		Temp	Frequency					
			1.0 GHz			3.0 GHz		
Solids	Water	(°C)	κ'	tan δ	κ''	κ'	tan δ	κ'
0.0	100	5	85.1	0.090	7.70	80.2	0.275	22.06
		25	77.1	0.052	3.99	76.7	0.157	12.04
		45	71.4	0.031	2.20	70.7	0.106	7.49
		65	65.0	0.022	1.40	64.0	0.076	4.90
0.57	99.43	5	84.6	0.092	7.80	79.6	0.281	22.4
		25	76.8	0.054	4.16	76.4	0.160	12.2
		45	71.3	0.037	2.61	70.5	0.106	7.5
		65	65.3	0.026	1.70	63.3	0.079	5.0
0.98	99.02	5	84.4	0.094	7.91	79.0	0.286	22.6
		25	76.6	0.056	4.29	76.1	0.159	12.1
		45	71.4	0.040	2.88	70.5	0.111	7.8
		65	65.7	0.030	2.01	63.8	0.080	5.1
1.81	98.19	5	84.0	0.096	8.05	77.4	0.297	23.0
		25	75.6	0.060	4.50	75.5	0.163	12.3
		45	71.0	0.051	3.62	70.2	0.114	8.0
		65	66.4	0.033	2.20	63.4	0.082	5.2
2.43	97.57	5	83.2	0.098	8.20	77.0	0.302	23.3
		25	75.2	0.063	4.75	73.2	0.164	12.0
		45	70.9	0.058	4.10	70.2	0.114	8.0
		65	66.2	0.041	2.72	63.2	0.084	5.3

[a] The dielectric constant (κ') and the dielectric loss factor (κ'') are related by the loss tangent (tan δ) where κ' tan $\delta = \kappa''$

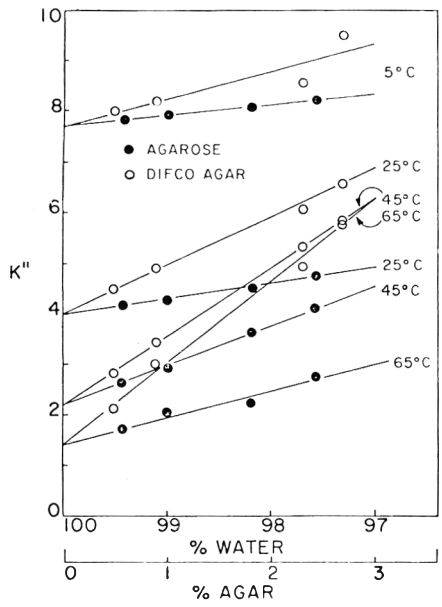


Fig. 1–Dielectric constant κ' of agar gels as a function of concentration at 1.0 GHz and at four temperatures.

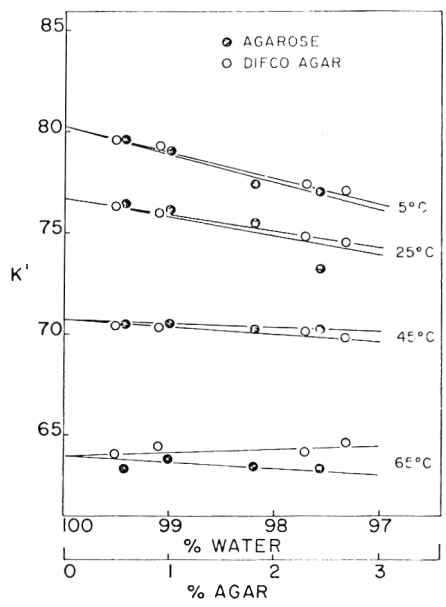


Fig. 2–Dielectric constant κ'of agar gels as a function of concentration at 3.0 GHz and at four temperatures.

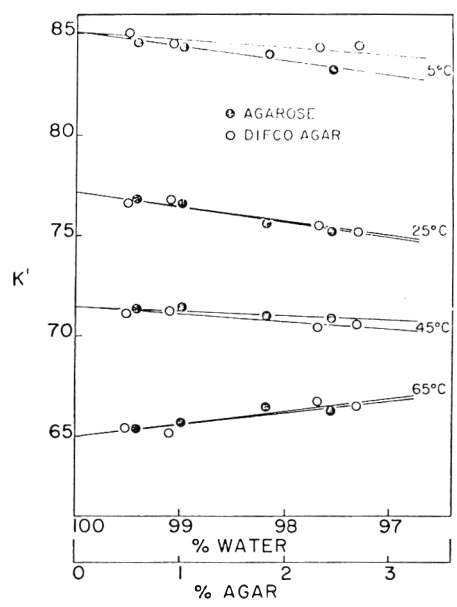


Fig. 3–Dielectric loss factor κ'' of agar gels as a function of concentration at 1.0 GHz and at four temperatures.

centration of agar increases. This behavior of κ' is unexpected and unexplained. In all instances, the dielectric constants κ' are similar for both types of agar gels and differ little from the dielectric constant of water.

Figures 3 and 4 clearly show that the dielectric loss factor κ'' increases linearly at both frequencies and for all four temperatures as the agar concentration increases. The rate of increase of κ'' is smaller for agarose than for Difco agar, especially at 1.0 GHz. Agarose is a more highly purified form of agar. The behavior of κ'' may be attributed to dielectric loss by ionic conductivity. As discussed by Hasted et al. (1948) and Lane and Saxton (1952) and as occurred in complex biologic systems by Goldblith and Pace (1967) and Roberts and Cook (1952), ionic conductivity becomes more significant as the microwave frequency decreases and as the temperature of the absorbing material increases. Ionic conductivity of the agar

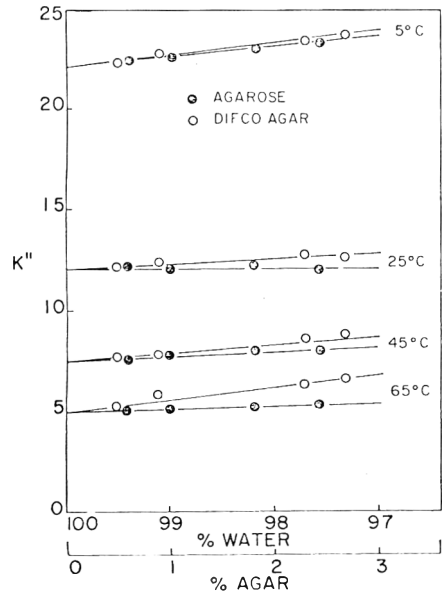


Fig. 4–Dielectric loss factor κ'' of agar gels as a function of concentration at 3.0 GHz and at four temperatures.

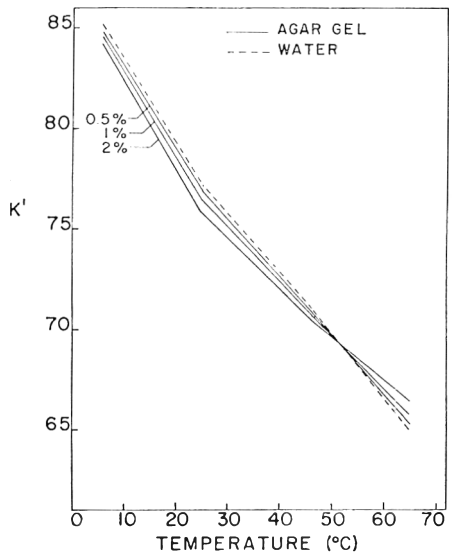


Fig. 5–Dielectric constant κ' of water and three concentrations of Difco agar as a function of temperature at 1.0 GHz.

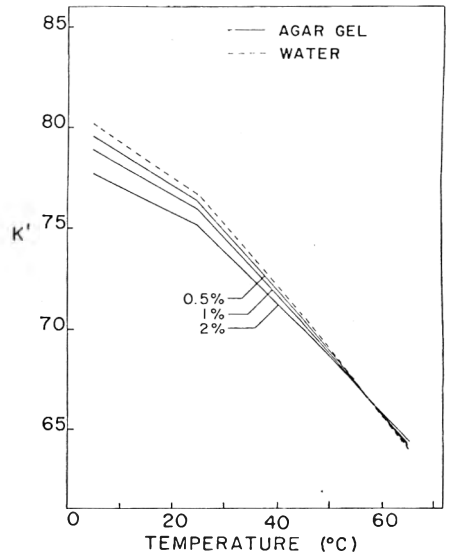


Fig. 6–Dielectric constant κ' of water and three concentrations of Difco agar as a function of temperature at 3.0 GHz.

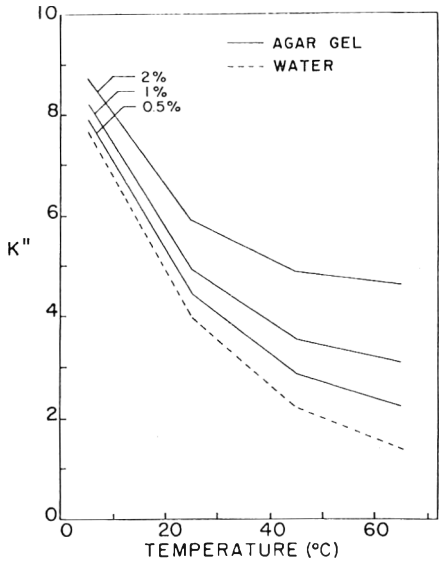


Fig. 7–Dielectric loss factor κ" of water and three concentrations of Difco agar as a function of temperature at 1.0 GHz.

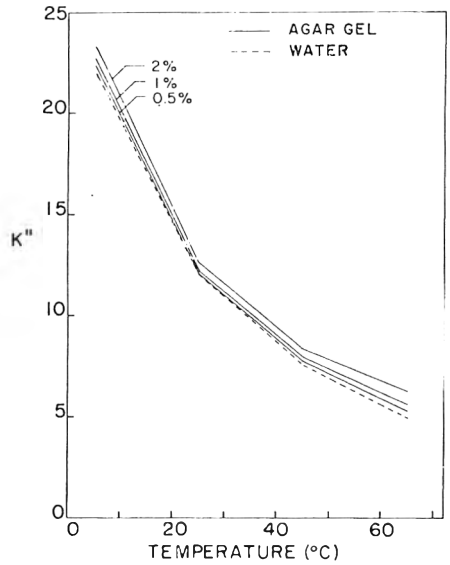


Fig. 8–Dielectric loss factor κ" of water and three concentrations of Difco agar as a function of temperature at 3.0 GHz.

gels was not measured. At 3.0 GHz, the κ'' values for the same concentrations of both agars are similar even at the higher temperatures (Fig. 4).

To clearly show the similarities of the dielectric properties of water and agar gels, the dielectric properties (κ' and κ'') of water and three selected concentrations (0.5, 1.0 and 2.0% by weight) of Difco agar were plotted as a function of the sample temperature (Fig. 5–8). The dielectric values (κ' and κ'') were determined from Figures 1–4 for the designated agar concentrations (0.5, 1.0 and 2.0%). The dielectric properties for the Agarose gels were similar to the properties of the Difco agar, shown in Figures 5–8 with one exception. For Agarose, the dielectric loss κ'' at 1.0 GHz and for the higher temperatures deviated less from the κ'' of water than did similar values for Difco agar. The lesser deviation of Agarose is attributed to its higher purity; hence, less ionic conductivity loss.

For both types of agar at 1.0 GHz, κ' of all concentrations of agar studies are very similar to the κ' of water at the appropriate temperature (Fig. 5). At 1.0 GHz, the percent difference between κ' of water and κ' of the 2% agar gels is not greater than 1.7%. For both types of agar gel at 3.0 GHz, the largest difference of κ' from the value of water is at 5°C and is only 2.2% different from water (Fig. 6).

The dielectric loss factor κ'' differs most from the corresponding value of water especially at higher temperatures, at the lower of the two frequencies, and for high concentrations of Difco agar (Fig. 7, 8).

The larger deviation of κ'' at 1.0 GHz compared to 3.0 GHz is undoubtedly due to the ionic conductivity as pointed out by Roebuck et al. (1972).

Thus, the use of these gels at concentration levels sufficient to result in firm gels for studies of the application of microwave power to process foods at 2.45 GHz should prove to be entirely feasible, whereas their use at 0.915 GHz could introduce a small error which should be correctable.

REFERENCES

Anonymous. 1951. Microwave Dielectrometer Instruction manual Central Research Laboratories, Red Wing, Minnesota.
Copson, D.A. 1956. Microwave energy in food procedures. IRE transactions of the Prof. Group on Medical Electronics, PGME 4: 24.
Copson, D.A. 1962. "Microwave Heating," Avi Publishing Co., Westport, Conn.
Goldblith, S.A. and Pace, W.E. 1967. Some considerations in the processing of potato chips. J. Microwave Power 3: 95.
Hasted, J.B., Ritson, D.M. and Collie, C.H. 1948. Dielectric properties of aqueous ionic solutions. Parts 1 and 2. J. Chem. Phys. 16(1): 1.
Lane, J.A. and Saxton, J.A. 1952. Dielectric dispersion in pure polar liquids at very high radio frequencies: 3. The effect of electrolytes in solution. Proc. Roy. Soc. A 214: 531.
deLoor, G.P. and Meijboom, F.W. 1966. The dielectric constant of foods and other materials with high water contents at microwave frequencies. J. Food Technol. 1: 313.
Roberts, J.E. and Cook, H.F. 1952. Microwaves in medical and biological research. Brit. J. Appl. Phys. 3: 33.
Pace, W.E. 1967. Dielectric properties of certain foodstuffs at selected frequencies and at different temperatures. Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, Mass.
Roebuck, B.D. 1970. Dielectric properties of carbohydrate-water mixtures at microwave frequencies. M.S. thesis, Massachusetts Institute of Technology, Cambridge, Mass.
Roebuck, B.D., Goldblith, S.A. and Westphal, W.B. 1972. Dielectric properties of carbohydrate-water mixtures at microwave frequencies. J. Food Sci. 37: 199.
Selby, H.H. and Selby, T.A. 1959. Agar. In "Industrial Gums," Ed., Whistler, R.L. and BeMiller, J.N. p. 15. Academic Press, New York.
Van Zante, H.J. and Nakayama, H. 1959. The effects of microwave energy on the internal temperatures of agar and food cylinders. J. Home Econ. 51: 454.
Von Hippel, A.R. (Ed.). 1954. "Dielectric Materials and Applications." M.I.T. Press, Cambridge, Mass.
Westphal, W.B. and Iglesias, J. 1970. Dielectric measurements on high-temperature materials. Technical Report AFML-TR-70-138, Air Force Materials Laboratory, Wright-Patterson AFB, Ohio.
Ms received 12/21/71; revised 5/23/75; accepted 5/28/75.

Contribution No. 2303 from the Dept. of Nutrition & Food Science, Massachusetts Institute of Technology.

This work was supported by Grant No. 5–RO1 FD-00166 of the Food and Drug Administration, entitled "Prediction of the Dielectric Properties of Foods," and by General Mills, Inc.

Mr. B.D. Roebuck was the recipient of a traineeship of the Food and Drug Administration under Grant No. 5-TO1-FD 00003-08.

S. E. GILLILAND and M. L. SPECK
Dept. of Food Science, North Carolina State University, Raleigh, NC 27607

INHIBITION OF PSYCHROTROPHIC BACTERIA BY LACTOBACILLI AND PEDIOCOCCI IN NONFERMENTED REFRIGERATED FOODS

INTRODUCTION

ANTAGONISTIC ACTIONS of lactic acid bacteria toward food-borne pathogens and spoilage microorganisms have been well documented and have been reviewed by Hurst (1973). The majority of work reported in this area has involved the inhibitory actions manifested during growth of the starter culture bacteria or those resulting from the characteristics of the fermented foods. We have shown that lactobacilli, lactic streptococci and *Leuconostoc citrovorum* exert antagonistic actions toward food-borne pathogens in associative cultures (Dahiya and Speck, 1968; Gilliland and Speck, 1972; Speck, 1972; Sorrells and Speck, 1970). Studies on the control of psychrotrophic spoilage bacteria and other undesirable bacteria in refrigerated cultured foods have focused primarily on the effect of inhibitory substances produced by starter culture bacteria during the manufacture of the foods (Goel et al., 1971; Geopfert and Chung, 1970; Mather and Babel, 1959). Little attention has been given to the possibility of controlling growth of psychrotrophs in foods such as ground beef and sweet milk during refrigerated storage. Reddy et al. (1970) have shown that inoculation of ground beef with mixtures of *Streptococcus lactis* and *L. citrovorum* retarded the growth of gram negative bacteria in the product during storage at 7°C. Daly et al. (1972) showed similar effects in ground beef, milk and cottage cheese inoculated with *S. diacetilactis*.

The objective of this study was to evaluate the possibility of using cells of selected starter culture bacteria which do not grow at normal refrigeration temperatures to control the growth of psychrotrophic bacteria in refrigerated foods.

EXPERIMENTAL

Bacterial cultures

All cultures used in this study were from the North Carolina State University Food Microbiology culture collection. *Pseudomonas fragi* and psychrotroph MC-60N, a gram negative rod isolated from raw milk in another study (Adams et al., 1974), were cultured in reconstituted 10% nonfat milk solids (NFMS) using 1% inocula and incubation at 22°C for 18 hr. *Lactobacillus bulgaricus* strains NCS1 and HWD, and *L. lactis* BYL1 were also maintained in the same manner but with incubation at 37°C for 18 hr. *Pediococcus cerevisiae* was cultured similarly using Lactobacilli MRS broth (Difco).

Preparation of concentrated cell suspensions

Lactobacilli and pediococci were subcultured in MRS broth once immediately prior to use in preparing the concentrated cell suspensions. The required volume of MRS broth was inoculated (1%) using fresh broth cultures and incubated at 37°C for 18 hr. The cells were centrifuged (4,100 × G at 2°C) from the broth, resuspended in cold, sterile 10% NFMS or 1% tryptone (Difco) and placed in a ice bath until used, normally within 1/2 hr.

Preparation of food samples

Cold sterile reconstituted 10% NFMS was inoculated with 500–1000 colony forming units (CFU) of *P. fragi* or psychrotroph MC-60N per ml. After thorough mixing, the inoculated milk was divided into portions of equal volume. One portion served as a control and the other(s) was inoculated with concentrated cell suspensions of lactobacilli (suspended in milk). The samples were subdivided into sterile containers whereby one could be used at each sampling period; these were placed in a low temperature incubator at 5 or 7°C.

Ground beef was purchased locally or was prepared in the laboratory by grinding raw beef roasts in a sterile hand grinder. After being thoroughly mixed in a sterile container to insure even distribution of the microbial flora, it was dispensed in sterile beakers in 100g quantities. The natural flora of the meat served as a source of psychrotrophic bacteria. Ten ml concentrated cell suspensions (in 1% tryptone) of lactobacilli or of pediococci were added to 100g samples of the meat and thoroughly mixed. Ten ml of sterile 1% tryptone was mixed with the control sample (100g). After mixing, 25g portions were placed in sterile petri plates and stored at 5°C.

Freshly picked crab meat was obtained from a N.C. seafood processor and prepared in a manner similar to that used for ground beef.

Effect of catalase on the antagonism

To determine if hydrogen peroxide was involved in the antagonism, experiments were conducted in which catalase (Sigma Chemical Co.) was added (3000 units/100 ml) to a sample of autoclaved 10% NFMS inoculated with psychrotroph MC-60N and *L. bulgaricus* NCS1. A similar sample containing heat inactivated (121°C for 15 min) catalase was also included. Active catalase was added to the control sample (without lactobacilli). All samples were incubated at 5°C on a rotary shaker (150 rpm) to insure continuous mixing during incubation.

Enumeration of bacteria

Distilled water containing 0.1% NFMS and 0.01% silicone antifoamer (Sigma Chemical Co.) was used as diluent. The initial dilutions (1:10) of meat samples were blended 2 min at low speed in chilled Waring Blendors. All other dilutions were made following procedures in *Standard Methods for the Examination of Dairy Products* (APHA, 1972). All counts were done in duplicate.

The growth of psychrotrophic bacteria was monitored by plate counts using either CVT agar (Olson, 1963) or Plate Count Agar (PCA) (Difco). The plates were incubated 5 days at 22°C. PCA was used to enumerate psychrotrophic bacteria in some experiments involving the lactobacilli since this organism would not form colonies on this medium at 22°C. The cells of lactobacilli included with the sample dilutions in plating did not appear to interfere with colony formation by the psychrotrophs on the agar media at 22°C.

Since lactobacilli or pediococci were added in greater numbers than other bacteria in the foods, they could be enumerated initially on nonselective media. Lactobacilli MRS broth plus 1.5% agar (MRS agar) was used for this purpose. In some experiments they were enumerated using Lactobacillus Selective (LBS) Agar (BBL). All plates of both media were incubated 72 hr at 37°C.

Hydrogen peroxide production

Cells from 20 ml of MRS broth cultures of the lactic acid bacteria were removed by centrifugation and resuspended in 50 ml aliquots of sterile 10% NFMS or a sterile broth containing 0.25% beef extract and 0.1% glucose. The samples were placed in 125 ml Erlenmeyer flasks and incubated 22 hr at 5°C on a rotary shaker (150 rpm). Hydrogen peroxide was measured by the method of Gilliland (1969). Results were recorded as optical density values at 400 nm.

RESULTS

L. bulgaricus NCS1 has a pronounced antagonistic action toward *P. fragi* in milk at 7°C (Fig. 1). The number of *P. fragi* (CVT agar plate counts) in the control increased rapidly during the 3-day period. The number detected initially in the sample containing *L. bulgaricus* NCS1 (8.4×10^7/ml) was approximately 1% of the number added and decreased further during storage. This indicated rapid inactivation of *P. fragi* by *L. bulgaricus*. The data in Table 1 shows the effect of two concentrations of *L. bulgaricus* NCS1 on psychrotroph MC-60N in milk at 5°C. The test organism did not appear to grow as rapidly as

P. fragi in the previous experiment. The number of psychrotroph MC-60N in the control milk increased over 100-fold in 10 days while decreases occurred in milk containing either level of *L. bulgaricus* NCS1; a larger decrease occurred in the presence of the higher number of lactobacilli. The pH values of the control and the sample containing 1×10^7 *L. bulgaricus* per ml were the same after 10 days; with 1×10^8 *L. bulgaricus* per ml it was only 0.5 units lower. The numbers of lactobacilli did not increase during incubation at 5°C. Based on this and similar observations in other experiments, the decreases in pH observed in such samples were not considered great enough to account for the antagonistic effect produced by the lactobacilli.

L. bulgaricus HWD (4.1×10^8/g) was inhibitory to psychrotrophic bacteria at 5°C in freshly picked crab meat. The initial counts on the crab meat were high (2.2 and 2.9×10^5/g). After 4 days of storage the count for the control had increased to 1.2×10^7/g, which was approximately four times higher than that for the sample containing the lactobacilli. Similar results were obtained in other experiments. In both samples the pH dropped from 8.0 to 7.4 during storage.

Results from preliminary experiments using ground beef purchased from a local supermarket indicated that some inhibition of psychrotrophic bacteria could be obtained by inoculating the meat with cells of *L. bulgaricus* or *P. cerevisiae* (Table 2). The initial CVT agar counts on these samples were quite high ($9.2–13 \times 10^6$/g) requiring that the number of lactic acid bacteria added also be higher than in other experiments. The CVT agar count for the control increased 10-fold during the first 2 days at 5°C while those for the samples containing lactobacilli or pediococci increased little or none. The counts for the latter samples increased during the period beyond day 2 but had not reached that of the control sample at day 6. To obtain ground beef containing fewer microorganisms initially, roasts at least 2 in. thick were ground in the laboratory. Cell suspensions of *L. bulgaricus* NCS1, *L. lactis* BYL1 and *P. cerevisiae* (5×10^8/g) were all inhibitory to the psychrotrophic bacteria in this meat (Table 3). The initial count was much lower than in the preliminary experiments. *L. bulgaricus* NCS1 and *L. lactis* BYL1 were more effective in controlling growth of the psychrotrophs than was *P. cerevisiae*, although in its presence the CVT count after 3 days was only 0.1 that in the control sample.

Results from experiment in which catalase was added to autoclaved 10% NFMS inoculated with psychrotroph MC-60N and *L. bulgaricus* NCS1 indicated that hydrogen peroxide produced by the lactobacilli was involved in the antagonism (Table 4). In this experiment all samples were incubated at 5°C with continuous agitation. Psychrotroph MC-60N was enumerated on PCA. Less inhibition was evident in the sample containing *L. bulgaricus* NCS1 with active catalase than in the one with the inactive enzyme. The PCA count for the sample containing the lactobacilli and inactive catalase decreased during the first 3 days and then increased during the last 2 days.

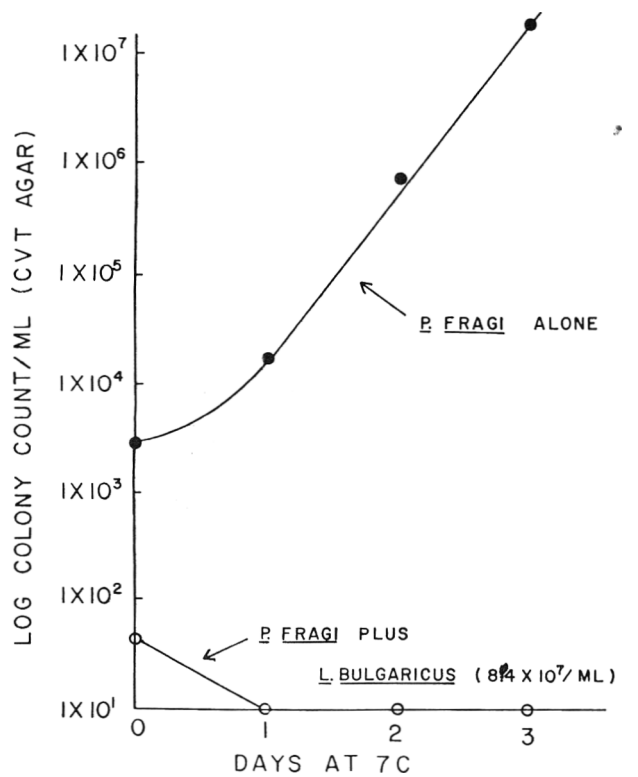


Fig. 1—Effect of L. bulgaricus NCS1 on growth of Pseudomonas fragi in autoclaved 10% NFMS at 7°C.

Table 1—Effect of cells of L. bulgaricus on growth of psychrotroph MC-60N in autoclaved 10% NFMS at 5°C

Days at 5°C	Control	L. bulgaricus NCS1 1×10^8 [a]	L. bulgaricus NCS1 1×10^7 [a]
	(Colony counts/ml on PCA)		
0	5.8×10^5	7.3×10^2	9.5×10^2
10	6.5×10^6	2.0×10^1	8.2×10^2
	(pH Values)		
0	6.7	6.7	6.7
10	6.5	6.0	6.5

[a] Number of L. bulgaricus NCS1 added per ml

Table 2—Effect of L. bulgaricus and P. cerevisiae on growth of psychrotrophic bacteria in ground beef[a] at 5°C

Days at 5°C	Control	L. bulgaricus HWD (2.7×10^9)[b]	L. bulgaricus NCS1 (2.2×10^9)[b]	P. cerevisiae (4.3×10^9)[b]
0	1.3×10^7 [c]	1.0×10^7	9.6×10^6	9.2×10^6
2	1.4×10^8	1.0×10^7	8.3×10^6	9.7×10^6
6	1.6×10^{10}	4.6×10^8	3.7×10^9	1.5×10^3

[a] Ground beef purchased from supermarket
[b] Colony forming units of starter culture bacteria added per gram of meat
[c] Colony forming units on CVT agar

Table 3—Effect of cells of L. bulgaricus, L. lactis and P. cerevisiae on growth of psychrotrophic bacteria in ground beef[a] at 5°C

Days at 5°C	Control	L. bulgaricus[b] NCS1	L. lactis[b] BYL1	P. cerevisiae[b]
0	6.7×10^2 [c]	8.3×10^2	8.5×10^2	8.4×10^2
3	1.5×10^5	5.3×10^3	4.7×10^3	1.5×10^4

[a] Ground beef prepared in laboratory
[b] Approximately 5×10^8 cells added per gram
[c] Colony counts per g on CVT agar

In this sample the final count was more than 100 times lower than that of the control and the sample containing active catalase.

A comparison of the ability of several cultures of lactobacilli and *P. cerevisiae* to produce hydrogen peroxide is shown in Table 5. In most cases, more peroxide was detected in the broth cell suspensions than in the milk. *L. lactis* BYL1 produced more H_2O_2 than any of the other cultures tested. Essentially no H_2O_2 was detected in the *P. cerevisiae* samples. Similar results were observed in replicate experiments.

DISCUSSION

THE APPARENT INACTIVATION of *P. fragi* and psychrotroph MC-60N in milk which occurred when *L. bulgaricus* was added suggested the rapid production of bactericidal substances by the lactobacilli. Dahiya and Speck (1968) showed that *L. lactis* cultures produce sufficient hydrogen peroxide to inhibit the growth of *Staphylococcus aureus* at 35°C. Although they indicated that maximum H_2O_2 was accumulated in cell suspensions of the lactobacilli at 5°C, the effects of the lactobacilli on growth of psychrotrophic bacteria at 5°C were not studied. Premi and Bottazzi (1972) have also shown that various species of lactobacilli produce H_2O_2 in broth and in milk at 5°C. Price and Lee (1970) reported that lactobacilli isolated from oysters produced sufficient H_2O_2 to inhibit *Pseudomonas* species. The antagonism apparently occurred at temperatures as low as 7°C. However, no experiments were conducted to determine if the addition of lactobacilli to refrigerated foods would retard the growth of the *Pseudomonas* species. Results from the present study show that hydrogen peroxide appears to be involved in causing inhibition of psychrotrophic bacteria (at 5–7°C) by the lactobacilli; other factors appear to be responsible for the inhibition by *P. cerevisiae*. Additional studies are underway to elucidate factors associated with the inhibitory actions produced by *P. cerevisiae*.

Most work concerning the antagonistic actions of starter culture bacteria toward undesirable microorganisms in foods has been done with actively growing cultures. The use of such methods in controlling undesirable microorganisms in many foods would not be acceptable due to the increased acidity produced by the growing cultures. On the other hand, the antagonistic actions reported in the present paper obtained using nongrowing cells of lactic acid bacteria were achieved with little or no alteration of the acidity of the food products. This suggests the possibility of using of starter culture bacteria as "biological control agents" in a wider variety of refrigerated foods. Psychrotrophic microorganisms pose a real threat to the preservation of nonsterile refrigerated foods. A need exists for GRAS (generally regarded as safe) type agents which can be added to foods for controlling these microorganisms. The incorporation of cells of starter culture bacteria into nonfermented refrigerated foods provides a possibility for meeting this need. Frozen concentrated starter cultures have been developed in recent years and are currently being used routinely in the manufacture of many cultured foods. The availability of these cultures provides the food processor with a source of highly concentrated suspensions of starter culture bacteria that could be added to nonfermented refrigerated foods to aid in the control of psychrotrophic microorganisms.

The numbers of lactic acid bacteria added to the foods in this study were high and at present would be rather costly. However, through the proper selection of cultures and more complete studies concerning the mechanism of the inhibition it should be possible to maximize the inhibition and thus required fewer cells.

Table 4—Effect of catalase on inhibition[a] of psychrotroph MC-60N by L. bulgaricus NCS1

Days at 5°C	Control	L. bulgaricus NCS1[b] Active catalase[c]	L. bulgaricus NCS1[b] Inactive catalase[c]
0	5.8×10^2	4.2×10^2	4.3×10^2
3	1.4×10^4	9.3×10^3	1.4×10^2
5	2.0×10^5	1.1×10^5	1.0×10^3

[a] In autoclaved 10% NFMS at 5°C and with continuous agitation (150 rpm)
[b] 2.5×10^7/ml
[c] 1 mg (3000 units) catalase/100 ml milk

Table 5—Peroxide production[a] by lactobacilli and pediococci at 5°C

Organism	H_2O_2 (OD at 400 nm)[b] 10% NFMS[c]	Broth[d]
L. bulgaricus NCS1	0.017	0.791
L. bulgaricus HWD	0.045	0.732
L. lactis BYL1	0.243	>2.000
P. cerevisiae	0.007	0.007

[a] With continuous agitation; 22 hr incubation
[b] The higher the optical density, the more peroxide is present
[c] Nonfat milk solids; autoclaved (15 min @121°C)
[d] 0.25% beef extract + 0.1% glucose; autoclaved (15 min @121°C)

REFERENCES

Adams, D.M., Barach, J.T. and Speck, M.L. 1974. Heat-resistant proteases produced in milk by psychrotrophic bacteria of dairy origin. J. Dairy Sci. 57: 592.

APHA. 1972. "Standard Methods for the Examination of Dairy Products," 13th ed. American Public Health Association, New York.

Dahiya, R.S. and Speck, M.L. 1968. Hydrogen peroxide formation by lactobacilli and its effect on Staphylococcus aureus. J. Dairy Sci. 51: 1568.

Daly, C., Sandine, W.E. and Elliker, P.R. 1972. Interactions of food starter cultures and food-borne pathogens: Streptococcus diacetilactis versus food pathogens. J. Milk Food Technol. 35: 349.

Gilliland, S.E. 1969. Enzymatic determination of residual hydrogen peroxide in milk. J. Dairy Sci. 52: 321.

Gilliland, S.E. and Speck, M.L. 1972. Interactions of food starter cultures and food-borne pathogens: lactic streptococci versus staphylococci and salmonellae. J. Milk Food Technol. 35: 307.

Goel, M.C., Kulshrestha, D.C., Marth, E.H., Francis, D.W., Bradshaw, J.G. and Read, R.B. Jr. 1971. Fate of coliforms in yogurt, buttermilk, sour cream and cottage cheese during refrigerated storage. J. Milk Food Technol. 34: 54.

Goepfert, J.M. and Chung, K.C. 1970. Behavior of Salmonella during the manufacture and storage of a fermented sausage product. J. Milk Food Technol. 33: 185.

Hurst, A. 1973. Microbial antagonisms in foods. Can. Inst. Food Sci. Technol. J. 6: 80.

Mather, D.W. and Babel, F.J. 1959. Inhibition of certain types of bacterial spoilage in creamed cottage cheese by the use of a creaming mixture prepared with Streptococcus citrovorus. J. Dairy Sci. 42: 1917.

Olson, H.C. 1963. Selective plating technique for detecting contamination is pasteurized milk. J. Dairy Sci. 46: 362.

Premi, L. and Bottazzi, V. 1972. Hydrogen peroxide formation and hydrogen peroxide splitting activity in lactic acid bacteria. Milchwissenschaft 27: 762.

Price, R.J. and Lee, J.S. 1970. Inhibition of Pseudomonas species by hydrogen peroxide producing lactobacilli. J. Milk Food Technol. 33: 13.

Reddy, S.G., Henrickson, R.L. and Olson, H.C. 1970. The influence of lactic cultures on ground beef quality. J. Food Sci. 35: 787.

Sorrells, K.M. and Speck, M.L. 1970. Inhibition of Salmonella gallinarum by culture filtrates of Leuconostoc citrovorum. J. Dairy Sci. 52: 239.

Speck, M.L. 1972. Control of food-borne pathogens by starter cultures. J. Dairy Sci. 55: 1019.

Ms received 2/11/75; revised 5/12/75; accepted 5/20/75.

Paper Number 4599 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, N.C.

P. F. GREENFIELD and R. L. LAURENCE
Dept. of Chemical Engineering, University of Massachusetts, Amherst, MA 01002

CHARACTERIZATION OF GLUCOSE OXIDASE AND CATALASE ON INORGANIC SUPPORTS

INTRODUCTION

AN ANALYSIS of the economics of immobilized enzyme processing has indicated that relatively low activities can be tolerated on catalysts of low cost. Because of pore and film diffusional restrictions, only a fraction of the immobilized enzyme is effectively utilized and this fraction becomes smaller as the loading increases. Hence, the relationship between the support cost and the required enzyme loading necessary to offset this cost cannot be described by a one to one relationship. Typically, a 100% increase in the overall cost of immobilization requires a threefold increase in enzyme loading to offset the increase (Greenfield et al., 1974). The overall cost of immobilization (CI) is given by

CI = Effective cost of support + Effective cost of enzyme + Cost of immobilization procedure

To keep this cost as low as possible for processing applications, it is desirable to consider the possible use of inexpensive supports, relatively crude enzyme preparations, and straightforward immobilizing schemes.

Inorganic supports of silica, silica-alumina, or titania are relatively inexpensive and possess a number of operational advantages over polymeric supports. These include improved dimensional stability, lower pressure drops, and improved flow characteristics when used in a packed bed reactor. The major disadvantage is that the enzyme loadings that are achieved on inorganic supports are generally not as large as those obtained for the more expensive supports.

From a preliminary series of tests in which glucose oxidase and beef liver catalase were immobilized on a number of low cost silica-alumina and diatomaceous earth supports, Markey et al. (1975) found that nickel impregnated kieselguhr and an attapulgites support were superior. A further series of tests showed that natural kieselguhr was of comparable activity for the two enzymes, glucose oxidase and catalase. The immobilization of glucose oxidase on nickel silica-alumina has been described in detail in a previous paper (Herring et al., 1972). The reported activities that were achieved using this support were generally quite low—less than 0.1 IU/g for glucose oxidase and less than 1 IU/g for catalase. The use of the natural clays represents a significant improvement yielding immobilized glucose oxidase activities of the order of 10 IU/g and 60 IU/g for catalase; these activities were achieved on particle sizes of 250/500 microns.

The enzymes, glucose oxidase and catalase, were immobilized because they form the basis of a number of applications studies, including the removal of trace glucose or oxygen from food products and the production of gluconic acid from glucose. Glucose oxidase catalyzes the oxidation of d-glucose to d-glucono-δ-lactone while catalase assists in the decomposition of hydrogen peroxide.

$$\text{d-glucose} + O_2 \xrightarrow{\text{GOX}} \text{d-glucono-}\delta\text{-lactone} + H_2O_2$$

$$\text{d-glucono-}\delta\text{-lactone} \xrightarrow{H_2O} \text{gluconic acid}$$

$$H_2O_2 \xrightarrow{\text{CAT}} H_2O + 1/2\ O_2$$

Glucose oxidase and catalase are both obtained from the mold, Aspergillus niger, and may be purchased either in the pure form or more economically as a mixture. Another form of catalase which is less expensive than the pure fungal catalase but which has inferior stability properties in the presence of hydrogen peroxide is extracted from beef liver.

The following sections describe the immobilization and characterization of these enzymes on diatomaceous earth materials.

EXPERIMENTAL

Support preparation

The supports which were used to immobilize the various enzymes were nickel oxide coated kieselguhr, natural kieselguhr, attapulgites, and a silica alumina catalyst. The nickel kieselguhr was an hydrogenation catalyst produced by Harshaw Chemical Co., Cleveland, Ohio (Cat. No. 0704). The 5/32-in. pellets were grey extrusions containing 18% nickel oxide. Natural kieselguhr was purchased from Eagle-Picher Industries, Inc., Cincinnati, Ohio (Cat. No. MP-76) and came as particles of between 16 and 40 mesh. Attapulgites was supplied by Englehard Minerals and Chemicals Corp., Edison, N.J., in the size range 18–40 mesh. Norton Co., Ravenna, Ohio, supplied the silica alumina (SA 5103) supports in the form of 1/8 in. × 1/8 in. cylindrical pellets. Where necessary, the supports were crushed in a hammer mill, sieved into various size fractions less than 1000 microns, washed, and allowed to dry. In the case of the silica alumina support, impregnation with a nickel salt and calcination were carried out as detailed by Herring et al. (1972). Weetall (1970) and Weetall and Hersh (1970) found that nickel oxide gave improved activity with glucose oxidase immobilization.

Immobilization procedure

The following enzymes were used in the characterization of the supports.

(i) Glucose oxidase (code GOP) from Worthington Biochemical Company, Freehold, N.J., 1.1×10^5 IU/gram.
(ii) Glucose oxidase/fungal catalase mixture (code GOC) from Searle Biochemics, Arlington Heights, Ill. 3.0×10^4 IU/gram of glucose oxidase activity, 1.4×10^5 IU/gram of catalase activity.
(iii) Beef liver catalase (code CTS) from Worthington Biochemical Co., Freehold, N.J., 1.05×10^5 IU/ml.
(iv) Fungal catalase from Searle Biochemics, Arlington Heights, Ill. 5.2×10^6 IU/gram.

Pure glucose oxidase was used for characterizing some of the properties of the immobilized enzyme. Its expense, however, precludes its use in large scale industrial processes and hence the less expensive glucose oxidase/catalase mixture was also investigated. The presence of catalase keeps the hydrogen peroxide concentration low and improves the stability of the glucose oxidase (Altomare et al., 1974a, b; Greenfield et al., 1975).

Three immobilization techniques were used—the isothiocyanate method, the glutaraldehyde method, and direct adsorption. In all cases, the support was pretreated with γ-aminopropyltriethoxy-silane (γ-APTES) from Union Carbide Chemicals, West Virginia. The support was refluxed for 24 hr with a 10% mixture of silane and toluene (5 ml solution/gram support), washed with toluene and acetone, and allowed to dry.

For coupling by the isothiocyanate method, the dry silanized support was refluxed for a further 24 hr in a 10% solution of thiophosgene in chloroform (5 ml solution/gram support). The thiophosgene was purchased from Apache Chemicals, Rockford, Ill. After refluxing, the support was washed thoroughly with chloroform, acetone, and 0.1M sodium borate-boric acid buffer solutions in turn. It was then ready for immobilization.

For the glutaraldehyde technique, the dry silanized support was

reacted for 90 min with a 2.5% solution of glutaraldehyde in distilled water (10 ml solution/gram support). The glutaraldehyde was purchased as a 50% w/w solution, biological grade, from Fisher Scientific Co., New Jersey. The solution was then decanted and the support washed thoroughly with distilled water and stored in buffer prior to immobilization.

When the enzyme was to be directly adsorbed onto the support, the silanized support was washed once with 0.1M sodium borate-boric acid buffer (pH 7.5) and stored.

Immobilization of glucose oxidase and/or catalase was carried out by dissolving the required amount of enzyme in pH 7.5 sodium borate-boric acid buffer (2 ml solution/gram support). For isothiocyanate coupling, the immobilization was carried out for 12 hr at 25°C for glucose oxidase, and for 12 hr at 5°C for catalase and for the glucose oxidase/catalase mixture. Glutaraldehyde and adsorptive coupling were allowed to proceed for 90 min at room temperature followed by 12 hr at 5°C.

Finally, the coupled enzyme was washed thoroughly and stored in buffer. For glucose oxidase, pH 5.5 citrate-phosphate buffer was used, while for catalase, pH 7 citrate-phosphate buffer was used.

Assay procedure

The activity of all enzymes was measured using a Yellow Springs Instrument Co. oxygen meter to measure the rate of oxygen uptake for glucose oxidase and the rate of oxygen production for catalase. Into a sample vial was placed 3 ml of the appropriate buffer and approximately 20 mg of immobilized enzyme. The water bath was adjusted to the appropriate temperature, normally 25°C. For glucose oxidase, the buffer solution was saturated with air yielding an oxygen concentration of 0.25 mM, while for catalase the oxygen level was reduced to zero by sparging with nitrogen. The oxygen probe was then inserted into place and the respective substrates injected into the sample. The oxygen level was monitored by a chart recorder. For glucose oxidase assays the resulting glucose concentration was 10 mM, while the hydrogen peroxide concentration for catalase assays was 0.6 mM. The initial rate of oxygen uptake for glucose oxidase or rate of oxygen production for catalase, expressed in IU/gram (i.e., micromoles of substrate consumed/min g support) was used as a measure of the immobilized enzyme activity.

When both glucose oxidase and catalase were immobilized on the same support, the catalase assay was carried out as previously described. To assay for glucose oxidase activity, however, sufficient catalase activity was introduced into the buffer solution to ensure the complete breakdown of any H_2O_2 that escapes from the particles. In this way one-half a mole of oxygen is consumed per mole of glucose which reacts.

All enzymes assayed by the YSI oxygen monitor were washed thoroughly for a number of hours with citrate-phosphate buffer solution to ensure that all soluble enzyme was removed. The immobilized enzymes were washed either in a packed bed flow reactor or in an agitated batch system with frequent changes of buffer.

Replicate assays were carried out for each test and the results averaged. The scatter between replicates was found to be small.

Effect of support

Glucose oxidase (GOP) was immobilized by the glutaraldehyde method on the 250/500 micron particle size range of each support. The activity of glucose oxidase in the immobilizing solution was 110 units/ml. The assay of immobilized glucose oxidase activity was carried out in 0.1M citrate-phosphate buffer at a pH of 5.5.

Beef liver catalase was immobilized also by the glutaraldehyde method on equivalently sized particles. The catalase activity in the immobilizing solution was 16,000 IU/ml. The assay of catalase activity was carried out in 0.1M citrate-phosphate buffer at a pH of 7.

Glucose oxidase-catalase mixture (GOC) was immobilized on the 841/1000 micron particle size range of natural kieselguhr and attapulgites by the glutaraldehyde procedure. The concentration of glucose oxidase in the immobilizing solution yielded an enzyme activity of 250 IU/ml while the catalase concentration was 14,000 IU/ml. All assays were carried out as previously described.

Effect of immobilization method

Glucose oxidase (GOP) and beef liver catalase were immobilized onto separate samples of attapulgites (841/1000 microns) and onto nickel-kieselguhr (420/595 microns) by the isothiocyanate method, the glutaraldehyde method, and by direct adsorption. The immobilized enzymes were assayed after thorough washing and prolonged soaking in buffer.

Glucose oxidase-catalase mixture (GOC) was immobilized on attapulgites and on natural kieselguhr (841/1000 microns) by the glutaraldehyde method and by direct adsorption. The particles were washed by placing them in a flow reactor and allowing 2M KCl to flow over them for a number of hours (Altomare et al., 1974a). Activity was measured regularly by determining the conversion of oxygen obtained when a buffered dextrose solution at pH 5.5 was interchanged with the KCl solution.

Effect of concentration of immobilizing solution

Glucose oxidase (GOP) was immobilized on the 420/595 micron size range of natural kieselguhr, nickel kieselguhr, and silica alumina at varying enzyme concentrations. The activity of glucose oxidase in the immobilizing solution was varied from 1–900 IU/ml. Assays were carried out as previously described.

Effect of operating pH on immobilized enzyme activity

Glucose oxidase (GOP) and beef liver catalase were immobilized onto separate samples of nickel kieselguhr (250/495 microns) by the isothiocyanate method. Glucose oxidase/catalase mixture (GOC) was immobilized on natural kieselguhr (841/1000 microns) and fungal catalase on attapulgites (841/1000 microns) by the glutaraldehyde method.

The effect of pH on the resultant activity of the immobilized enzymes was determined by assaying the activity at pH values ranging from 2–9. Solutions of 0.1M citric acid and 0.1M disodium phosphate were mixed to yield the various pH values.

Effect of operating temperature

The effect of temperature on immobilized glucose oxidase (GOP) and beef liver catalase was determined by immobilizing the respective enzymes on nickel kieselguhr (420/595 microns) by the isothiocyanate method and measuring the initial activities at temperatures of 2°C, 15°C, 25°C and 35°C. The temperature dependence of fungal catalase has been determined previously (Altomare et al., 1974a, b).

Storage stability of immobilized enzymes

Storage stability tests were carried out for glucose oxidase (GOP) and beef liver catalase immobilized by the isothiocyanate method on nickel kieselguhr (420/595 microns). After immobilization and washing, the enzymes were stored at temperatures of 5°C, 25°C, and 35°C in pH 5.5 and pH 7 citrate-phosphate buffers respectively. At specified intervals, the enzymes were removed and assayed at a temperature of 25°C in the respective buffer solutions.

RESULTS & DISCUSSION

THE PROPERTIES of immobilized glucose oxidase and catalase that have been measured were chosen because of their relation to process economics. Since low activity is the limiting factor in the case of inexpensive inorganic supports, it is desirable to know of possible improvements in technique which bring about increased activity in the immobilized enzyme without destroying the cost advantages.

The activities achieved by immobilizing the enzymes on the various supports are reported in Table 1. As a reference, the

Table 1–Immobilized enzyme activities achieved on inorganic supports

Support	Immobilized glucose oxidase activity IU/gram	Immobilized catalase activity IU/gram
Ni-Kieselguhr	11[a]	45[b]
Kieselguhr	18[c]	60[b]
	16[a]	32[a]
Attapulgites	16[c]	90[b]
	12[a]	53[a]
Silica-alumina	0.04	0.6
Porous glass (Herring et al., 1972)	0.10	1.6

[a] GOC
[b] Beef liver
[c] GOP

results which were obtained by Herring et al. (1972) using porous glass and the isothiocyanate coupling method are also reported. The superiority of the natural clay supports—kieselguhr and attapulgites—is noticeable. Although the presence of a nickel oxide coating improves the activity when using the silica alumina supports (Herring, 1972), there is no advantage when kieselguhr is used. The relatively high activities achieved using the mixed glucose oxidase-catalase suggest that this is a particularly suitable enzyme for industrial processing.

The effect of immobilization method on resultant immobilized enzyme activity is shown in Table 2. It is apparent that there is negligible effect as far as the two enzymes, glucose oxidase and catalase, are concerned when the glutaraldehyde method is used compared to isothiocyanate coupling. For cost and safety reasons, therefore, the glutaraldehyde coupling procedure is being used in the applications studies. Adsorption leads to activities which are of the order of 15% of the covalently coupled enzyme in the case of glucose oxidase and up to 50% in the case of catalase for nickel-kieselguhr. In the case of natural kieselguhr and attapulgites, the initial activities that were achieved by adsorption in the flow studies were significantly higher, this being due to the much higher internal surface area, and to the fact that the enzymes were not washed as thoroughly as in the batch tests before being placed in the flow reactor. Attapulgites has an internal surface area of approximately 100 m^2/g while nickel kieselguhr has an area of 4 m^2/g. Figure 1 shows, however, that this activity is lost quite readily under flow conditions especially from the attapulgites which has a relatively open pore structure. Although adsorption is the simplest and least expensive means of attaching an enzyme to a support, and for that reason is industrially attractive, with inorganic supports such as natural clays the use of glutaraldehyde coupling appears to give superior performance.

Another operating parameter is the optimum enzyme concentration to be used in the immobilizing solution. Figure 2 shows the effect of varying the glucose oxidase concentration in the immobilizing solution on the resultant activity. It is apparent that a point is reached where increasing concentration achieves a relatively small effect. This is a function of the number of available binding sites and the diffusional restrictions which exist because of the geometry of the support and the size of the enzyme molecule. Although the efficiency of immobilization for inorganic supports is relatively low, this can be improved somewhat by reuse of the immobilizing solution for successive immobilizations.

The effect of substrate pH on immobilized glucose oxidase is shown in Figure 3 and for immobilized catalase in Figure 4. For immobilized glucose oxidase there is relatively little change from the soluble form except that the bell shaped

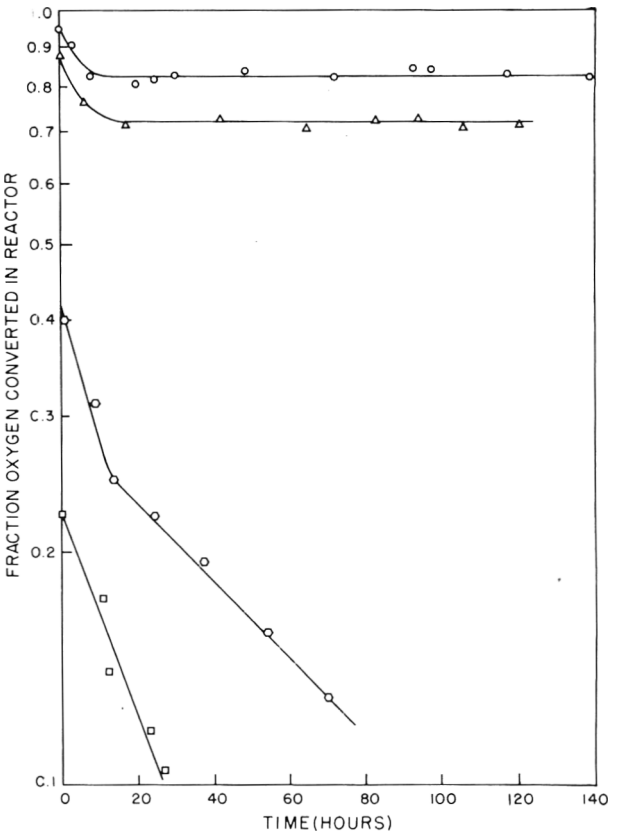


Fig. 1—Comparison of direct adsorption to glutaraldehyde coupling for glucose oxidase/catalase immobilized on attapulgites and natural kieselguhr. Samples were washed in flow reactor with 2M KCl. [△ attapulgites, glutaraldehyde coupling; ○ kieselguhr, glutaraldehyde coupling; ○ attapulgites, adsorption; □ kieselguhr, adsorption]

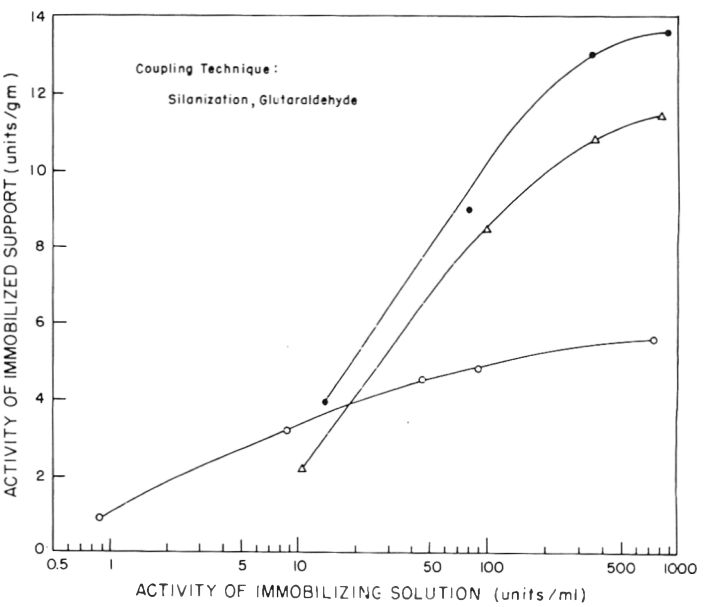


Fig. 2—Effect of glucose oxidase concentration in immobilizing solution on initial immobilized glucose oxidase activity. [● kieselguhr; △ nickel kieselguhr; ○ silica-alumina]

Table 2—Immobilized enzyme activities achieved with different immobilization techniques

Support	Immobilization method	Glucose oxidase activity IU/g	Catalase activity IU/g
Ni-Kieselguhr (420/595μ)	Glutaraldehyde	14	41
	Isothiocyanate	12	36
	Adsorption	1.8	15
Attapulgites (841/100μ)	Glutaraldehyde	15	45
	Isothiocyanate	12	42
	Adsorption	3	25

curve is widened, as would be expected in the presence of the diffusional restrictions which exist for the immobilized enzyme. The similarity in shape of the curves for the soluble and immobilized forms of both enzymes and the fact that there is negligible displacment of the pH optimum suggest that there is little charge interaction between the surface of the support and the microenvironment of the enzyme. The pH profile of the fungal catalase suggests that it is to be preferred to the beef liver form in cases where the pH drops into the acid region.

Temperature effects on the initial activities of the immobilized enzymes are shown in Figure 5. The effect of temperature on the activity of fungal catalase was reported by Altomare et al. (1974). The apparent activation energies for glucose oxidase and for beef liver catalase are respectively 6.2 kcal/mole and 11.8 kcal/mole. These values compare with 4.7 kcal/mole found for immobilized fungal catalase (Altomare et al., 1974b). It should be recognized that such values are diffusion limited and hence are a function of the temperature dependence of the diffusivity (Smith, 1970).

It is noticeable, however, that the storage stability of these immobilized enzymes is adversely affected by the higher storage temperatures (Fig. 6, 7). The kieselguhr support is slightly superior to the glass supports which were used in previous experiments (Herring et al., 1972). It should be noted that immobilized catalase and glucose oxidase undergo substrate and product inactivation respectively by hydrogen peroxide. This has been investigated in some depth and the results reported in a number of publications (Altomare et al., 1974a, b; Greenfield et al., 1975); the conclusion is that industrial performance of these immobilized enzymes will be determined

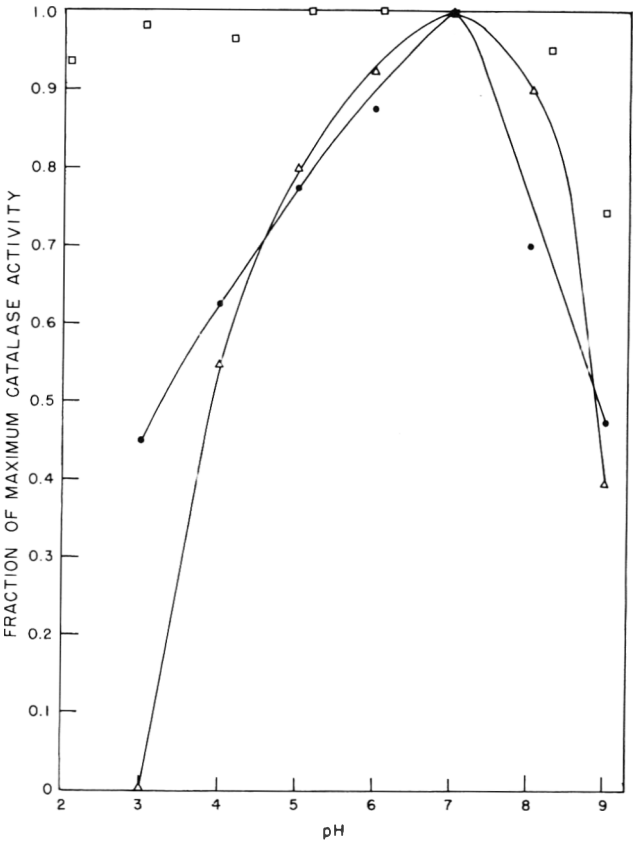


Fig. 4–Effect of substrate pH on immobilized catalase activity. [△ soluble beef liver catalase; ● immobilized beef liver catalase; □ immobilized fungal catalase]

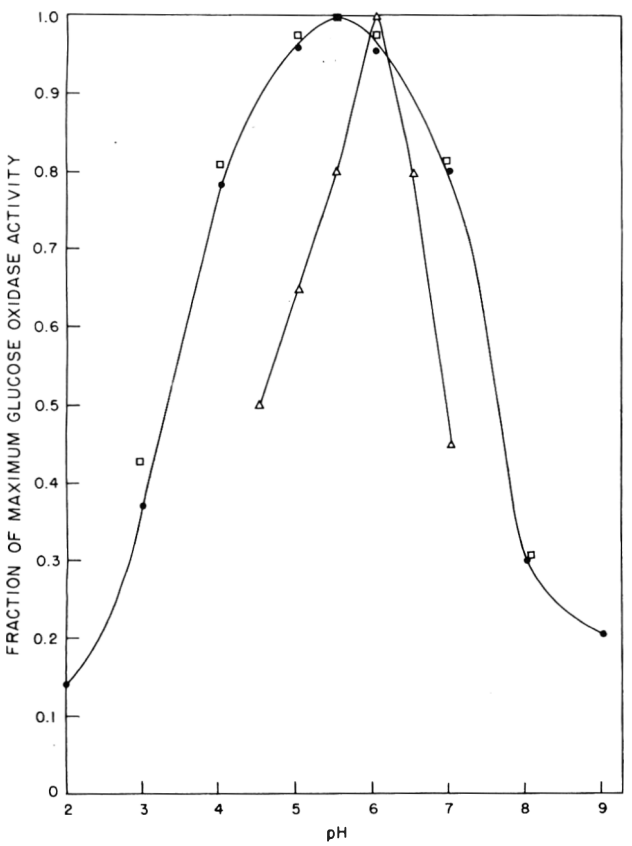


Fig. 3–Effect of substrate pH on immobilized glucose oxidase activity. [△ soluble glucose oxidase (GOP); ● immobilized glucose oxidase (GOP); □ immobilized glucose oxidase (GOC)]

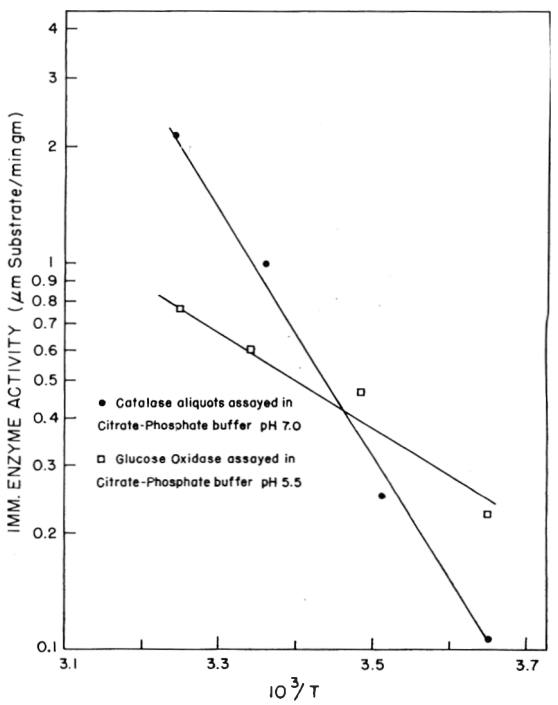


Fig. 5–Effect of operating temperature on initial immobilized enzyme activity. [□ immobilized glucose oxidase (GOP); ● immobilized beef liver catalase]

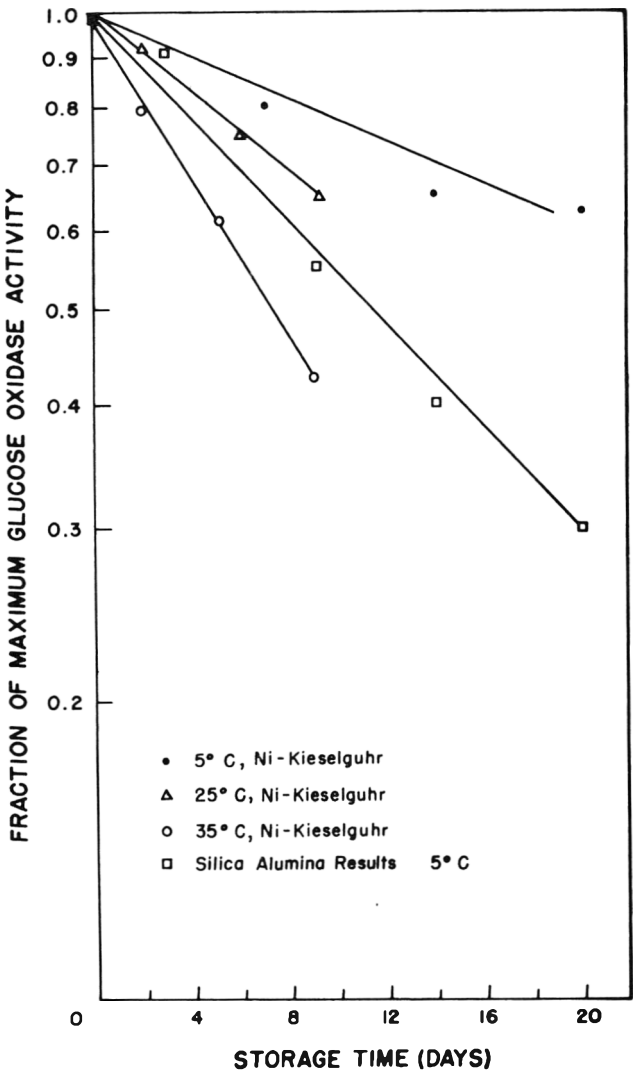


Fig. 6—Storage stability tests for immobilized glucose oxidase.

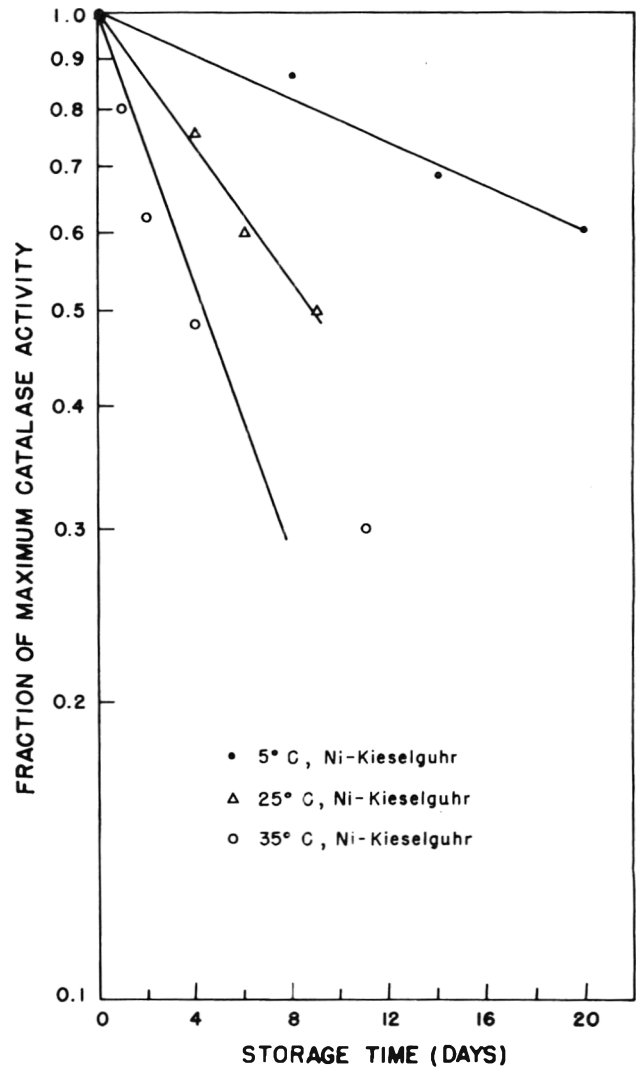


Fig. 7—Storage stability tests for immobilized beef liver catalase.

from such operational stability studies rather than from storage tests.

It was found by the above authors that fungal catalase is markedly superior to beef liver catalase from the aspect of stability to hydrogen peroxide. With the improved performance at low pH values, it appears that fungal catalase is to be preferred from a processing point of view. Likewise, it was found that extended use of immobilized glucose oxidase could only be achieved if catalase was immobilized simultaneously to decompose the hydrogen peroxide withir. the particles. The fact that a glucose oxidase-fungal catalase mixture can be purchased at a lower cost than either of the pure enzymes and can be immobilized by a simple technique to inexpensive supports suggests that this enzyme-support combination might prove viable in applications studies.

REFERENCES

Altomare, R., Kohler, J., Greenfield, P.F. and Kittrell, J.R. 1974a. Deactivation of immobilized beef liver catalase by hydrogen peroxide. Biotechnol. Bioeng. 16: 1659.

Altomare, R., Greenfield, P.F. and Kittrell, J.R. 1974b. Inactivation of immobilized fungal catalase by hydrogen peroxide. Biotechnol. Bioeng. 16: 1675.

Greenfield, P.F., Kittrell, J.R. and Laurence, R.L. 1974. Immobilized enzymes on silica-alumina supports. Presented at A.C.S. meeting, Atlantic City, September.

Greenfield, P.F., Kittrell, J.R. and Laurence, R.L. 1975. Inactivation of immobilized glucose oxidase by hydrogen peroxide. Anal. Biochem. 65: 109.

Herring, W.M. 1972. Immobilization of enzymes onto insoluble supports. M.Sc. thesis, Univ. of Mass., Amherst.

Herring, W.M., Laurence, R.L. and Kittrell, J.R. 1972. Immobilization of glucose oxidase on nickel-silica alumina. Biotechnol. Bioeng. 14: 1975.

Markey, P.E., Greenfield, P.F. and Kittrell, J.R. 1975. Studies of immobilized enzymes on silica-alumina supports. Biotechnol. Bioeng. 17: 285.

Messing, R.A. 1974. Simultaneously immobilized glucose oxidase and catalase in controlled-pore titania. Biotechnol. Bioeng. 16: 897.

Smith, J.M. 1970. "Chemical Engineering Kinetics." McGraw-Hill Book Co., New York.

Weetall, H.H. 1970. Storage stability of water-insoluble enzymes covalently coupled to inorganic carriers. Biochim. Biophys. Acta 212: 1.

Weetall, H.H. and Hersh, H.H. 1970. Preparation and characterization of glucose oxidase covalently linked to nickel oxide. Biochim. Biophys. Acta 206: 54.

Ms received 3/6/75; revised 5/14/75; accepted 5/20/75.

The investigation was supported by Grant GI 39476 RANN, National Science Foundation. Paul Greenfield was supported by a postdoctoral fellowship from CSIRO, Aust. The investigators acknowledge Peter Markey for carrying out the flow reactor experiments.

O. G. VITZTHUM, P. WERKHOFF and P. HUBERT
Research Dept., Hag Aktiengesellschaft Bremen, Hagstrasse, 28 Bremen, Western Germany

VOLATILE COMPONENTS OF ROASTED COCOA: BASIC FRACTION

INTRODUCTION

THE USE OF modern analytical techniques, particularly coupled gas chromatography-mass spectrometry has revealed the complexity of aroma in heat-processed foodstuffs. Roasted cocoa beans possess a characteristic pleasant aroma, but little is known on compounds responsible for the aroma, although approximately 310 volatile compounds have been identified in roasted cocoa beans and products made therefrom (Landschreiber and Mohr, 1974). The characteristic cocoa aroma is developed in two fundamentally important stages during processing of cocoa beans. The first of these is the fermentation step in which flavor precursors are formed, since Knapp (1937) has shown that aroma precursors are not present in unfermented cocoa beans. Secondly roasting of fermented beans is essential for the development of characteristic chocolate flavor. Much work has been done on possible cocoa aroma precursors which form the characteristic aroma of chocolate while being heated (Rohan and Stewart, 1967a, b; Rohan, 1967). Previous work on cocoa flavor has aimed at isolating and identifying volatile key aroma compounds. Many of the identified constituents contribute something to cocoa flavor, but no compound could be isolated which is responsible for the typical roasted cocoa character.

Some of the aroma components of cocoa have been studied by Dietrich et al. (1964) and Rizzi (1967). Marion et al. (1967) listed 126 cocoa flavor constituents, 35 of which had not been reported before. Flament et al. (1967) identified 62 components in roasted cocoa, 42 of which were formerly unknown. Steam volatile components from cocoa nibs were examined by van Praag et al. (1968). van der Wal et al. (1971) isolated 181 compounds from solvent extracts of commercial cocoa powder, 112 of which were not previously reported to be present in cocoa flavor. These authors also attempted reconstitution of chocolate flavor by evaluating their gas chromatograms in order to estimate the proportions and amounts of the constituents involved. The resulting synthetic mixture was reminiscent of cocoa, but was easily distinguishable from natural cocoa extract. New developments in coffee and cocoa aroma research are outlined by Watanabe (1969). The effect of fermentation and roasting on certain chemical properties related to flavor in chocolate is reviewed by Keeney (1972). Furthermore, there have been a few reports on GC or combined GC-MS on the analysis of headspace samples (Bailey et al., 1962; Reymond et al., 1966; Pinto and Chichester, 1966). Recently, Reineccius et al. (1972) have pointed out factors effecting the concentration of pyrazines in cocoa beans.

The above mentioned papers report on compounds obtained from roasted cocoa by well-known isolation techniques, e.g., organic solvent extraction, high vacuum distillation, pumping off volatile compounds under high vacuum followed by isolation on cold-finger traps. Atmospheric steam distillation and vacuum steam distillation combined with solvent extraction of steam distillate or codistillation with propylene glycol or ethanol and subsequent extraction of the water-diluted distillate with pentane have also been reported. The present paper deals with the problem of separation of aroma volatiles from cocoa mass and reports on GC-MS analysis of basic cocoa components.

It is known that substances in several compressed gases which are supercritical with respect to temperature and pressure show concentrations which are much higher than could be expected from the vapor pressures of the mentioned material. This effect is growing with rising pressure due to increasing density of the gases. This effect has been studied by the Studiengesellschaft (1964) and by HAG AG (1974, a,b,c,d). The latter has applied it to the extraction of various foods, e.g., cocoa, tobacco, spices and coffee aroma oil. Peter and Wenzel (1973) have been looking for mathematical methods describing phase equilibria. The use of CO_2 has great advantages in comparison with other extraction media, since this solvent is free of interfering contaminants and has great dissolving capacity, by being variable with respect to parameters e.g., temperature and pressure. Moreover, extracts obtained by this method possess full and complete flavor showing that very volatile components are encompassed as well. This is possible because CO_2 has high vapor pressures, resulting in wide differences between boiling points of CO_2 and volatile compounds. Randall et al. (1971) and Schultz and Randall (1970) have performed extraction with liquid CO_2, e.g., CO_2 under subcritical conditions, in order to concentrate fruit essences.

This paper is concerned with the extraction of lipophilic fractions from cocoa mass with supercritical CO_2, followed by steam distillation. Substances with hydrophilic behavior e.g., sugars, amino acids and proteins are not extracted, thus avoiding artefact formation during steam distillation.

EXPERIMENTAL

Source of cocoa mass

The original material was cocoa mass manufactured from African cocoa beans (Ghana).

CO_2-extraction procedure

The apparatus used for separation of the aroma-containing cocoa butter from the cocoa mass is shown schematically in Figure 1. 750g cocoa mass are introduced into vessel A and the initially liquid extraction medium is drawn off from the tank and forced by a pump into the extraction vessel after being heated to supercritical temperatures (e.g., 50°C) until a pressure of 320 at gauge is reached. By passing the molten cocoa mass the compressed gas is charged with cocoa aroma and cocoa butter. The charged, supercritical gas phase or "solution" expands through an expansion valve into a separating vessel B, the pressure decreases from 320 to approximately 65 at gauge and a separation of cocoa aroma and butter from the gas current takes place. The reason for using this procedure depends on the variability of solvent characteristics of CO_2, which cannot solve cocoa butter and aroma under the conditions of decompression in vessel B, where CO_2 is subcritical and partially liquefied. The pure gas is drawn off from B by the pump and again forced into vessel A, initiating a second extraction step and so on. If the desired degree of extraction is reached the solvent is pumped back into the tank and the CO_2, which is part of the contents of B is blown off slowly after additional cooling of B, whereby no loss of flavor occurs because of the decreasing temperature. In this manner 147g of a slightly yellowish cocoa butter with a very intensive cocoa odor have been obtained. Experimental details concerning operating parameters were previously described by HAG AG (1974a).

Sample preparation for analysis

The CO_2-extraction product obtained was made into a slurry with distilled water. This slurry was placed into a 2-liter round bottom flask and the volatiles were separated by atmospheric steam distillation. Isolation and enrichment procedures of volatile aroma components were carried out in the same manner as described previously on coffee by Vitzthum and Werkhoff (1974a, b).

GC equipment

The method used was similar to that previously described by two of us. Investigations were carried out using a Carlo Erba GI 450 model gas chromatograph, which is specially designed for direct injection onto capillary columns (Grob and Grob, 1969a, b). The column was a 85m × 0,31 mm i.d. glass capillary column coated with polypropylene glycol (UCON HB 5100), details of which are in the legend to Figure 2.

GC-MS equipment

The GC-MS was carried out on a modified Varian MAT 111 instrument, in which the original gas chromatograph had been replaced by a homemade unit equipped with a splitless-injection unit (Grob and Grob, 1969a, b) and a high-resolution glass capillary column to analyze very dilute aroma mixtures. A 200m × 0,31 mm i.d. glass capillary column was coupled to the single-focussing mass spectrometer. Four commercial glass columns were connected by means of platinum capillaries (Neuner-Jehle et al., 1973). A 30 cm long pressure restriction platinum capillary of 0,15 mm i.d. was put at the end of the column to keep the column end near atmospheric pressure in order not to lose separation efficiency. The UCON HB 5100 column was temperature programmed from 20–180°C at 1°C per min. Helium carrier gas flow: 4 ml/min. MS-conditions: ionization voltage, 80 eV; emission current, 270 μA; ion source temperature, 300°C; ion source pressure, 3×10^{-6} torr; separator temperature, 220°C; inlet line, 230°C. The GC-MS instrument was equipped with a mass marker with upper limit m/e 999.

Synthetic routes

Many authentic samples of organic compounds were obtained from reliable commercial sources (in the case of commercially available compounds, the abbreviation c.a. is used in Table 1).

Alkyloxazoles were synthesized by procedures described in the literature or by simple modifications thereof (Vitzthum and Werkhoff, 1974a). Cyclopentapyrazines and tetrahydroquinoxalines which were also found previously in coffee aroma were prepared as reported earlier (Vitzthum and Werkhoff, 1975). Additionally, 2-ethyl-6,7-dihydro-5H-cyclopentapyrazine was obtained by condensation of ethyl glyoxal and 1,2-diaminocyclopentane in toluene and subsequent oxidation of the bicyclic intermediate with KOH in ethylene glycol at 180°C for 60 min according to Nakatani and Yanatori (1973).

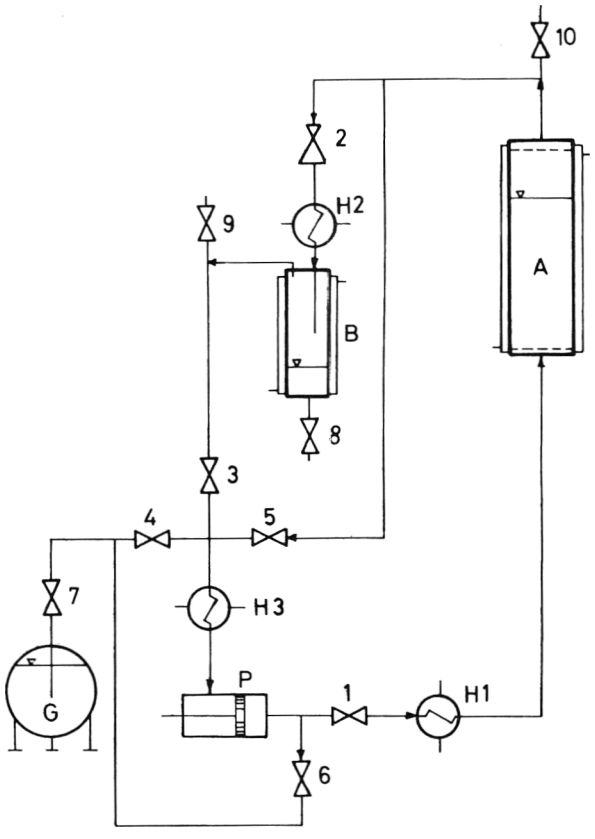


Fig. 1—Schematic of apparatus used for separation of aroma-containing cocoa butter from cocoa mass. A = pressure tube (= extraction vessel), equipped with a jacket for heating fluids, partially filled with molten cocoa mass; B = Separating vessel, equipped with a jacket for cooling or heating fluids, partially filled with a mixture of cocoa butter, containing cocoa aroma and liquid CO_2; P = Pump for liquefied gas; G = Liquid gas tank; H1–H3 = Heat exchanger; 1,3–8 = normal valves; 2 = expansion valve; and 9 + 10 = purge valves.

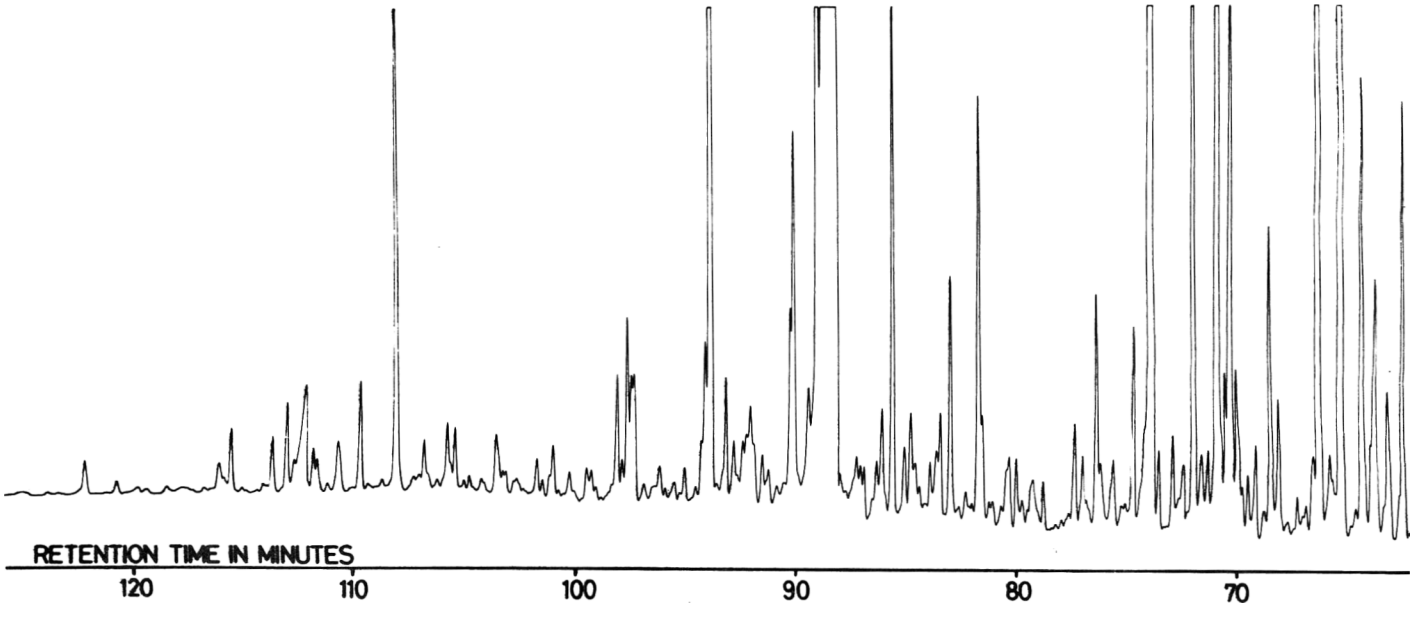


Fig. 2—Glass capillary GLC analysis of cocoa aroma volatiles obtained from cocoa mass by extraction with compressed CO_2 and subsequent steam distillation at atmospheric pressure. Direct injection of 0,5 μl of an ether solution of basic aroma constituents on a 85m X 0,31 mm i.d. column coated

RESULTS & DISCUSSION

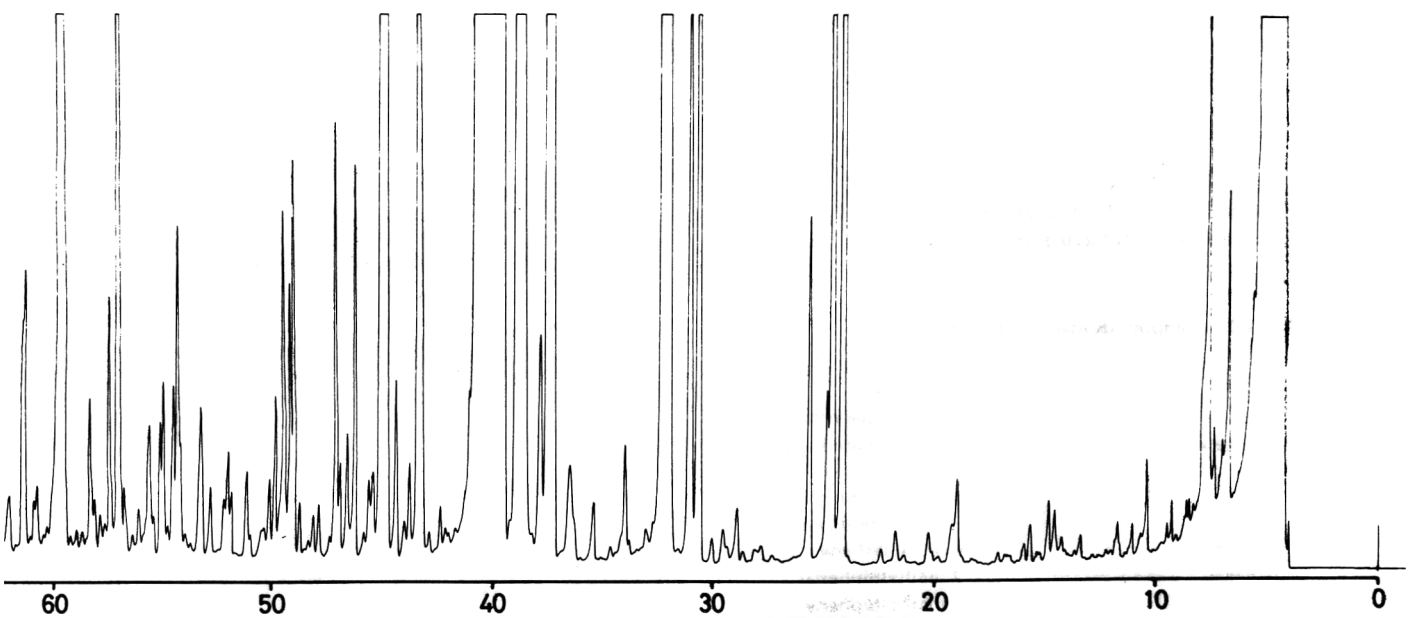
THE RESULTING CO_2-cocoa extract exhibited a full cocoa aroma and flavor. Preparation of a concentrated aroma possessing the desirable aroma and being suitable for gas chromatographic separation, represents a major problem in cocoa research (van der Wal et al., 1971). An attractive new isolation method in food odor research appears to be the extraction with compressed i.e., supercritical CO_2. As expected gas chromatographic analysis of the total aroma fraction shows that cocoa extract was an extremely complex mixture. In order to simplify GC patterns, the total extract was separated into basic and neutral fractions. This gave better GC-resolution for many of the components listed in Table 1, which are present to a large extent in traces only. A typical FID-chromatogram showing resolution of the basic cocoa components is presented in Figure 2. Glass capillary columns were preferred because of their high-resolution capability.

Table 1 lists the components characterized by the combination of glass capillary GLC and mass spectrometry. 59 nitrogen-containing compounds not previously reported as constituents of roasted cocoa have been identified. The newly reported heterocyclic compounds of the basic fraction of cocoa aroma are common to other roasted products which include coffee, peanuts, filberts, almonds, pecans, sesame seed, etc.

The identified compounds include 4 oxazoles, 34 pyrazines, 9 cycloalkapyrazines, 7 pyridines, 3 quinoxalines and quinoline. The presence of methyl o-aminobenzoate in the cocoa volatiles is also noteworthy, because this component has also been reported as a constituent of black tea (Cazenave and Horman, 1974). Certain classes of compounds, e.g., alicyclic pyrazines, oxazoles, pyridines, quinoxalines and quinoline are reported for the first time in roasted cocoa. By adding the new compounds resulting from the present investigation to those mentioned in an earlier review (Landschreiber and Mohr, 1974), it can be concluded that at least 369 volatile constituents occur in cocoa aroma. Many of the identified products are expected to be organoleptically significant, but none was isolated which could be considered to have the typical roasted cocoa character.

Some peaks in earlier GC runs were due to compounds from the Porapak-adsorption material found in blank runs with the FID. Therefore, mass spectrometry in combination with glass capillary GC was also carried out on a Porapak Q-ether-extract and led to the identification of many compounds on the basis of their mass spectra (Table 2). For that reason, Porapak Q was extracted (Soxhlet) twice with benzene and diethyl ether for 15 hr, before using it for cocoa aroma adsorption.

Removal of volatile organic compounds from adsorption material can be accomplished by means of desorption at elevated temperature (Kinlin et al., 1972; Tassan and Russell, 1974) or by solvent extraction (Roeraade and Enzell, 1972; Vitzthum and Werkhoff, 1974a, b, 1975). Since desorption at elevated temperature is more likely to induce chemical reactions, solvent extraction seems to be preferable for aroma research in order to avoid artefact formation as much as possible. Volatiles collected from cocoa sample via CO_2 extraction, with subsequent steam distillation using the Porapak method, thus excluding water soluble amino acids and carbohydrates, had a strong characteristic cocoa aroma. The authors had previously carried out studies on volatiles in roasted coffee. Qualitatively, the patterns of basic cocoa components characterized are similar to those found in roasted coffee. But in contrast to basic coffee volatiles, the basic cocoa fraction was found to contain a majority of pyrazine components. As a result of this work, 57 pyrazines were discovered in the basic cocoa fraction, 23 of which were formerly reported by other investigators and are therefore not listed in Table 1. Identification was based on mass spectra and GLC retention times. Final comparison of GLC and MS data with those of reference substances was carried out in a number of cases. Compounds were considered positively identified if their mass spectra and their retention times agreed with those of the reference compounds. Furthermore, naturally occurring compounds were identified by comparing their mass spectra with published spectra. The structure of some compounds listed in Table 1 with the annotation "tentative" could not be conclusively established due to incomplete GC-separations (mixed spectra) or lack of



with polypropylene glycol (UCON HB 5100). Carrier gas flow 2,5 ml/min hydrogen. The temperature was held for 5 min at 20° C, 8 min at 50° C, then programmed at 1,5° C/min up to 180° C and held at the upper limit. Injection port, 200° C. FID, 200° C.

Table 1—Basic cocoa volatiles identified in the present investigation (first report in roasted cocoa)

Compound	MS	GLC	Synthesis[a]	MS-reference
Oxazoles				
2,5-dimethyloxazole	positive	+	+	Vitzthum and Werkhoff (1974a)
4,5-dimethyloxazole	positive	+	+	Vitzthum and Werkhoff (1974a)
2,4,5-trimethyloxazole	positive	+	+	Vitzthum and Werkhoff (1974a)
2n-propyl-5-methyloxazole	positive	+	+	Vitzthum and Werkhoff (1974a)
Pyridines				
Pyridine	positive	+	c.a.	Cornu and Massot (1966)
2-methylpyridine	positive	+	c.a.	Cornu and Massot (1966)
3-vinylpyridine	positive			Neurath and Duenger (1969) and MSDC[b]
2-methyl-5-ethylpyridine	positive	+	c.a.	Cornu and Massot (1966)
2-acetylpyridine	positive	+	c.a.	Ferretti and Flanagan (1971) and MSDC[b]
3-phenylpyridine	positive			Neurath and Duenger (1969)
3-phenyl-methylpyridine (two isomeres)	tentative			
Cycloalkapyrazines				
6,7-dihydro-5H-cyclopentapyrazine	positive	+	+	Flament et al. (1973), Pittet et al. (1974) and Vitzthum and Werkhoff (1975)
2-ethyl-6,7-dihydro-5H-cyclopentapyrazine	positive	+	+	Pittet et al. (1974) and Vitzthum and Werkhoff (1975)
2(or 3),5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine	positive	+	+	Flament et al. (1973), Pittet et al. (1974) and Vitzthum and Werkhoff (1975)
2-methyl-6,7-dihydro-5H-cyclo-pentapyrazine	positive	+	+	Flament et al. (1973), Pittet et al. (1974) and Vitzthum and Werkhoff (1975)
2,3-dimethyl-6,7-dihydro-5H-cyclo-pentapyrazine	positive	+	+	Flament et al. (1973), Pittet et al. (1974) and Vitzthum and Werkhoff (1975)
2-methyl-3-ethyl-6,7-dihydro-5H-cyclopentapyrazine	positive	+	+	Flament et al. (1973) and Vitzthum and Werkhoff (1975)
5,6,7,8-tetrahydroquinoxaline	positive	+	+	Pittet et al. (1974) and Vitzthum and Werkhoff (1975)
2-methyl-5,6,7,8-tetrahydroquinoxa-line	positive	+	+	Pittet et al. (1974) and Vitzthum and Werkhoff (1975)
5-methyl-5,6,7,8-tetrahydroquinoxa-line	positive			Pittet et al. (1974) and Vitzthum and Werkhoff (1975)
Pyrazines				
2-methyl-3-ethylpyrazine	positive	+	c.a.	Kinlin et al. (1972)
2-methyl-6-isopropylpyrazine	positive			Flament (1974)
n-propylpyrazine	positive			Kinlin et al. (1972)
2-methyl-3-isopropylpyrazine	positive			Friedel et al. (1971)
2,3-dimethyl-5-ethylpyrazine	positive			Demole (1972)
2-methyl-5-vinylpyrazine	positive			Kinlin et al. (1972)
2-methyl-5n-propylpyrazine	positive			Demole (1972)
2,5-dimethyl-3-isopropylpyrazine	positive			Flament (1974)

authentic material. Moreover, insufficient material for mass spectral analysis rendered identification more difficult depending on the fragmentation behavior and strength of background.

Table 2—Compounds identified from Porapak Q

toluene	n-heptadecane
ethyl benzene	naphthalene
styrene	1-methyl naphthalene
2,6-dimethylstyrene	2-methyl naphthalene
o-xylene	diphenyl
m-xylene	dimethylnaphthalene
p-xylene	diphenylmethane
1,2-diethylbenzene	diphenylethane
1,3-diethylbenzene	2-methylbiphenyl
1,4-diethylbenzene	3-methylbiphenyl
n-tetradecane	1,2-diphenylethane
n-hexadecane	1,1-diphenyl ethylene

Mass spectral evidence was obtained for a large number of higher molecular weight polysubstituted alkylpyrazines (M^+ 164, 178, 192 and mol wt greater than 192), which were very difficult to characterize, because there are a great number of possible structures all with very similar mass spectra and GLC retention times. This problem has made structure assignment impossible, because reference compounds were not yet available.

The pathways by which alkylpyrazines, acylpyrazines, furylpyrazines, alicyclic pyrazines and oxazoles are formed can be readily explained on the basis of known precursors. Suggested pathways for the formation of some of the newly identified components were discussed in previous papers on roasted foodstuffs such as peanuts (Walradt et al., 1971), roasted sesame seed (Manley et al., 1974) and roasted coffee (Vitzthum and Werkhoff, 1974b; 1975). A general review covering the hypotheses and possible mechanisms for the formation of pyrazines in foods has been published by Maga and Sizer (1973).

Table 1 (continued)–Basic cocoa volatiles identified in the present investigation (first report in roasted cocoa)

Compound	MS	GLC	Synthesis[a]	MS-reference
2-methyl-5-isobutylpyrazine	positive			Demole (1972)
2-methyl-6-isobutylpyrazine	positive			Demole (1972)
2,6-dimethyl-3-isopropylpyrazine	positive			Flament (1974)
2-ethyl-5-isopropylpyrazine	positive			Demole (1972)
2,5-diethyl-3-methylpyrazine	positive			Friedel et al. (1971), Ferretti et al. (1971) and Kinlir et al. (1972)
2,6-diethyl-3-methylpyrazine	positive			Kinlir et al. (1972)
2,3-diethyl-5-methylpyrazine	positive			Kinlir et al. (1972)
Isopropenylpyrazine	positive			Walradt et al. (1971)
2-ethyl-6n-propylpyrazine	positive			Demole (1972)
2,5-dimethyl-3n-butylpyrazine	positive			Wheeler and Blum (1973)
Triethylpyrazine	positive			Kinlin et al. (1972)
2,6-dimethyl-3n-butylpyrazine	positive			Wheeler and Blum (1973)
2-methyl-5-acetylpyrazine	positive			Kinlin et al. (1972) and Takei et al. (1974)
2-ethyl-5-acetylpyrazine	positive			Kinlin et al. (1972) and Takei et al. (1974)
2n-pentyl-3-methylpyrazine	positive			Friedel et al. (1971)
2n-pentyl-5-methylpyrazine	positive			Friedel et al. (1971)
2-(2'-methylbutyl)-3-methyl-pyrazine	positive			Friedel et al. (1971)
2-isoamyl-3-methylpyrazine	positive			Friedel et al. (1971)
2,5-dimethyl-3n-pentylpyrazine	positive			Wheeler and Blum (1973)
2,6-dimethyl-3n-pentylpyrazine	positive			Wheeler and Blum (1973)
2,6-dimethyl-3-(2-methylbutyl)-pyrazine	positive			Wheeler and Blum (1973)
2-(2'-furyl)-pyrazine	positive			Friedel et al. (1971) and Kinlin et al. (1972)
2-methyl-5-(2'-furyl)-pyrazine	positive			Neurath and Duenger (1969) and Friedel et al. (1971)
2-methyl-6-(2'-furyl)-pyrazine	positive			Friedel et al. (1971)
2-(2'-furyl-5'-methyl)-5(6)-methyl-pyrazine	tentative			
2-(2'-furyl-3'(4'),5'-dimethyl)-5(6)-methylpyrazine	tentative			
Quinoxalines				
2-methylquinoxaline	positive	+	c.a.	Karjalainen and Krieger (1970)
2,3-dimethylquinoxaline	positive	+	c.a.	Koch and Markgraf (1970) and Karjalainen and Krieger (1970)
2,5-dimethylquinoxaline	tentative			
Miscellaneous				
Quinoline	positive	+	c.a.	Cornu and Massot (1966)
Methyl-o-aminobenzoate	positive	+	c.a.	Cazenave and Horman (1974)

[a] c.a. = commercially available
[b] MSDC = Mass Spectrometry Data Centre, AWRE, Aldermaston Reading, RG 7 4 PR, U.K.

REFERENCES

Bailey, S.D., Mitchell, D.G., Bazinet, M.L. and Weurman, C. 1962. Studies on the volatile components of different varieties of cocoa beans. J. Food Sci. 27: 165.

Cornu, A. and Massot, R. 1966. "Compilation of Mass Spectral Data." Heyden and Son, London.

Cazenave, P. and Horman, I. 1974. Sur la composition de l'arome de the noir. Helv. Chim. Acta 57: 209.

Demole, E.P. 1972. Use of aromatic ingredients in tobacco. German Patent Appl. 2,202,065.

Dietrich, P., Lederer, E., Winter, M. and Stoll, M. 1964. Flavors. 11. Cocoa flavor. Helv. Chim. Acta 47: 1581.

Ferretti, A. and Flanagan, V.P. 1971. The lactose-casein (maillard) browning system: volatile components. J. Agr. Food Chem. 19: 245.

Flament, I., Willhalm, B. and Stoll, M. 1967. Research on aromas. 16. Cocoa aroma. Helv. Chim. Acta 50: 2233.

Flament, I., Sonnay, Ph. and Ohloff, G. 1973. Pyrazines. 2. Synthesis of alkyl-5H-6,7-dihydrocyclopenta [b] pyrazines. Helv. Chim. Acta 56: 610.

Flament, I. 1974. New pyrazine compounds. German Patent Appl. 2,257,122.

Friedel, P., Krampl, V., Radford, T., Renner, J.A., Shephard, F.W. and Gianturco, M.A. 1971. Some constituents of the aroma complex of coffee. J. Agr. Food Chem. 19: 530.

Grob, K. and Grob, G. 1969a. Splitless injection on capillary columns. Part 1. The basic technique; steroid analysis as an example. J. Chromatogr. Sci. 7: 584.

Grob, K. and Grob, G. 1969b. Splitless injection on capillary columns. Part 2. Conditions and limits, practical realization. J. Chromatogr. Sci. 7: 587.

HAG AG. 1974a. Method of producing cocoa butter. Brit. Pat. 1.356.750.

HAG AG. 1974b. Method of extracting nicotine from tobacco. Brit. Pat. 1.357.645.

HAG AG. 1974c. Method of producing spice extracts with a natural composition. Brit. Pat. 1.336.511.

HAG AG. 1974d. Method of extracting coffee oil containing aroma constituents from roasted coffee. Brit. Pat. 1.336.276.

Karjalainen, A. and Krieger, H. 1970. Mass-spectrometric fragmentation of quinoxalines. Suom. Kemistilehti B 43: 273.

Keeney, P.G. 1972. Various interactions in chocolate flavor. J. Amer. Oil Chem. Soc. 49: 567.

Kinlin, T.E., Muralidhara, R., Pittet, A.O., Sanderson, A. and Walradt, J.P. 1972. Volatile components of roasted filberts. J. Agr. Food Chem. 20: 1021.

Knapp, A.W. 1937. "Cocoa Fermentation," p. 124. John Ball, Sons and Curnow Ltd., London.

Koch, Ch.W. and Markgraf, J.H. 1970. The mass spectrum of 1,2,2a,3-tetrahydrocyclobuta [b] quinoxaline. J. Heterocycl. Chem. 7: 235.

Landschreiber, E. and Mohr, W. 1974. Uber Kakaoaroma und seine Zusammensetzung. 1. Internationaler Kongress über Kakao- und Schokoladeforschung, München.
Maga, J.A. and Sizer, Ch.E. 1973. Pyrazines in foods. A review. J. Agr. Food Chem. 21: 22.
Manley, C.H., Vallon, P.P. and Erickson, R.E. 1974. Some aroma components of roasted sesame seed. J. Food Sci. 39: 73.
Marion, J.P., Müggler-Chavan, F., Viani, R., Bricout, J., Reymond, D. and Egli, R.H. 1967. Composition of cocoa aroma. Helv. Chim. Acta 50: 1509.
Nakatani, Y. and Yanatori, Y. 1973. Simple synthesis of 2,3-disubstituted pyrazines. Agr. Biol. Chem. 37: 1509.
Neuner-Jehle, N., Etzweiler, F. and Zarske, G. 1973. Platinum capillaries as interface in the direct coupling of glass capillary columns to a mass spectrometer. Chromatographia 6: 211.
Neurath, G. and Duenger, M. 1969. Isolation of weakly basic heteroaromatic compounds from tobacco smoke. Beitr. Tabakforsch. 5: 1.
Peter, S. and Wenzel, H. 1973. Calculation of chemical gas equilibria at high pressures. Chem. Ing. Tech. 45: 573.
Pinto, A. and Chichester, C.O. 1966. Changes in the content of free amino acids during roasting of cocoa beans. J. Food Sci. 31: 726.
Pittet, A.O., Muralidhara, R., Walradt, J.P. and Kinlin, T. 1974. The synthesis and properties of alkylated five- and six-membered alicyclic pyrazines. J. Agr. Food Chem. 22: 273.
Praag, M. van, Stein, H.S. and Tibbetts, M.S. 1968. Steam volatile aroma constituents of roasted cocoa beans. J. Agr. Food Chem. 16: 1005.
Randall, J.M., Schultz, W.G. and Morgan, A.I. 1971. Extraction of fruit juices and concentrated essences with liquid carbon dioxide. Confructa 16: 10.
Reineccius, G.A., Keeney, P.G. and Weissberger, W. 1972. Factors affecting the concentration of pyrazines in cocoa beans. J. Agr. Food Chem. 20: 202.
Reymond, D., Müggler-Chavan, F., Viani, R., Vuataz, L. and Egli, R.H. 1966. Gas chromatographic analysis of steam volatile aroma constituents: application to coffee, tea and cocoa aromas. J. Gas Chromatogr. 4: 28.
Rizzi, G.P. 1967. The occurrence of simple alkylpyrazines in cocoa butter. J. Agr. Food Chem. 15: 549.
Roeraade, J. and Enzell, C.R. 1972. Tobacco Chemistry. 14. Sampling, concentration, and examination of tobacco headspace vapors. J. Agr. Food Chem. 20: 1035.
Rohan, T.A. 1967. The precursors of chocolate aroma: application of gas chromatography in following formation during fermentation of cocoa beans. J. Food Sci. 32: 402.
Rohan, T.A. and Stewart, T. 1967a. The precursors of chocolate aroma: production of free amino acids during fermentation of cocoa beans. J. Food Sci. 32: 395.
Rohan, T.A. and Stewart, T. 1967b. The precursors of chocolate aroma: production of reducing sugars during fermentation of cocoa beans. J. Food Sci. 32: 399.
Schultz, W.G. and Randall, J.M. 1970. Liquid Carbon Dioxide for selective aroma extraction. Food Technology 24: 94.
Studiengesellschaft Kohle m.b.H. 1964. Separation process. Brit. Pat. 1.057.911.
Takei, Y., Shimada, K., Watanabe, S. and Yamanishi, T. 1974. Volatile components of roasted almonds: basic fraction. Agr. Biol. Chem. 38: 645.
Tassan, C.G. and Russell, G.F. 1974. Sensory and gas chromatographic profiles of coffee beverage. Headspace volatiles entrained on porous polymers. J. Food Sci. 39: 64.
Vitzthum, O.G. and Werkhoff, P. 1974a. Oxazoles and thiazoles in coffee aroma. J. Food Sci. 39: 1210.
Vitzthum, O.G. and Werkhoff, P. 1974b. Newly discovered nitrogen-containing heterocycles in coffee aroma. Z. Lebensm. Unters.-Forsch. 156: 300.
Vitzthum, O.G. and Werkhoff, P. 1975. Cycloalkapyrazines in coffee aroma. J. Agr. Food Chem. In press.
Wal, B. van der, Kettenes, D.K., Stoffelsma, J., Sipma, G. and Semper, A. Th. J. 1971. New volatile components of roasted cocoa. J. Agr. Food Chem. 19: 276.
Walradt, J.P., Pittet, A.O., Kinlin, Th.E., Muralidhara, R. and Sanderson, A. 1971. Volatile components of roasted peanuts. J. Agr. Food Chem. 19: 972.
Watanabe, S. 1969. New development in coffee and cocoa aroma research. Koryo 92: 53.
Wheeler, J.W. and Blum, M.S. 1973. Alkylpyrazine alarm pheromones in ponerine ants. Science 182: 501.
Ms received 11/12/74; revised 2/11/75; accepted 2/18/75.

The authors are greatly indebted to Dr. A. Haag, Dept. of Organic Chemistry, University of Erlangen, for discussions of mass spectra. They also thank H.R. Wienges for his skilled technical assistance, M. Möhlenbrock and W. Gutmann for obtaining GC and mass spectral data and H. Kiank and B.v.d. Kammer for preparing reference compounds.

The authors gratefully acknowledge the gifts of cocoa mass from Cacaofabriek de Zaan b.v., Holland.

SHAWKY M. DAGHER and ILYAS G. RUHAYYIM
National Council for Scientific Research, Beirut, Lebanon
and Dept. of Food Technology & Nutrition,
Faculty of Agricultural Sciences, American University of Beirut, Beirut, Lebanon

FUSEL OIL AND METHANOL CONTENT OF LEBANESE ARAK

INTRODUCTION

ARAK is an alcoholic beverage distilled from fermented grape juice and flavored with aniseed extract. It is the national alcoholic drink in Lebanon and is also consumed to a limited extent in the neighboring countries of the Middle East.

While there have been extensive studies on the composition of many alcoholic beverages, no data are available on the composition of Lebanese Arak. Grape brandy distillates which are manufactured in a similar way to Arak except for the manner in which the flavor is added, were reported by Guymon (1970) to contain 70–300 ppm (w/v) of n-propyl alcohol, 70–250 ppm (w/v) of isobutyl alcohol and 220–880 ppm (w/v) of amyl alcohol.

Askew and Lisle (1971) analyzed a number of brandy samples from various parts of the world. They found methanol to be present in the range of 20–8750 ppm (w/v), propyl alcohol 20–13320 ppm, isobutyl alcohol 10–1080 ppm and amyl alcohols in the range of 40–3000 ppm (w/v). Webb et al. (1952) reported that isoamyl alcohol made up more than 50% of grape brandy fusel oil and Connell and Strauss (1974) found that fusel oil distilled from Australian grape wines contained a large number of aliphatic esters in addition to propanol, isobutanol, active and isoamyl alcohols. Other distilled alcoholic beverages have been analyzed for their fusel oil content by Carroll (1970) and more recently by Martin and Caress (1971).

The pathway for the formation of higher aliphatic alcohols by deamination and decarboxylation of amino acids during the alcoholic fermentation process is already well established (Castor and Guymon, 1952; Guymon, 1966; Äyräpää, 1971). Using C^{14}-labelled amino acids, Spanyer and Thomas (1957) and Reazin et al. (1970, 1973) have shown that valine, leucine and isoleucine were the precursors of isobutyl, active and isoamyl alcohols, respectively.

In the present report we determine the fusel oil and methanol content of Lebanese Arak and the level of the precursor amino acids in the fermentable material.

EXPERIMENTAL

Fusel oil analysis

A Perkin Elmer-900 gas chromatograph equipped with automatic linear temperature programmer and flame ionization detector was used for the determination of higher alcohols. Chromatogram recordings were traced on a Hewlett Packard strip chart recorder. A 6 ft glass column with 2.2 mm i.d. was used. The column was packed with 80/100 mesh chromosorb W coated with 15% carbowax 20 M. Injection port temperature was 200°C and the flame ionization detector was maintained at 225°C. Oven temperature was 60°C and temperature programming at the rate of 6°C/min, was started 4 min after sample injection. Final temperature was maintained at 150°C for 8 min. Nitrogen flow rate was 50 ml/min and the hydrogen and air flow rates to the detectors were 30 and 300 ml/min respectively.

For quantitative determination of fusel oil components, known concentrations of alcohols to be measured were prepared in 40% (v/v) aqueous ethanol to span the range of concentrations expected in the samples. 1-Pentanol was used as the internal standard, since a preliminary survey showed that Arak samples were void of this alcohol. A 10% (w/v) solution of internal standard was prepared in 40% (v/v) aqueous ethanol and kept as a stock solution. This was then mixed with the standard solutions 1:100 prior to injection. The Arak samples were likewise mixed 100:1 with internal standard stock solution before direct injection for gas chromatographic analysis.

The chromatogram recordings were evaluated by measuring peak heights from the local baseline for propanol, isobutanol and amyl alcohol, with respect to the height of the internal standard peak. The recorded response versus concentration was linear for all the alcohols studied within the range of concentrations used.

Methanol determination

Methanol was determined by the AOAC method (1970). Absorbance of samples was measured in a Bausch and Lomb Spectronic 20 spectrophotometer. Efficient distillation of Arak samples before analysis was found necessary in order to separate completely the alcohol fraction from anethole which was found to interfere with color development when chromotropic acid was added.

Amino acid analysis

Samples of grape juice used for the preparation of Arak were freeze dried and assayed for their amino acid content by gas liquid chromatography according to the method of Gherke et al. (1968). Dowex 50 – X, H^+ form was used as the ion exchange resin and tranexamic acid was used as an internal standard.

Preparation of Arak

Three local varieties of *Vitis vinifera* (Mirwahi, Miksasi and Obeidi) served as the source of juice samples used in this study. These grape varieties are commonly used by commercial distillers for the manufacture of Arak in Lebanon. The juice was filtered through a double layer of cheesecloth and held at 30°C for 7–10 days to allow complete fermentation of the grape sugars as judged by measurements with a brix hydrometer. The fermented juice was then distilled and the proof of the alcohol distillate adjusted to 40° by the addition of distilled water. To every 500 ml of the diluted alcohol, 25g of aniseed were added and the mixture distilled again. The "head" fraction consisting of the first 12 ml of distillate was discarded and the following 85 ml were collected and diluted with water to proof 110° and used as Arak samples.

RESULTS & DISCUSSION

FIGURE 1 shows a gas chromatogram of a typical sample of Lebanese Arak. Of the 9 peaks appearing in most chromatograms, 5 were tentatively identified by their retention times when compared with pure reference compounds. The compound emerging in peak 9 had the same retention characteristics of anethole, the major component of anise oil.

The results of the quantitative determination of the various alcohols in Arak are presented in Table 1. Methyl alcohol which is primarily produced during fermentation by the demethylation of pectins, is very toxic for human consumers (Rõe, 1955). The variation in the level of this alcohol among the different samples tested may be due to differences in the distillation techniques employed by commercial distillers. Methyl alcohol is the most volatile component of the fusel alcohols and efficient fractional distillation of fermented fruits can reduce its level appreciably. The levels of longer chain alcohols varied considerably between the samples analyzed. Although differences in the fermentation conditions such as temperature, aeration and yeast strain do affect the formation

Table 1—Methanol and fusel oil content of Arak[a]

Sample No.	Proof	Methanol (ppm, v/v)	Propanol (ppm)	Isobutanol (ppm)	Amyl alcohol (ppm)
1	86	90	0	0	0
2	106	180	0	0	0
3	99	60	210	135	86
4	97	220	171	84	277
5	101	200	202	296	394
6	101	150	94	155	410
7	103	380	101	98	422
8	102	230	187	104	437
9	100	60	131	205	422
10	108	480	104	168	609
11	106	400	135	265	679
12	100	490	244	357	759
13	93	370	182	355	765
14	100	300	190	338	867
15	111	750	184	377	990
16	106	500	150	438	1246

[a] Samples were purchased locally and represented all the major brands of commercial manufacturers.

Table 2—Analysis of juice and Arak prepared from local grape varieties

Grape variety	Fusel oil content (ppm)			Precursor amino acids in juice (% w/v)[a]		
	Propanol	Isobutanol	Amyl alcohol	Valine	Leucine	Isoleucine
Miksasi	72	572	1558	0.075	0.085	0.060
Mirwahi	113	424	1085	0.075	0.075	0.055
Obeidi	64	77	362	0.085	0.075	0.060

[a] Valine is the precursor of isobutanol and leucine and isoleucine produce active and isoamyl alcohols, respectively.

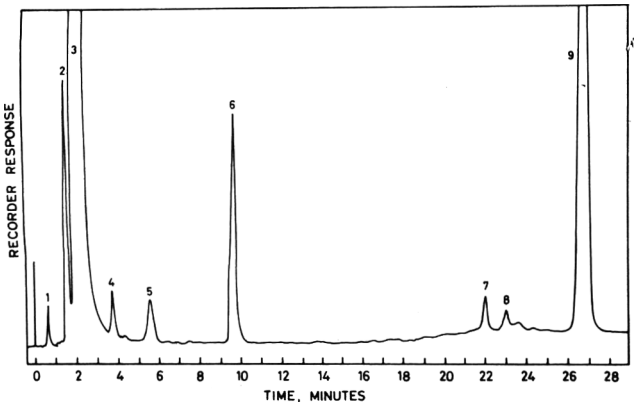


Fig. 1—Chromatogram of an Arak sample. Peak no. 3 is ethanol; no. 4, propanol; no. 5, isobutanol; and no. 6, amyl alcohol. Packed column 6 ft × 2.2 mm i.d. filled with Carbowax 20 M (15% by wt) on chromosorb W, 80—100 mesh. Starting temperature 60°C programmed to 150°C at 6°C/min. Final temperature maintained for 8 min.

of fusel oils (Guymon, 1972), the distillation technique remains the more important factor that controls the level of these alcohols in the final product. According to Guymon (1972), when highly efficient rectifying columns are used and if the proof of the alcohol distillate collected is 190 or above, such distillate will be almost void of fusel oils regardless of the source of the fermenting material. It is suspected that some manufacturers have deviated from the procedure described above for the preparation of Arak, and have utilized high proof alcohol for the anisation step. This may be economically attractive to commercial producers because they can utilize cheaper carbohydrate sources for fermentation.

The levels of higher alcohols present in Arak prepared from various Lebanese grape varieties under laboratory conditions are shown in Table 2. The amino acid precursors of the higher alcohols are also reported. It is evident that the levels of the alcohols did not vary in tandem with those of their amino acid precursors. This may be due to variations in the yeast populations responsible for the fermentation, since these experiments relied on the yeast naturally carried on the fruit skins, and to possible differences in the availability of some yeast nutrients in various samples of grape juice (Ayräpää, 1971).

The results of this study indicate that the fusel oil and methanol content of Arak is affected by variations among grape varieties, by conditions prevailing during fermentation and by the technique used for distillation.

Since consistency in flavor and quality of the final product requires a constant desirable level of these constituents, and since methanol can have harmful effects particularly on chronic alcoholics, it seems necessary that further studies be carried out to investigate the factors responsible for such variations and to establish standardized levels of these compounds in the alcoholic beverage.

REFERENCES

AOAC. 1970. "Official Methods of Analysis." Ed. Horwitz, W. Assoc. of Off. Anal. Chemists, Washington, D.C.
Askew, B. and Lisle, D.B. 1971. Variation in the concentrations of higher alcohols, methanol and ethyl acetate in brandies. J. Sci. Fd. Agric. 22: 102.
Ayräpää, T. 1971. Biosynthetic formation of higher alcohols by yeast. Dependence on the nitrogenous nutrient level of the medium. J. Inst. Brew. 77: 266.
Carroll, R.B. 1970. Gas liquid chromatography of whiskies by direct injection. Quart. J. Stud. Alc., Suppl. 5: 6.
Castor, J.G.B. and Guymon, J.F. 1952. On the mechanism of formation of higher alcohols during alcoholic fermentation. Science 15: 147.
Connell, D.W. and Strauss, C.R. 1974. Major constituents of fusel oils distilled from Australian grape wines. J. Sci. Fd. Agric. 25: 31.
Gherke, C.W., Roach, D., Zumwalt, R.W., Stalling, D.L. and Wall, L.R. 1968. "Quantitative Gas Liquid Chromatography of Amino Acids in Proteins and Biological Substances." Analytical Biochemistry Laboratories Inc., Columbia, Mo.
Guymon, J.F. 1966. Mutant strains of Saccharomyces cerevisiae applied to studies of higher alcohol formation during fermentation. Develop. Ind. Microbiol. 7: 88.
Guymon, J.F. 1970. Composition of California commercial brandy distillates. Am. J. Enol. and Vitic. 21: 61.
Guymon, J.F. 1972. Higher alcohols in beverage brandy. Feasibility of control of levels. Wines & Vines 53: 37.
Martin, G.E. and Caress, E.A. 1971. Ethyl acetate and fusel oil determination in distilled spirits by gas liquid chromatography and confirmation by mass spectrometry. J. Sci. Food Agric. 22: 587.
Reazin, G., Scales, H. and Andreason, A. 1970. Mechanism of major congener formation in alcoholic grain fermentations. J. Agr. Food Chem. 18: 585.
Reazin, G., Scales, H. and Andreason, A. 1973. Production of higher alcohols from threonine and isoleucine in alcoholic fermentations of different types of grain mash. J. Agr. Food Chem. 21: 50.
Röe, O. 1955. The metabolism and toxicity of methanol. Pharmacol. Reviews. 7: 399.
Spanyer, J.W. Jr. and Thomas, A.T. 1957. Utilization of C^{14} leucine and C^{14} glycine by Saccharomyces cerevisiae. J. Agr. Food Chem. 5: 702.
Webb, A.D., Kepner, R.E. and Ikeda, R.M. 1952. Composition of a typical grape brandy fusel oil. Anal. Chem. 24: 1944.
Ms received 12/13/74; revised 3/6/75; accepted 3/10/75.

A partial report was presented before the Fifth Science Meeting of the Lebanese Association for the Advancement of Science, Dec. 13, 1973. Contribution from the Faculty of Agricultural Sciences, American University of Beirut as Journal Number 443.

D. E. CARROLL, W. B. NESBITT and M. W. HOOVER
Departments of Food Science and Horticultural Science
North Carolina State University, Raleigh, NC 27607

CHARACTERISTICS OF RED WINES OF SIX CULTIVARS OF Vitis rotundifolia Michx.

INTRODUCTION

COMMERCIAL PRODUCTION of muscadine grapes (*Vitis rotundifolia* Michx.) has increased rapidly in the Piedmont and Coastal Plain of North Carolina. The principal market outlet for these grapes is in the manufacture of sweet, white specialty wines containing under 14% of alcohol by volume. A smaller quantity of fortified white dessert wine is also produced. For this reason, the light-skinned cultivars of muscadines are more widely planted than are the dark-skinned cultivars. However, in order to promote the continued growth of the muscadine grape industry, suitable dark-skinned cultivars are needed for the manufacture of red wines, especially dry and semidry table wines.

The present study reports on the comparative quality and the chemical and physical characteristics of red wines produced from six commercial, dark-skinned muscadine cultivars.

MATERIALS & METHODS

Grape cultivars

Fruits of six black-skinned cultivars were harvested at the Central Crops Research Station, Clayton, N.C., and/or at the Sandhills Agricultural Research Station, Jackson Springs, N.C., in the years 1968 through 1972. Hunt and Noble were tested at both locations for 5 yr; Albemarle at both locations for 4 yr; Tarheel at Clayton, N.C., for 4 yr; Magoon and Thomas at Clayton, N.C., for 3 yr. Each cultivar was harvested when judged to be at optimum ripeness by visual observation and refractometer tests for sugar concentration of free-run juice of berry samples.

After harvesting, 16–50 kg composite fruit samples of each cultivar were transported the same day to the Plant Products Lab., Dept. of Food Science, N.C.S.U., and either processed immediately or held under 1.7°C refrigeration for a maximum of 4 days prior to processing.

Wine production

Grapes were crushed with a small roller crusher-stemmer unit. The musts were treated with 100 ppm SO_2 in the form of potassium metabisulfite and, after 3 hr, inoculated with 1% (v/v) of an actively fermenting pure culture of *Saccharomyces cerevisiae* Montrachet strain (No. 522). Vigorous fermentation was underway in 24 hr with all samples. Fermentation was allowed to proceed on the skins at 21°C and the grapes were mixed several times daily to facilitate pigment extraction.

Musts were lightly pressed about 4 days after initiation of fermentation when pigment extraction was judged virtually at a maximum for most cultivars. Sugar levels of the musts were adjusted to 21° Brix with sucrose as based on the original °Brix of the must prior to inoculation with yeast. Musts were ameliorated with 21° Brix sucrose sirup based on the total titratable acidity of the must after fermentation on the skins for the 4 day period. The following schedule was used:

Total acidity of must (g/100 ml)	Amelioration (% by wt)
<0.7600	0
0.7600 to 0.8099	10
0.8100 to 0.9599	15
0.9600 to 1.0999	20
1.100 and higher	25

Wines were fermented to dryness (< 0.25% reducing sugar) at 21°C in 2- to 5-gal glass carboys fitted with fermentation locks. After settling, wines were racked into clean glass carboys and stored at 12.8°C for several months. The carboys were transferred to −3°C refrigerated storage for 5 wk to precipitate argols (insoluble potassium bitartrate crystals) and to encourage natural clarification of the wine. Wines were racked into clear glass carboys and returned to storage at 12.8°C. Approximately 1 yr after completion of fermentation, the wines were treated with 20 ppm of SO_2, filtered under nitrogen through a Millipore filter unit, filled into 1/5 gal wine bottles and corked. Wines were bottle-aged at 12.8°C for at least 6 months before being organoleptically evaluated and subjected to chemical and physical analysis.

Analytical tests

The following determinations were made in duplicate on filtered grape juice samples or wine samples: °Brix by table refractometer; pH value by glass electrode pH meter; total titratable acidity as tartaric acid by titration to pH 8.2 with 0.1N NaOH; % alcohol by volume by immersion refractometer with distillation (Amerine, 1965); extract by immersion refractometer of the dealcoholized sample (Amerine, 1965); tannin by the PRO method (Amerine, 1965). Color was evaluated as the tristimulus values using a D 25 sphere Hunterlab Color and Color Difference Meter by the method of Robinson et al. (1966). In tristimulus color values, "L" is lightness where 0 is black and 100 is white. Saturation is $(a^2 + b^2)^{1/2}$ and is proportional to the strength of the color. Hue is expressed as the hue angle, θ (arccot. $\theta = a/b$). The range of 0–60° is equivalent to a color change of violet red to orange.

Organoleptic evaluations were made on the wines by a technical panel composed of 8–12 judges experienced in evaluating muscadine wines and other wines. Presentation of samples was randomized and all samples were identified by code only. Tastings were done in the evenings and 10 samples were evaluated at one sitting. Wines were presented in 6 oz clear wine glasses and were served at a temperature of 12.8°C. Evaluations were recorded using a standard score card which was a modification of the one described by Amerine et al. (1972). Evaluations were made on important sample attributes, each worth a given number of points. Maximum numbered points for a wine is 20. The distribution of points is:

Sample attribute	Max no. points
Appearance	2
Color	2
Aroma	4
Flavor	3
Acid	2
Sugar	1
Astringency	2
General quality	4
	20

RESULTS & DISCUSSION

STATICTICAL ANALYSIS of data showed that within a given year and at the same location, virtually all parameters measured on both musts and wines were significantly different ($P < 0.01$) when individual cultivars were compared. However, seasonal variations were very large for a given cultivar. Therefore, the data in Tables 1 and 2 are presented without notations of statistical significance. These tables summarize the total data obtained on each cultivar at each location over the 3–5 yr test period. Both the mean value and the range for individual parameters are given. The authors believe this

format accurately indicates the variability in field samples when they are obtained over a period of years.

The muscadine cultivars lacked sufficient sugar to make balanced and stable wines (Table 1). Lack of sufficient sugar is a common occurrence for grapes grown in the Eastern United States. Therefore, sugar (sucrose) was added to all musts in order to increase the sugar contents to 21° Brix. Albemarle averaged the highest sugar content (16.24° Brix) and Hunt the lowest (14.51° Brix) while sugar contents of the musts of the other cultivars were similar (ca. 15° Brix).

Total titratable acidity of the fresh musts of Albemarle, Noble and Tarheel were lower than for musts of the other cultivars and lower than desirable for wine manufacture (Table 1). Total titratable acidity of 0.5% or higher is usually required as wines with too little acidity tend to have a flat taste. However, after fermentation on the skins for 4 days, considerable increase in titratable acidity resulted (Table 1). The largest increases occurred with Albemarle (167%) and with Noble (95%). In contrast to grapes of *V. vinifera* species, the skins of muscadine grapes are thick and tough. Furthermore, a distinct, thick layer of tissue is firmly bound to the skin. The combination of skin and attached tissue layer constitute the "hull" of the muscadine grape. The pulp of the grape is found directly underneath the hull. Apparently the hulls of muscadines contain appreciable amounts of acid which are released into the fermenting wine. After fermentation on the skins and pressing, most musts were high enough in acidity to require amelioration with 21° Brix sugar sirup in order to reduce the total acidity of the finished wines to palatable levels (ca. < 0.8%). However, Noble and Tarheel averaged considerably less acid than the other four cultivars although their acid contents were well above the minimum acceptable value.

Alcohol and extract values for all the wines were within an acceptable range for red table wines (Table 2). Alcohol con-

Table 1—Composition of the musts of six black-skinned cultivars of Vitis rotundifolia Michx.[a]

Determination		Cultivar					
		Albemarle	Hunt	Magoon	Noble	Tarheel	Thomas
°Brix	Avg	16.24	14.51	15.28	15.12	15.02	15.18
	Range	15.30–17.45	13.40–16.00	14.80–16.35	13.15–16.40	13.25–17.80	14.30–16.35
pH	Avg	3.47	3.28	3.28	3.46	3.47	3.30
	Range	3.40–3.69	3.15–3.50	3.15–3.40	3.40–3.70	3.22–3.80	3.15–3.50
Total acidity	Avg	0.368	0.628	0.788	0.392	0.453	0.750
(g/100 ml)	Range	0.316–0.468	0.443–1.312	0.511–1.091	0.279–0.629	0.366–0.595	0.495–1.000
Intermediate total	Avg	0.983	1.053	1.125	0.765	0.743	0.965
acidity[b] (g/100 ml)	Range	0.908–1.129	0.839–1.391	1.094–1.141	0.698–0.869	0.704–0.818	0.923–1.125
Increase in total	Avg	167	68	45	95	64	29
acidity (%)	Range	105–206	51–131	5–123	38–171	19–106	12–94

[a] The number of yr and location(s) for which each cultivar was tested are specified under Materials & Methods.
[b] On the lightly pressed juice after fermentation in the presence of the skins for 4 days

Table 2—Chemical composition, color specification and wine score for wines from six black-skinned cultivars of Vitis rotundifolia Michx.[a]

Determination		Cultivar					
		Albemarle	Hunt	Magoon	Noble	Tarheel	Thomas
pH	Avg	3.19	3.09	2.97	3.29	3.44	3.04
	Range	3.00–3.35	2.92–3.35	2.95–3.00	3.14–3.37	3.20–3.63	2.85–3.15
Total acidity	Avg	0.750	0.776	0.860	0.687	0.700	0.771
(g/100 ml)	Range	0.650–0.801	0.641–0.890	0.790–0.929	0.632–0.771	0.645–0.724	0.751–0.801
Alcohol	Avg	13.3	13.7	12.2	12.7	12.4	12.2
(by vol)	Range	12.4–13.9	12.6–13.9	12.5–13.2	12.5–13.8	12.6–12.9	12.6–13.3
Extract	Avg	2.24	2.35	2.07	2.35	2.39	2.35
(g/100 ml)	Range	2.08–2.33	2.08–2.58	1.90–2.20	2.23–2.53	2.32–2.43	2.33–2.69
Tannins	Avg	78	125	74	208	271	100
(mg/100 ml)	Range	74–84	61–106	68–78	165–236	204–320	74–136
"L" value	Avg	60.1	44.3	62.0	24.9	17.5	58.9
	Range	54.8–68.0	35.4–66.9	57.1–66.9	21.7–32.0	10.8–25.6	57.7–61.2
Saturation	Avg	45.3	55.0	49.9	55.4	47.6	53.1
	Range	35.5–52.0	32.5–64.5	43.4–56.6	52.3–61.2	36.41–60.8	49.3–57.6
Hue	Avg	39.1	24.6	36.0	17.6	14.6	36.8
	Range	38.0–46.2	23.5–31.3	31.6–41.4	16.6–19.6	13.0–16.2	34.4–37.8
Wine score	Avg	9.7	11.8	10.3	16.8	15.8	11.1
	Range	7.2–13.4	5.3–16.0	9.7–11.3	14.9–19.2	13.6–17.2	10.5–12.0

[a] The number of yr and location(s) for which each cultivar was tested are specified under Materials & Methods.

tent is largely a function of the amount of sugar available to the yeast cells during fermentation. In the case of muscadine cultivars, final alcohol content of the wine can be controlled by the amount of sugar added to correct for sugar deficiencies in the musts. Extract is a rough measure of the "body" of a wine, the higher the extract value the heavier the body of the wine.

Color is an important attribute of red wines and a good red, ruby red or purple red color is desirable. Of the six cultivars tested, only Noble and Tarheel always yielded wines having desirable color characteristics. With the exception of one sample of Hunt in 1 yr (which yielded a wine of borderline color acceptability), the other four cultivars always yielded wines having various orange, orange-brown or brown discolorations. Because they produce wines having poor and unstable color characteristics, Albemarle, Hunt, Magoon and Thomas are not recommended for red wine production.

The wines having desirable color characteristics, i.e., those from Noble and Tarheel, averaged much lower "L" and hue values than those wines having poor color (Table 2). The color of muscadine wines is due primarily to the presence of anthocyanin pigments, specifically the 3,5-diglucosides of malvidin, peonidin, petunidin, cyanidin and delphinidin (Ballinger et al., 1973). Furthermore, good red wine color in muscadines is apparently associated with large amounts of malvidin-3,5-diglucoside and poor wine color with smaller or trace amounts (Nesbitt et al., 1974). A more detailed study confirmed the importance of malvidin-3,5-diglucoside and also indicated that petunidin-3,5-diglucoside contributed significantly to good red wine color in muscadine wines (Ballinger et al., 1974). Both Noble and Tarheel contain large quantities of malvidin and petunidin-3,5-diglucosides.

The tannin contents of wine samples from Noble and Tarheel were consistently higher than for the other four cultivars (Table 2). This is expected as tannin is more accurately reported as "tannin and coloring matter" (Amerine, 1972) and Noble and Tarheel contain more coloring matter than the other cultivars tested as shown by their lower "L" values (Table 2). Tannin content in a finished wine in excess of about 250 mg/100 ml tends to make a wine excessively astringent. Tannin content can be most effectively controlled by reducing the time in contact with the skins during primary fermentation and/or by blending with wines of lower tannin contents.

In organoleptic evaluations, wines of Noble and Tarheel were consistently judged superior to wines of the other four cultivars and produced good quality dry table wines of the muscadine type (Table 2). Over the test period, wines from Noble and Tarheel averaged scores of 16.8 (5 yr avg) and 15.8 (4 yr avg), respectively. Athough wines of these cultivars are similar in overall quality, Noble wines are distinctly "varietal" in flavor characteristics while wines from Tarheel are more "neutral." It should be noted, howeve, that all of the muscadine wines which we have tested are different in flavor and aroma characteristics from wines of *Vitis vinifera, Vitis labrusca* and French-hybrid cultivars. Muscadine wines have high "fruity" flavor and aroma notes which are different from the "foxy" characteristics typical of wines from many *V. labrusca* cultivars.

REFERENCES

Amerine, M.A. 1965. "Laboratory Procedure for Enologists." Assoc. Students Store. Davis, Calif.

Amerine, M.A., Berg, H.W. and Cruess, W.V. 1972. "The Technology of Wine Making," 3rd ed. Avi Pub. Co., Inc., Westport, Conn.

Ballinger, W.E., Maness, E.P., Nesbitt, W.B. and Carroll, D.E. Jr. 1973. Anthocyanins of black grapes of 10 clones of Vitis rotundifolia Michx. J. Food Sci. 38: 909.

Ballinger, W.E., Maness, E.P., Nesbitt, W.B., Makus, D.J. and Carroll, D.E. Jr. 1974. A comparison of anthocyanins and wine color quality in black grapes of 39 clones of Vitis rotundifolia Michx. J. Amer. Soc. Hort. Sci. 99: 338.

Nesbitt, W.B., Maness, E.P., Ballinger, W.E. and Carroll, D.E. Jr. 1974. Relationship of anthocyanins of black muscadine grapes (Vitis rotundifolia Michx.) to wine color. Amer. J. Enol. Vitic. 25: 30.

Robinson, W.B., Bertino, J.J. and Whitcombe, J.E. 1966. Objective measurement and specification of color in red wines. Amer. J. Enol. Vitic. 17: 18.

Ms received 3/3/75; revised 5/17/75; accepted 5/20/75.

Paper no. 4613 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, NC 27607.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named, nor criticism of similar ones not mentioned.

The technical assistance of Lula S. Paschal and John B. Earp is gratefully acknowledged. Appreciation is given to Mr. Herbert J. Kirk for advice and assistance in statistical analysis of data.

D. R. PETRUS, R. L. HUGGART and M. H. DOUGHERTY
State of Florida Department of Citrus, Lake Alfred, FL 33850

CORRELATION OF CAROTENOID VISIBLE ABSORBANCE AND NUMERICAL COLOR SCORE OF ORANGE JUICE

INTRODUCTION

PRODUCT COLOR has a profound effect on consumer preferences and purchases of orange juices and related products. A consumer survey (CECO, 1965) revealed that the color of orange juices or orange drinks directly influenced the consumers' opinion concerning flavor, body, sweetness and other characteristics associated with the quality of these products. The importance of color as a quality factor is evident in that it is included in the U.S. Standards for Grades of Frozen Concentrated Orange Juice (USDA, 1968). The grading system allows up to 40 quality points, out of a total of 100, for color. The importance of color is further indicated in that some processors are now paying growers a premium for fruit having high internal color.

The color of orange juice varies from pale yellow for early season varieties to deep orange for late season varieties. Previously, color had been scored visually by comparing the orange juice samples with USDA plastic color standards (USDA, 1963). As a result of the work of Huggart and Wenzel (1955), Huggart et al. (1966) and Hunter (1967), the Hunter Citrus Colorimeter is used exclusively to determine the color scores of Florida processed orange juices.

The investigations of carotenoid pigments in citrus fruits are many and complex. Most methods, such as those of Higby (1962), Benk (1961) and Ting (1961) require extraction and separation of the carotenoids and involve procedures requiring a series of operations. Ting (1961) investigated the relationship between total carotenoid content and color of orange concentrates and obtained a coefficient of correlation of +0.903. Francis (1969) reviewed the work of many other investigators who studied the relationship between pigment content and color of various fruits and vegetables.

Petrus and Dougherty (1973) reported on the visible and ultraviolet absorption characteristics of alcoholic solutions of orange juices. They observed that absorption curves for each variety were similar and visible absorption intensity increased as the Hamlin and Pineapple varieties matured. The visible absorbance of Valencia varied only slightly with maturity. Absorption intensity also increased in the order of Hamlin to Pineapple to Valencia.

This study was undertaken to determine the relationship between the visible absorption of alcoholic solutions of orange juices and their color scores as determined by the Hunter Citrus Colorimeter.

EXPERIMENTAL

Juice sample preparation

An approximate 3000-lb fruit sample each of Hamlin (Oct. to March), Pineapple (Dec. to April), and Valencia (Feb. to June) oranges was harvested at 2-wk intervals during the 1973–74 season. Each sample was washed, graded and divided into random lots. Each lot was then extracted by two types of commercial extractors (Ex-1 and 2) and an official State test house (Ex-3) extractor (State of Florida Dept. of Citrus Regulations, 1970). Each type of commercial extractor was set to give a soft squeeze-soft finish and a hard squeeze-hard finish juice. All juices were immediately heat stabilized (90.6°C for approximately 28 sec), rapidly cooled to 6–8°C, canned and stored at 0°C. These juices were prepared by personnel of the Florida Dept. of Citrus and Agricultural Research & Education Center for the Juice Definition Program initiated in 1970 by the State of Florida Department of Citrus (Attaway and Carter, 1971; Attaway et al., 1972).

Preparation of alcoholic solution for spectrophotometric analyses

A volume of the juice sample was diluted with an equal volume of water and 5 ml of the diluted juice were made to 50 ml with absolute alcohol. The alcoholic solution was placed in the dark until the flocculent precipitate formed. The precipitate was removed by centrifugation to obtain a crystal clear solution.

A Coleman Model 124 Recording Spectrophotometer was used to scan the clear alcoholic solution from 600–200 nm. A 90% ethanol solution was used as the reference. Since only the visible absorption is to be discussed, only that portion of the spectra will be presented.

To obtain the visible and ultraviolet absorption from the same alcoholic solution necessitated a 1:20 dilution of the orange juice. The dilution resulted in a weak visible absorbance which was then compensated for by recorder scale expansion. Therefore, if one wishes to investigate only the visible portion of the spectrum 5 ml of orange juice should be made to 50 ml with absolute alcohol. The results were observed to obey Beer's law.

Color score measurement

The Hunter Citrus Colorimeter was used to measure the CR and CY color components of the single strength orange juice samples. The color score (CS) was then calculated from the equation: CS = 22.150 + 0.165 CR + 0.111 CY (State of Florida, Dept. of Citrus, 1972).

RESULTS & DISCUSSION

THE AUTHORS point out that the absorbance data and color score data were investigated independently with different goals or objectives. There was no prior agreement for a correlation study.

Typical visible absorption spectra of alcoholic solutions of heat stabilized mature Hamlin (H), Pineapple (P) and Valencia (V) orange juices are shown in Figure 1. Absorption maxima

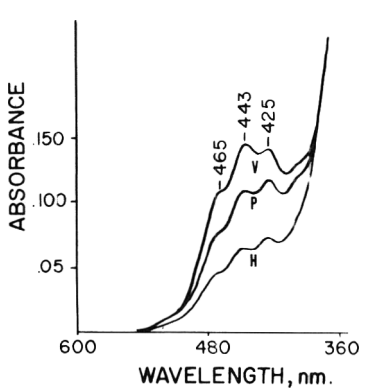


Fig. 1—Visible absorption spectra of solutions of Hamlin (H), Pineapple (P) and Valencia (V) orange juices.

were observed at 465, 443 and 425 nm. A slightly broad shoulder was also observed at about 395–398 nm. The maxima were in accordance with previous investigations (Petrus and Dougherty, 1973). It is evident from the spectra that absorbance increased in the order of Hamlin, Pineapple and Valencia.

Figure 2 is the visible absorption spectrum, of a solution of Valencia showing the absorbed and transmitted wavelengths as they apply to orange juice. Colors are seen by means of either transmitted or reflected light. If white light passes through a medium which absorbs some wavelengths and is transparent with respect to certain wavelengths, then the medium appears colored to the eye. Since only the transmitted waves reach the eye, their wavelengths dictate the color of the medium. This color is said to be complementary to the color being absorbed. From Figure 2 it can be seen that from 400 to 435 nm violet (V) light is absorbed permitting a quantity of the complementary color yellow-green (YG) to be transmitted or reflected to the eye. From 435–480 nm blue (B) is absorbed and yellow (Y) is seen. From 480–490 nm green-blue (GB) is absorbed and orange (O) is observed and from 490–500 nm blue-green (BG) is absorbed and red (R) is observed. The preceding analogy of the Valencia variety would also apply to the Hamlin and Pineapple varieties. As the season progresses for each variety or, as Figure 1 reveals, from Hamlin to Pineapple to Valencia, the absorbance increases. This increase in absorption removes more and more of the effect of the absorbed color (violet, blue, etc.) on the complementary color, allowing more of the pure colors to be transmitted or reflected to the eye. The interaction of these complementary colors then give orange juice its characteristic color. Since "total absorbance" should be related to transmittance or reflectance, it appeared reasonable to correlate the sum of the carotenoid absorbance at 465, 443 and 425 nm with the color score obtained with the Hunter Citrus Colorimeter.

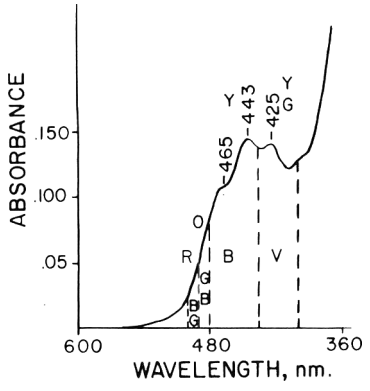


Fig. 2–Visible absorption spectrum, of a solution of Valencia orange juice, showing the absorbed and transmitted colors as they apply to orange juice. Absorbed colors: violet (V), blue (B), green-blue (GB) and blue-green (BG). Transmitted colors: yellow green (YG), yellow (Y), orange (O) and red (R).

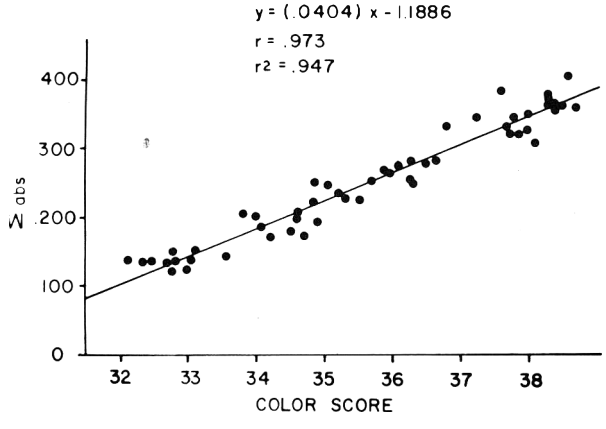


Fig. 3–Correlation of the sums of absorption (Σ abs.) intensities and color scores of the combined data obtained from the various extractors and finisher settings.

Correlations of carotenoid visible absorbance with color score are listed in Table 1. The correlations cover a range of orange juice samples of varying °Brix, percent sinking pulp, maturity, variety and extraction procedures. From the table it can be seen that the coefficients of correlation and determination were high considering all the variables mentioned above. The minimum and maximum percent sinking pulp, obtained for each extractor throughout the season, has been included to illustrate that the amount of pulp present did not appear to effect the correlations. The results were significant at the 1% level.

Figure 3 is a plot of the sum of absorption intensities against color score of the combined data obtained from the various extractors and extractor settings. The results show a coefficient of correlation r = 0.973 and a coefficient of determination revealing that 94.7% of the variation in the sum of absorbance is associated with variation in color score. The results were significant at the 1% level.

Table 1–Correlation of carotenoid visible absorbance with color score

Extractor[b]	Coefficient Correlation[a] r	Coefficient Detm r^2	% Sinking pulp Min	% Sinking pulp Max
Ex-1a	0.989	0.979	7.0	15.0
Ex-1b	0.996	0.992	8.0	18.5
Ex-2a	0.991	0.981	6.0	14.5
Ex-2b	0.992	0.984	10.5	22.0
Ex-3	0.983	0.967	11.0	23.5

[a] Results significant at the 1.0% level

[b] Extractor setting: a = soft squeeze-soft finish; b = hard squeeze-hard finish; 3 = State test house extractor.

CONCLUSIONS

A SIMPLE PROCEDURE utilizing alcoholic solutions of orange juices has been used to investigate the visible absorption characteristics of these juices.

The results showed that the carotenoid visible absorbance increased with maturity and variety, Hamlin having the weakest and Valencia having the strongest absorption.

It has been shown what colors are absorbed by the carotenoids and what effect this absorption may have on the transmittance or reflectance of the complementary colors. The stronger the absorption the less will be its interfering effect on the complementary colors, allowing more color to reach the eye or to be recorded numerically as color score.

The correlation of the sum of carotenoid absorbance with color score are shown to be high (significant at the 1% level) regardless of extractor type or setting and finisher setting. Samples of varying °Brix, percent sinking pulp, maturity and variety did not appear to effect the correlation. The coefficient of correlation was r = 0.973 or greater with a coefficient of determination of r^2 = 0.947 or greater.

The procedure may be utilized by members of the citrus industry to monitor product quality with existing equipment or inexpensive instrumentation which is available.

REFERENCES

Attaway, J.A. and Carter, R.D. 1971. Some new analytical indicators of processed orange juice quality. Proc. Fla. State Hort. Soc. 84: 200.

Attaway, J.A., Barron, R.W., Blair, J.G., Buslig, B.S., Carter, R.D., Dougherty, M.H., Fellers, P.J., Fisher, J.F., Hill, E.C., Huggart, R.L., Maraulja, M.D., Petrus, D.R., Ting, S.V. and Rouse, A.H. 1972. Some new analytical indicators of processed orange juice quality, 1971—72. Proc. Fla. State Hort. Soc. 85: 192.

Benk, E. 1961. Uber den Nachweis des Zusatzes von β-Carotin in Orangensaften und Deren Zubereitungen Deutsche Lebensmittle-Rundschaw. Heft 12: 324.

CECO (1965) Marketing Consulting Research Inc. The influence of color on consumer preference for orange juice. Survey prepared for the Florida Citrus Commission.

Francis, F.J. 1969. Pigment content and color in fruits and vegetables. Food Technol. 23: 32.

Higby, W.K. 1962. A simplified method for determination of some aspect of the carotenoid distribution in natural and carotene fortified orange juice. J. Food Sci. 27: 42.

Huggart, R.L. and Wenzel, F.W. 1955. Color differences of citrus juices and concentrates using the Hunter color difference meter. Food Technol. 9: 27.

Huggart, R.L., Barron, R.W. and Wenzel, F.W. 1966. Evaluation of the Hunter citrus colorimeter for measuring the color of orange juices. Food Technol. 20: 109.

Hunter, R.S. 1967. Development of the citrus colorimeter. Food Technol. 21: 906.

Petrus, D.R. and Dougherty, M.H. 1973. Spectral characteristics of three varieties of Florida orange juice. J. Food Sci. 38: 659.

State of Florida Dept. of Citrus Regulations. 1970. Regulation 105-1.18, Sec. (9), (a)1, p. 7, Nov. 25.

State of Florida Dept. of Citrus Regulations. 1972. Regulation 105-1.19, Sec. (1) (r).

Ting, S.V. 1961. The total carotenoid and carotene content of Florida orange concentrate. Proc. Fla. State Hort. Soc. 74: 262.

USDA. 1963. Scoring color of orange juice products with the USDA 1963 orange juice color standards. Agr. Marketing Ser., Washington, D.C.

USDA. 1968. U.S. standards for grades of frozen concentrated orange juice. U.S. Dept. Agr., Agr. Marketing Service, Washington, D.C.

Ms received 12/18/74; revised 3/18/75; accepted 3/22/75.

Florida Agricultural Experiment Stations Journal Series No. 5727. Cooperative research by the State of Florida, Dept. of Citrus and the University of Florida Agricultural Research & Education Center.

I. SHOMER and I. BEN-GERA
Institute for Technology & Storage of Agricultural Products
Agricultural Research Organization, The Volcani Center, Bet Dagan
and A. FAHN
Dept. of Botany, Hebrew University of Jerusalem, Jerusalem, Israel

EPICUTICULAR WAX ON THE JUICE SACS OF CITRUS FRUITS: A POSSIBLE ADHESIVE IN THE FRUIT SEGMENTS

INTRODUCTION

ONE OF THE MAIN PROBLEMS in the preparation of citrus fruit segments for canning or freezing is the loss of their compact fine macrostructural organization. It is well known that the quality of the canned grapefruit depends, among other criteria, on the percentage of broken segments (U.S. Standards, 1973). The quality standard refers to the finished product only. Additional serious damage to the raw material may occur during early stages of processing (before canning, during peeling and removal of the segment "skin") and continue throughout the processing operations during exhausting and pasteurization, and even through gravity labeling. Results of a survey made in several factories in Israel, as well as our experiments, indicate that the loss of grapefruit segments as a result of breakage and disintegration along the processing line can amount to 20–50% of the total weight of segments processed. It was shown (Levi, 1972) that structural damage to the peeled segments tends to be higher at the beginning of the harvest season and in over-ripe fruits. However, no explanation was offered as to any biological or agrotechnical factors which might have an effect on, or increase the damage to, the integrity of peeled citrus fruits segments.

The citrus fruit develops from a syncarpous gyneecium with axial placentation. The fruit locules are filled with stalked spindle-shaped juice sacs. These sacs develop from cells of the inner epidermis and subepidermal layers of the pericarp, i.e., from the endocarp. Each juice sac consists of an external layer of elongated epidermal cells which enclose large, thin-walled juice cells. The juice sacs of each locule adhere to each other, and form–together with endocarps of each carpel–the fruit segment (Schneider, 1968; Fahn et al., 1974). Information regarding the factors responsible for the preservation of the compact fine macrostructure of the citrus fruit segments in general, and of grapefruits in particular, could lead to improved methods of handling and processing, and to considerable savings both in raw material and in production costs. The first report of a study made by the present authors, of the factors responsible for adhesion of the juice sacs of each segment, was based mainly on morphological methods. The results led us to suggest that the binding agent is an epicuticular wax (Fahn et al., 1974).

The present study included an investigation of the ultrastructure of citrus segments. To obtain information related to the inter-juice sacs area (including the epidermal region of the juice sacs), chemical analyses of lipids extracted from the outer surface of the juice sacs, and tests of the disintegration rate of citrus segments placed under conditions which promote disintegration, were carried out.

MATERIALS & METHODS

SEGMENTS were obtained from Marsh Seedless grapefruit (*C. paradisi* Macf) and Shamouti and Valencia oranges (*C. sinensis* (L.) Osbeck). The fruit was peeled and the segment "skin" (carpellary membrane) was removed by hand.

Transmission electron microscopy (TEM)

Segment cross sections of about 1–2 mm thickness, which contained cross sections of 3–10 juice sacs altogether, were handcut using a razor blade. The material was fixed in 3.5% glutaraldehyde buffered with 0.1M sodium cacodylate (pH 6.9) for 2–3 hr, and post fixed for 2 hr with 2% osmium tetroxide buffered as above. Dehydration was done with ethanol at increasing concentrations of 30, 50, 70, 90 and 100%. The sections were left for 15 min in each stage, and for at least 1 hr in pure ethanol. In the absolute alcohol, connected juice sac cross sections were separated very carefully by forceps under a dissecting microscope. The embedding of the separated cross sections was done with Spurr's resin (Spurr, 1969) for 3 days, and polymerized at 70°C for 12 hr. The embedded material was sectioned with an LKB ultramicrotome. The sections were stained with uranyl acetate and lead citrate, and examined in a Philips-300 electron microscope.

Scanning electron microscopy (SEM)

The fixation and dehydration were done as for TEM except that the sections were slightly larger (ca. 10 × 10 × 10 mm), with 8–10 juice sac cross sections. After fixation, the wax was fixed on the outer surface of the juice sacs, and hence no disintegration occurred. Sections were held in absolute ethanol until they were coated with gold in a rotating vacuum evaporator. Before applying vacuum, the sections of adjacent juice sacs were separated by forceps and new, clean and undamaged outer surfaces of the cuticle of the inner juice sacs were exposed. Pieces containing 1–5 juice sacs altogether were placed on the SEM stubs, so that their outer surfaces were exposed to observation in a Cambridge Stereoscan S4 microscope.

Removal of the waxy materials

About 3 kg of whole and undamaged peeled citrus fruit segments was immersed in chloroform for wax removal. No evidence of internal extraction or damage to the juice sacs was observed by TEM. This procedure was repeated three times and slight shaking was applied for about 3 min, by which time the segments had disintegrated completely. A final rinse was done with fresh analytical chloroform. The solvent was removed from the extract in a rotary avaporator at 60°C (in vacuo).

Fractionation of the waxy material' mixture

Saponification and determination of the unsaponifiable matter, were done according to the procedure of Morice and Shorland (1973). 0.2g of the unsaponifiable matter was dissolved in hexane (boiling range 67–70°C, free of aromatic hydrocarbons) and boiled under reflux for about 5 min. After cooling, the solution was transferred to an aluminum oxide (Brockman, activity II of BDH) column, 90 cm long and 1.2 cm in diameter. The column was rinsed five times with 10 ml of hexane. The same procedure was followed with benzene, diethyl ether and 20% methanol in diethyl ether (v/v). The fractions of hydrocarbons, primary alcohols, secondary alcohols, and unidentified components were obtained accordingly. Each fraction was studied by thin-layer chromatography (TLC). Hydrocarbons were analyzed by gas chromatography (GLC) using a Varian Aerograph series 2400, with flame ionization detector, on a SS column of 3% SE 30 on 100/120 Var-A-Port 30.

Thin-layer chromatography

TLC was done according to Holloway and Challin (1966). Silica gel-G precoated plates were used. Three solvent systems were used: benzene:chloroform (3:7 v/v); benzene:chloroform:ethyl acetate

(1:2:2 v/v/v); and benzene:acetic acid:methanol (45:8:2 v/v/v). Several spots, each with 50–100 μg of waxy material, were applied to the plates. Detection was done with a Rhodamine-6G spray. Observation was done under UV light of 365 nm and 254 nm.

Disintegration tests

One peeled citrus fruit segment was immersed in 100 ml of one of various solvents in a 250-ml Ehrlenmeyer flask, which was shaken gently under controlled shaking and temperature conditions. The time required for complete disintegration of the segment was measured. The solvents used were chloroform, ethanol, xylene, hexane, benzene, diethyl ether and water. Samples immersed in 100 ml of water in a 250 ml Ehrlenmeyer flask were shaken at various temperatures (room temperature, 50°, 60°, 70°, 80° and 90°C) and at different controlled shaking intensities. Disintegration by freezing was studied at various rates of freezing by liquid N in a biological freezer apparatus (Union Carbide), and by Freon-12 in a laboratory freezer.

RESULTS & DISCUSSION

THE CITRUS SEGMENT is naturally compact. Under certain conditions, and following the removal of its "skin" envelope, loss of the segment's fine macrostructure can occur. This work demonstrates the fact that both the chemical nature and temperature conditions of the segment's environment can affect the segment's macrostructure, and promote its destruction.

Earlier studies of the ultrastructure of the juice sacs' epidermal cells (Fahn et al., 1974), revealed that their outer walls are rather thick in comparison with their other walls, and even more so in relation to the walls of the inner cells. When observed through a dissecting microscope, the outer surface of the juice sac is smooth and shiny. TEM (Fig. 1G) confirms the presence of a cuticle with a moderately undulant topography (Fig. 3D) of its outer surface. TEM reveals that at least part of this distinct cuticle of the juice sacs was covered with an osmiophilic material. Study of this electron dense-stained material suggests it to be a wax or waxy material. The occurrence of wax secretion on the outer surface of leaves and fruits of many plants is well known (Martin and Juniper, 1970; Albrigo, 1972a). The occurrence of wax deposits or layers in the inner part of fruits has not been recorded in the literature, to the best of our knowledge. Even Schneider (1968), when stating that the epidermal cells of the juice sacs "have a waxy cuticle on their outer side," does not suggest an accumulation of wax secretion, or a wax layer over the outer surface of the juice sacs cuticle in citrus fruit segments.

Wax or waxy layers were found in this study on the outer surface of juice sacs in both oranges and grapefruits. In the two orange varieties studied—Valencia and Shamouti—the secreted wax which was observed by TEM has both a homogeneous and granular intermix (Fig. 1G). However, in Marsh Seedless grapefruit there appears only a layer of homogeneous structure with regular protrusions (Fahn et al., 1974). At some sites the wax is torn possibly as a result of mechanical damage by external forces which may have acted during peeling.

Observations with the SEM reveal a structure of secreted deposits on the outer surface of the juice sacs of all citrus fruit segments studied (Fig. 2A, E; Fig. 3A, D). Various morphological patterns were observed in the different citrus species and varieties. Generally, more than one form of wax structure was found in the same variety. When the two juice sacs were separated through the middle of the wax layer, rather than along the cuticle of one of them, the internal structure of the wax layer was exposed. Details of the formation of the connecting structures between adjacent juice sacs will be discussed in a separate paper.

Oranges

Wax secretion, in the form of a three-dimensional meshwork of thread-shaped structures, was observed on the juice sacs of orange segments (Fahn et al., 1974). The meshwork pattern is sometimes continuous, with flat homogeneous layers, but may also occur both in continuation of and in alternation with such layers, forming a complicated storied structure of several strata (Fig. 3A, C). Several layers of plates with irregular borders were observed, especially in Shamouti oranges (Fig. 3B); they gave the impression that they may have been the result of breakage of larger sheaths. Some of these structural patterns are similar to the wax layer of the outer surface of oranges (Albrigo, 1972a, b). Certain structural patterns resemble that of the wax of some other plant leaves, as discussed by Fahn et al. (1974).

Grapefruit

Wax accumulations, with three main types of structure, were observed on the outer surface of the juice sacs of grapefruits:

(1) Continuous layers with papillae-like protrusions, which, as seen in cross section (Fahn et al., 1974), were local inflations of the wax layer, and large bizarre-shaped protrusions (Fig. 2D, E). In some cases the papillae-like protrusions formed aggregate structures (Plate 2D, E). Sometimes flat sheaths of wax occurred on top of the papillae-like protrusions (Fahn et al., 1974).

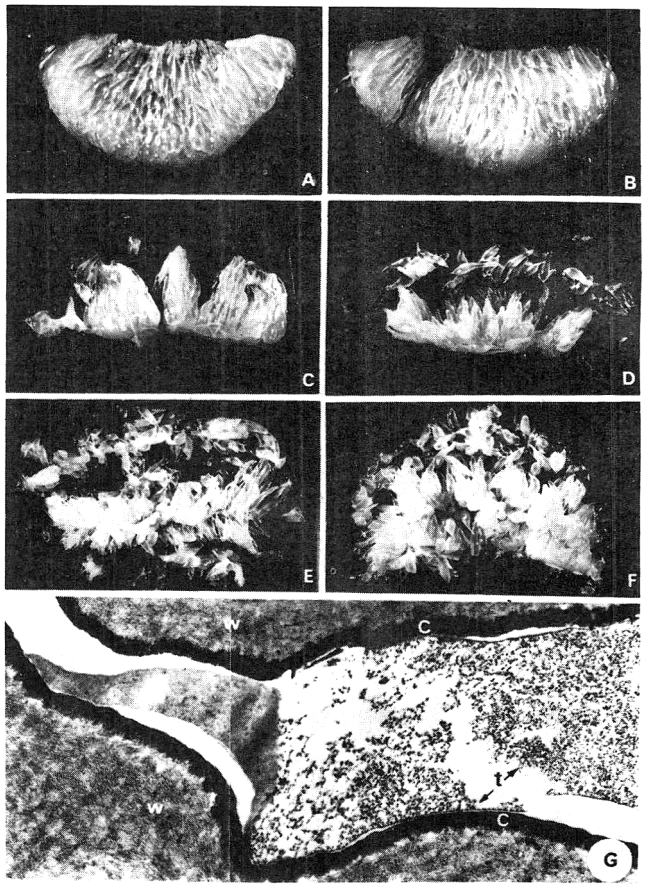


Fig. 1—Various rates of disintegration as a result of controlled destruction conditions (segments of Marsh Seedless grapefruit were used). [A–E a segment which was shaken for 5 min in water at room temp, 50–60°C, 70°C and above 70°C, (X0.5), respectively; F = a segment immersed for a few seconds in chloroform, (X0.5) (Fahn et al., 1974); G = Electron micrograph of part of a cross section of juice sacs showing epidermal walls (W), cuticle (C), and epicuticular wax (Wa) in the space between the juice sacs of Valencia orange, and tearing (t) region in it (X9640) (Fahn et al., 1974).]

(2) Dispersed groups of rod-like structures (Fig. 2A, B). These structures sometimes appeared to have melted and fused to form bizarre masses.
(3) Aggregate coating which consisted of superimposed fibrillar, rod-shaped or thread-shaped structures forming a complicated three-dimensioned meshwork (Fig. 2C). In 4–5-month-old grapefruit fruits (age from blossom time), initiations of granular structure were observed, which cover large areas of the outer juice sacs' surfaces. Between these areas, flat planes were found.

It is assumed that all these structures in both the ripe oranges and grapefruits, are derivatives of the basic structure which appears in the early stage of the citrus fruit segment's development. The presence of various forms of structures (papillae, rod-like protrusions, flat sheaths, bizarre masses, fibrillar constructions, or others), sometimes on the same area of the juice sac surface, may be created as a result of certain physical conditions that act on the wax layer during the accumulation. A mixture of several structural types could be observed, especially where the wax structures between adjacent juice sacs touch each other, and where no damage through dislocation occurred. Details related to these observations and a possible explanation regarding the large variability observed, will be discussed in a separate paper.

These last observations show that both thickness and structure of the wax layer may be influenced by the amount of secretion and by the available spaces between the juice sacs that permit wax accumulation. It is assumed that several factors, like pressure or disconnecting forces arising from turgor pressure, or deformation of the outer surface topography during development of the juice sacs, may influence the original structure of the accumulated wax. Such actions may result in an increase in wax density. Extreme pressures may push excess wax to relatively empty space (Fig. 3C). Tension can also create a porous structure and disconnection of the continuity of the wax layer (Fig. 3A, Fig. 1G). Several planes of wax with different densities were sometimes observed (Fig. 3C). This situation may arise from the deformation of the wax structure by pressure acting from several directions.

As mentioned above, at some sites the outer surface of the juice sacs is smooth and the wax cover has no apparent structure. This may occur in two cases: (a) when the entire wax layer is removed and the cuticle is exposed, when the juice sacs are separated from one another (Fig. 3A); and (b) when two juice sacs are separated mechanically, and the entire wax layer between them remains attached to one sac (Fig. 3A). The surface will appear smooth, since only where the layer of wax is broken can the internal structure of the wax be observed. The TEM observations also revealed that the contact area between the outer surface of the juice sacs' cuticle and the wax layer was seen as structureless but rather smooth (Fig. 1G).

Age was found to influence the establishment of physical contact between wax layers of two adjacent juice sacs, as such contact will occur only at a certain age of the fruit. It was assumed that the occurrence of the cementation between adjacent juice sacs depends mainly on two factors: outer surfaces of adjacent juice sacs draw closer together as a result of a development which causes an increase in their volume (Bain,

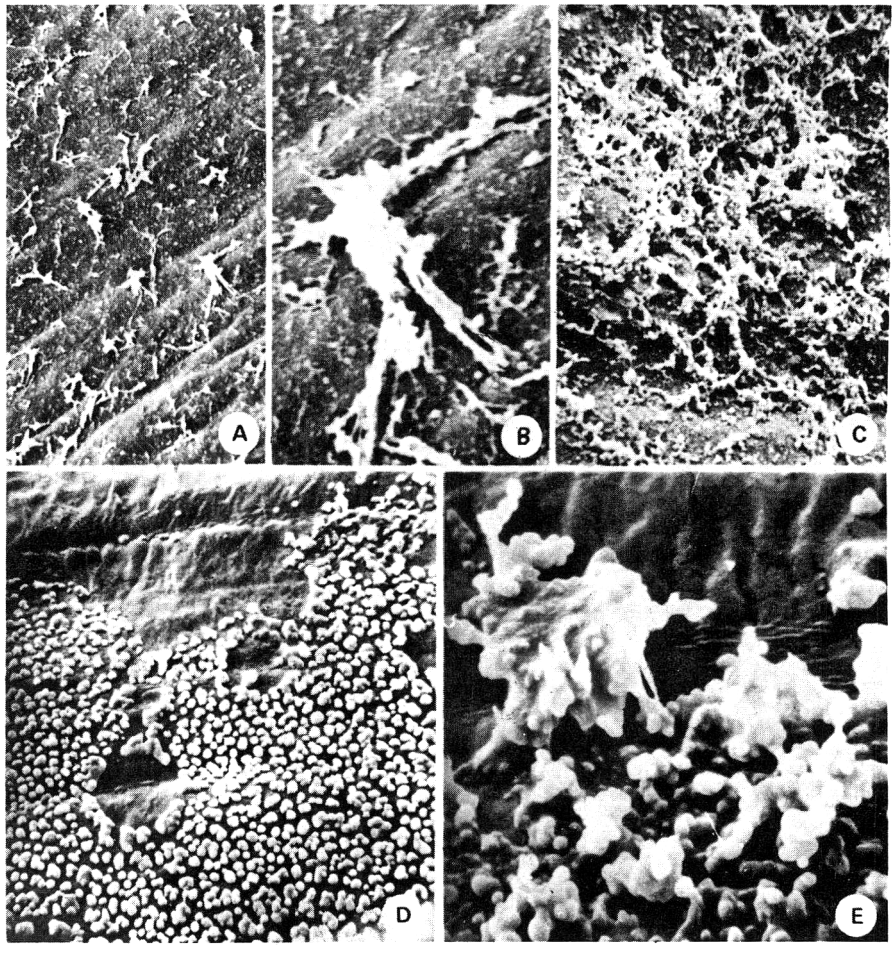


Fig. 2–Scanning electron micrographs of the outer surface of juice sacs of Marsh Seedless grapefruit (Fahn et al., 1974). (A, X1320; B, X7062; C, X1188; D, X3663).

1958); and the secretion of wax on the outer surface of the cuticle, which continues after the adjacent juice sacs already touch one another.

The composition of the wax constituents of the juice sacs

TLC analysis of chloroform extraction of juice sacs (Fig. 4) established the presence on the outer surface of the juice sacs, of materials composed of the same constituents as those found on the outer surface of citrus fruits (Schulman and Monselise, 1970). These materials contained the major wax constituents of the outer surface of cabbage leaves or bee's wax (Holloway and Challin, 1966; Martin and Juniper, 1970). It was found that this wax material which was removed from the outer surface of the juice sacs of grapefruit contains 63.25% of unsaponifiable and 35.53% of saponifiable matter (by weight). The unsaponifiable matter was divided by aluminum oxide column into four gropus: iso- and normal paraffins, 64.63%; primary alcohols, 17.23%; secondary alcohols, 13.34%; and unidentified matter, 4.81%. The saponifiable matter was divided into 19.9% petrol ether-soluble fatty acids and 16.63% petrol ether-insoluble fatty acids (of the total recovered wax). The results of studies carried out by Fernandez et al. (1964), Holloway and Challin (1966), Morice and Shorland (1973), Schulman and Monselise (1970), and Martin and Juniper (1970), on waxes of leaves and fruits of various plants, are similar to those obtained by us from the chloroform extract of the outer surface of the juice sacs. There were two reasons for analyzing the paraffins: One is that the paraffins are prominent in wax of the outer surface of juice sacs; and the second more important one, is that this group of hydrocarbons is supposed to be the major material used as an adhesive agent between the outer surfaces of the juice sacs. It was found by GLC analysis that the hydrocarbons between the tricosane and the octatriacontane were present. The iso- and normal tricosane, tetracosane and pentacosane constituted the highest relative amount, each between 18% and 24% of the total alkanes. More details about the composition of the wax constituents will be given in a separate paper. Nagy and Nordby (1971, 1972) and Nordby and Nagy (1971) analyzed lipid constituents of freeze-dried powder of juice sacs of several citrus fruit varieties and species. From our present knowledge it appears that this freeze-dried powder included not only lipids from the juice sacs' outer surface, but also from the inner part of the juice sacs as well.

Schneider (1968), referring to the work of Dodd (1944) and Davis (1932), proposed that "oil droplets" or "deposits of oil" may occur in the central region of the juice sacs. In the present work, using TEM, lipids were found not only on the outer surface of the juice sacs as waxy layers, but in other regions of the juice sac tissue of oranges and grapefruits. Relatively large osmiophilic drops were observed in various parts of the cell, in the inner central region of the juice sac, generally in the plastides and the cyloplasma, and also between the plasmalema and the wall. A detailed description of these observations will be given in a separate paper.

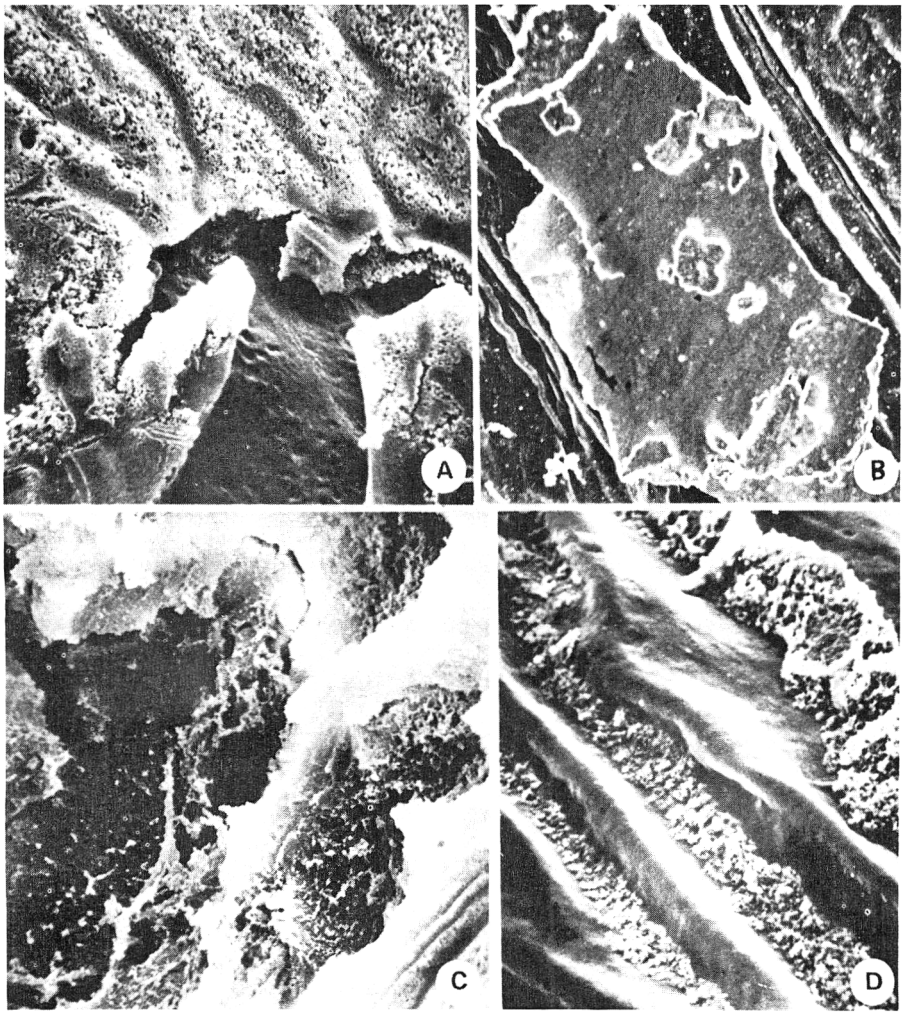


Fig. 3–Scanning electron micrographs of the outer surface of juice sacs of Valencia orange (A) and Shamouti orange (B, C, D) (Fahn et al., 1974). (A, X1551; B, X1452; C, X1584; D, X3456).

Segments disintegrations parameters

Chloroform and diethyl ether are the best wax solvents, according to Holloway and Challin (1966) and Schulman and Monselise (1970). Other solvents, like ethanol, benzene and hexane, dissolve wax more slowly. Immersion of peeled segments in these solvents resulted (at room temperature) in complete disintegration of the segment. While immersion in chloroform, hexane or xylene for example, resulted in complete disintegration within a few seconds (Fig. 1F), with the other solvents mentioned, like ethanol, complete disintegration required slight shaking and was achieved only after at least 1–2 hr, if at all.

A study of grapefruit wax properties under a dissecting microscope revealed that at ca. 50°C the wax begins to melt, and at ca. 70°C melting is completed. Shaking of peeled grapefruit segments immersed in a water bath, at temperatures lower than 50°C, did not result in structural damage to the segments. With increasing water temperature (in the range 50–70°C) disintegration advanced rapidly, promoted by shaking; it increased in rate as the temperature was increased (Fig. 1A–E). At 70°C or above, with even slight shaking, disintegration took place immediately. The kinetics of segment disintegration will be published at a later date.

Three research methods were used to prove the assumption that the wax is the adhesive agent between the juice sacs: ultrastructure observations of the outer surfaces of the juice sacs; chemical analysis of the material which was removed from the outer surface of the juice sacs; and disintegration tests of the segments under various controlled conditions. Generally, observations which were done by TEM and SEM both before and after the removal of the wax by organic solvents, and after melting the wax by immersing the segments in hot water above 50°C, showed that the cuticle and the epidermal wall structure were not damaged by the treatments. The wax deposits on the outer surface of the juice sacs disappeared after treatment of the segments with chloroform. The melting of the wax by hot water above 70°C was absolute and the wax structure was destroyed, but the wax remained on the outer surface of the cuticle as a homogenic smooth layer. In the same treatments in which the wax was removed or melted, the segments disintegrated to single but complete juice sacs. On the other hand, if the immersion of the segment cross sections (which were prepared to the SEM observations) in chloroform was done after the fixation and the dehydration procedures, there was neither removal of the wax from the outer surface of the cuticle nor disintegration of the sections into separate sections of single juice sacs. These findings will be discussed in more detail in a future publication. The disintegration tests show that the cementing ability of the wax may depend on its elasticity and plasticity, in addition to its adhesiveness and cohesiveness. These properties of the wax may be influenced by pressure, tension, temperature, chemical factors, etc.

The effect of freezing on the adherence of juice sacs

With certain freezing methods, the disintegration problem is very serious. Segment structure, and adherence of juice sacs, were influenced by both the type of freezant and the rate of freezing, in cryogenic freezing of peeled grapefruit segments in liquid nitrogen and liquid Freon-12. Apparently, these two freezing media represent two different possible pathways to structural damage.

In freezing experiments with liquid nitrogen, disintegration advanced with increased speed of freezing. Figure 5 shows three freezing rates used in the freezing experiments. An increased rate of freezing resulted in structural damage of the type and magnitude shown in Figure 1B, C and D, respectively. Freezing by immersion in Freon-12 resulted in rapid structural damage of the type and magnitude shown in Figure 1E.

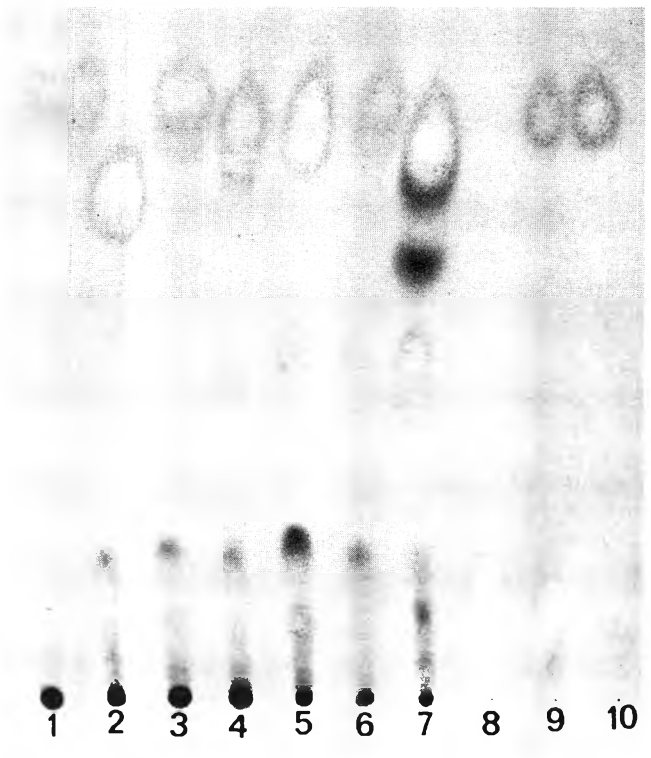


Fig. 4–Thin-layer chromatography of wax removed from the outer surface of juice sacs of Marsh Seedless grapefruit. From left to right: 1–6, wax from progressively later dates of harvesting; 7 = wax removed by Freon-12; reference constituents about 10 µg each of: 8 = 1,1-eicosanol, 9 = n-eicosanol:octadecanoic acid:docosane, 10 = docosene:stigmasterin.

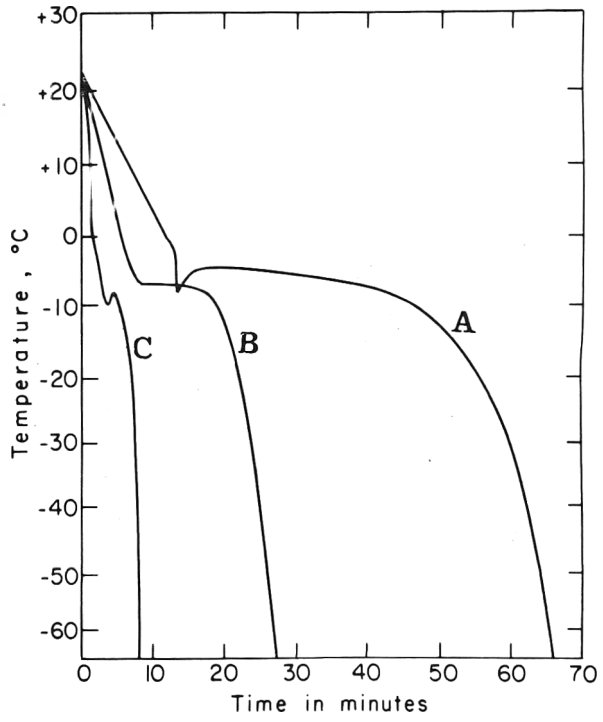


Fig. 5–Various rates of freezing which were measured (by copper constantan thermocouple) in the center of the peeled segment of grapefruit. After freezing at the rate of Curve A the segments were undamaged. Freezing rate B caused some breakage which was similar to that snown in Figure 1C. After freezing at rate C, the segment disintegrated, as shown in Figure 1D.

Liquid-nitrogen freezing damage can be explained through volume changes of the juice sacs (Fennema and Powrie, 1964; Idle and Hudson, 1968), where, because of the fast temperature drop and the subsequent immediate freezing, no connecting ice bridges were formed between the juice sacs. These bridges may form during slower freezing processes (Mazur, 1969). The liquid Freon-12 was found to act as a wax-removing agent. All of the wax components found in the chloroform extract were found in the liquid Freon-12 used for contact freezing of the grapefruit segments. This can be seen by the TLC analysis in Figure 4.

CONCLUSIONS

THE OBSERVATIONS and results recorded here demonstrate the vital role played by the wax coating on the outer surface of the juice sacs, in preserving the structure of peeled citrus segments. In all cases removal of such coating results in a rapid and complete disintegration.

REFERENCES

Albrigo, L.G. 1972a. Distribution of stomata and epicuticular wax on oranges as related to stem and rind breakdown and water loss. J. Am. Soc. Hort. Sci. 97(2): 220.

Albrigo, L.G. 1972b. Ultrastructure of cuticular surfaces and stomata of developing leaves and fruit of the "Valencia" orange. J. Am. Soc. Hort. Sci. 97(6): 761.

Bain, J.M. 1958. Morphological, anatomical and physiological changes in the developing fruit of the Valencia orange, Citrus sinensis (L.) Osbeck. Aust. J. Bot. 6: 1.

Davis, W.B. 1932. Deposits of oil in the juice sacs of citrus fruits. Am. J. Bot. 19(2): 101.

Dodd, J.D. 1944. Three-dimensional cell shape in the carpel vesicles of Citrus grandis. Am. J. Bot. 31(2): 120.

Fahn, A., Shomer, I. and Ben-Gera I. 1974. Occurrence and structure of epicuticular wax on the juice vesicles of citrus fruits. Ann. Bot. 38(157): 869.

Fennema, O. and Powrie, W.D. 1964. Fundamentals of low temperature food preservation. In "Advances in Food Research," Vol 13: 220. Ed. Chichester, C.O. and Mrak, E.M. Academic Press, New York.

Fernandez, A.M.S., Baker, E.A. and Martin, J.T. 1964. Studies on plant cuticle. 6. The isolation and fractionation of cuticular waxes. Ann. Appl. Biol. 53: 43.

Holloway, P.J. and Challin, S.B. 1966. Thin-layer chromatography in the study of natural waxes and their constituents. J. Chromatog. 25: 336.

Idle, D.B. and Hudson, M.A. 1968 Physical effects during ice formation in plants. In "Low Temperature Biology of Foodstuffs," Ed. Hawthorn, J. and Rolfe, E.J., p. 153. Pergamon Press, Oxford, London.

Levi, A. 1972. (Correlation between physical properties and chemical composition of fresh and canned grapefruit segments.) M.Sc. thesis, Technion—Israel Institute of Technology, Haifa, Israel.

Martin, J.I. and Juniper, B.E. 1970. "The Cuticle of Plants." E. Arnold, London.

Mazur, P. 1969. Freezing injury in plants. Ann. Rev. Plant Physiol. 20: 419.

Morice, I.M. and Shorland, F.B. 1973. Composition of the surface waxes of apple fruits and changes during storage. J. Sci. Fd. Agric. 24: 1331.

Nagy, S. and Nordby, H.E. 1971. Comparative long chain hydrocarbon profiles of orange and tangerine juice sacs. Lipids 6(11): 826.

Nagy, S. and Nordby, H.E. 1972. Saturated and mono-unsaturated long chain hydrocarbons from lemon juice sacs. Phytochemistry 11: 3249.

Nordby, H.E. and Nagy, S. 1971. Fatty acid profiles of orange and tangerine juice sacs lipids. Phytochemistry 10: 615.

Schneider, H. 1968. The anatomy of citrus. In "The Citrus Industry," Ed. Reuther, W., Batchelor, L.D. and Webber, H.J. Univ. of California, Berkeley.

Schulman, J. and Monselise, S.P. 1970. Some studies on the cuticular wax of citrus fruits. J. Hort. Sci. 45: 471.

Spurr, A.R. 1969. A low viscosity epoxy resin embedding medium for electron-microscopy. J. Ultrastructure Res. 26: 31.

U.S. Standards for Grades of Canned Grapefruit. 1973. In "The Almanac of the Canning, Freezing, Preserving Industries," p. 299. Edward E. Judge & Son, Inc.

Ms received 1/5/75; revised 3/17/75; accepted 3/22/75.

Contribution No. 128-E from the Agricultural Research Organization, 1975 Series.

J. W. KESTERSON and R. J. BRADDOCK
University of Florida, Institute of Food & Agricultural Sciences
Agricultural Research & Education Center, P.O. Box 1088, Lake Alfred, FL 33850

TOTAL PEEL OIL CONTENT OF THE MAJOR FLORIDA CITRUS CULTIVARS

INTRODUCTION

THE COLD-PRESSED oils obtained from the peel of Florida citrus fruits return an estimated $5–6 million annually to the Florida citrus industry. Most citrus processors recover only 10–20% of the total available oil from the fruit and for this reason it is important that data be presented to show the maximum amount of recoverable oil from different commercial citrus varieties. Such information should enable citrus processors around the world to develop processing techniques that result in greater recoveries of these flavoring materials. Citrus processors utilize the oil contained in the peel of the fruit to impart a natural fresh citrus flavor to concentrate and juice products. The food, flavor and beverage industries use large quantities of various citrus essential oils to impart flavor and aroma to their products. The potential amounts and availability of these flavoring materials are important to all segments of the food and flavor industry.

Hood (1916) probably made the first attempt to determine the peel oil content of Florida oranges. Later, Giacometti (1952) sought to morphologically classify the various strains of Parson Brown oranges by studying the primary, secondary and tertiary oil glands in these fruits. He found that thin-skinned fruit contained fewer oil glands than thick-skinned fruit. Hendrickson et al. (1969, 1970) have more recently demonstrated that budwood and rootstocks are variables which influence the peel oil content of oranges. Bartholomew and Sinclair (1946a, b) and Bitters and Scora (1970) studied California citrus fruit and found that peel oil content is directly correlated with the surface area of the fruit and that rootstock influenced the amount and type of oil found in the rind of oranges. Data are presented in the present study which show the total peel oil contents of 12 different citrus cultivars over a period of 3–5 yr.

EXPERIMENTAL

PEEL OIL CONTENT studies were made on four-tree plots with three replications per selection. The same plots were used throughout this study. The 12 cultivars used in this survey were typical commercial plantings on rough lemon rootstock receiving recommended fertilizer applications and normal rainfall. Florida citrus processors were polled to determine processing dates for the cultivars used in this study. Sixteen fruit samples were collected from each four-tree plot on a monthly basis throughout the normal processing season. This entailed picking four fruit from each tree. These four fruit were picked at each cardinal point on the compass (N, E, S & W) equidistant between the top and bottom of the tree.

Peel oil content was determined in accordance with the procedure of Hendrickson et al. (1969). In this method, two discs (2 cm diam) were cut from 16 fruit at the equatorial section of the fruit which had previously been sprayed with a clear plastic (No. 2X723 Acrylic, Dayton Elec. Mfg. Co., Chicago) to prevent oil loss during cutting of the discs. The fruit were weighed and the longitudinal and equatorial diameters were measured. Pounds of peel oil per ton of fruit were calculated, by determining the volume of oil per unit weight of fruit, extrapolating for the equivalent volume in a ton of fruit and finally converting to weight by using the density of the oil.

RESULTS & DISCUSSION

CITRUS OR CITRUS FLAVORED (orange, lemon and lime) products constitute one of the largest groups of flavors for the food industry. Since citrus oils serve as raw materials to impart citrus flavors to foods and beverages, they become an important commodity to both citrus and food processors.

Orange cultivars

Orange cultivars were sampled to include early-season, mid-season, Valencia and Temple oranges. The specific cultivars used in this study were: Hamlin, Parson Brown, Pineapple, Valencia and Temple orange. Data in Table 1 show the maximum and minimum values in total oil content for four different processing seasons. Four-year averages show the following order of oil contents (lb oil/ton fruit): Valencia (13.5), Parson Brown (10.6), Pineapple (9.7), Temple (7.9) and Hamlin (7.8). These data indicate that fruit variety has an influence on oil content and that the amount varies from season to season. Variability in oil content is also due in part to fruit size. Kesterson et al. (1971) have reported that the quantity of oil per unit surface area of fruit increased as the fruit increased in size. Increase in fruit size with maturity is normally greater than the oil increase resulting in a lower oil content on a per ton of fruit basis.

Grapefruit cultivars

The grapefruit cultivars used in this study were Duncan, Marsh and Ruby Red, the primary varieties used for processing in Florida. Data in Table 2 show the maximum and minimum total oil content for four different processing seasons. Grapefruit contained approximately one-half of the oil found in Valencia oranges. The amount of oil present in the above cultivars followed the order of Ruby Red (6.5), Marsh (6.2) and Duncan (5.6 lb/ton). As was demonstrated for oranges, variety also influenced the total oil content of grapefruit. Fruit size and climatic factors are variables which could influence the season to season oil content.

Miscellaneous citrus cultivars

The miscellaneous cultivars investigated were: Dancy tangerine, Orlando tangelo, Persian lime and lemons. Data in Table 3 show the maximum and minimum peel oil content of these fruits for either three, four or five different processing seasons. The peel oil content, listed from highest to lowest, was tangerine (15.5), lemon (15.1), tangelo (11.3) and lime (8.1 lb/ton). Dancy tangerine and lemons had the highest oil content of all the citrus fruits studied. Persian lime was similar to Hamlin orange, while Orlando tangelo was comparable to Parson Brown in total oil content. These data clearly demonstrate the variability in oil content with variety. Climatic or seasonal variations were found to influence the oil content of these cultivars. Of all the citrus fruits studied, lemons showed the greatest seasonal variability in oil content.

Potential yield of citrus essential oils

Table 4 contains information derived from the oil content

data in the present study and from published information (Citrus Summary, 1973) about the annual processed tonnage of each variety of Florida citrus fruit. The important commodity, cold-pressed oil, may only be obtained by processing the fruit. Since the percentage of the total crop sold to processing plants in Florida is quite large, this amounts to a considerable quantity of available oil. For example, 98% of the lemons, 92% of all oranges and 63% of the grapefruit are processed for juice and, in most cases, some peel oil is recovered. The yield of peel oil recovery is limited by technology and manufacturing practices. However, utilizing the most up-to-date and efficient technology available for the recovery of essential oils, the citrus industry should be able to recover 65–70% of the total oil in the fruit. This would amount to some 60–65 million lb annually from the potential of 92 million lb listed in Table 4. Using present technology, the Florida Citrus Industry is pro-

Table 1—Peel oil content of various orange cultivars

Cultivars	Season	Processing dates	No. of samples		Peel oil (lb oil/ton of fruit) Max	Min	Avg
Hamlin	1968–69	Oct.–Feb.	4		9.2	8.9	9.1
	1969–70		27		6.5	4.8	5.8
	1970–71		15		7.9	7.1	7.4
	1971–72		18		10.4	7.2	8.9
				Avg =	8.5	7.0	7.8
Parson Brown	1968–69	Oct.–Feb.	24		17.6	12.5	14.7
	1969–70		15		9.1	7.1	7.9
	1970–71		15		11.3	8.1	9.6
	1971–72		18		11.8	8.6	10.2
				Avg =	12.5	9.1	10.6
Pineapple	1968–69	Dec.–Apr.	36		17.6	8.4	11.1
	1969–70		35		14.9	6.1	9.3
	1970–71		15		10.7	7.5	8.8
	1971–72		14		12.6	7.6	9.7
				Avg =	14.0	7.4	9.7
Valencia	1968–69	Mar.–July	113		18.8	9.2	16.0
	1969–70		118		18.7	9.8	13.5
	1970–71		12		13.1	11.3	12.1
	1971–72		12		14.4	11.3	12.2
				Avg =	16.3	10.4	13.5
Temple	1969–70	Dec.–Mar.	11		8.2	6.2	7.2
	1970–71		12		8.2	6.1	7.4
	1971–72		15		10.0	7.7	8.5
	1972–73		9		10.0	7.3	8.4
				Avg =	9.1	6.8	7.9

Table 2—Peel oil content of various grapefruit cultivars

Cultivars	Season	Processing dates	No. of samples		Peel oil (lb oil/ton of fruit) Max	Min	Avg
Duncan	1968–69	Oct.–June	14		7.1	5.1	6.1
	1969–70		18		6.8	4.8	6.0
	1970–71		27		7.2	4.8	5.4
	1971–72		24		6.6	4.3	4.9
				Avg =	6.9	4.8	5.6
Marsh	1968–69	Oct.–June	18		7.8	6.2	6.9
	1969–70		24		7.5	5.1	5.9
	1970–71		27		6.8	5.1	5.8
	1971–72		18		7.0	5.5	6.1
				Avg =	7.3	5.5	6.2
Ruby Red	1968–69	Oct.–June	1		—	—	7.2
	1970–71		27		7.9	4.8	6.1
	1971–72		27		7.5	5.3	6.2
	1972–73		27		8.1	5.2	6.5
				Avg =	7.8	5.1	6.5

Table 3–Peel oil content of miscellaneous citrus cultivars

Cultivars	Season	Processing dates	No. of samples		Peel oil (lb oil/ton of fruit) Max	Min	Avg
Dancy tangerine	1968–69	Nov.–Feb.	7		15.4	13.0	14.6
	1970–71		12		18.4	15.2	16.8
	1971–72		18		20.4	15.0	17.2
	1972–73		9		15.3	10.9	13.3
				Avg =	17.4	13.5	15.5
Orlando tangelo	1970–71	Dec.–Mar.	9		11.6	10.0	11.0
	1971–72		15		13.0	8.7	10.8
	1972–73		12		13.6	10.4	12.1
				Avg =	12.7	9.7	11.3
Persian lime	1968	May–Aug.	3		7.0	6.6	6.8
	1971		54		9.9	7.4	8.5
	1972		64		10.7	7.9	9.0
				Avg =	9.2	7.3	8.1
Lemons	1968	Aug.–Nov.	4		14.4	11.5	12.5
	1969		8		22.8	11.5	17.6
	1970		120		23.0	10.3	14.9
	1971		52		15.5	10.7	13.2
	1972		86		20.3	15.6	17.5
				Avg =	19.2	11.9	15.1

Table 4–Total potential of citrus essential oils from processed fruit during the 1972–73 season

Variety	Total crop processed (%)	Fruit processed (1000 tons)	Oil content (lb/ton)	Total oil available (lb)
Oranges:				
Early & mid-season	92.6	3,749	9.5[a]	35,615,500
Valencia	93.0	3,337	13.5	45,049,500
Temple	52.0	119	7.9	940,100
Grapefruit:				
Duncan	97.3	422	5.6	2,363,200
Marsh	59.5	595	6.2	3,689,000
Pink	38.0	189	6.5	1,228,500
Tangerines	32.7	44	15.5	682,000
Tangelos	51.2	81	11.3	915,300
Murcotts	39.2	16	13.4	214,400
Limes	53.0	26	8.1	210,600
Lemons	98.0	79	15.1	1,192,900

[a] 25% Hamlin, 25% Parson Brown, 50% Pineapple

ducing approximately 30 million lb of cold-pressed citrus oils and d-limonene annually, or about 50% of the maximum potential. This suggests that improvements in equipment, processing and handling techniques are needed to realize the optimum yield of essential oil.

REFERENCES

Bartholomew, E.T. and Sinclair, W.B. 1946a. Volatile oil content of the peel of oranges. Calif. Citrograph 31: 293.

Bartholomew, E.T. and Sinclair, W.B. 1946b. The factors influencing the volatile oil content of the peel of immature and mature oranges. Plant Physiol. 21(3): 319.

Bitters, W.P. and Scora, R.W. 1970. The influence of citrus rootstocks upon the volatile rind oil content of Valencia oranges. Bot. Gaz. 131(2): 105.

Citrus Summary, 1973. Fla. Agric. Statist., Fla. Dept. of Agric., Tallahassee, Fla.

Giacometti, D.C. 1952. Fruit characteristics of sweet orange varieties. Univ. Fla. thesis (unpublished).

Hendrickson, R., Kesterson, J.W. and Cohen, M. 1970. The effect of rootstock and budwood selections on the peel oil content of Valencia oranges. Proc. Fla. State Hort. Soc. 83: 259.

Hendrickson, R., Kesterson, J.W. and Ting, S.V. 1969. Peel oil content of Valencia oranges. Proc. Fla. State Hort. Soc. 82: 192.

Hood, S.C. 1916. Relative oil yield of Florida oranges. Ind. Eng. Chem. 8: 709.

Kesterson, J.W., Hendrickson, R. and Braddock, R.J. 1971. Florida citrus oils. Bull. 749, Fla. Agric. Exp. Stn. Tech. 180 p.

Ms received 1/29/75; revised 5/1/75; accepted 5/6/75.

Florida Agricultural Experiment Stations Journal Series No. 5747.

JOSEPH J. JEN and CHARLES S. GRAHAM
Departments of Food Science and Biochemistry, Clemson University, Clemson, SC 29631

GLUTAMIC OXALOACETIC TRANSAMINASE ACTIVITIES IN PEACHES DURING MATURATION

INTRODUCTION

GLUTAMIC oxaloacetic transaminase (GOT; E.C.2.6.1.1.) is ubiquitous in mammalian tissues and is implicated as maintaining balance between aspartate and glutamate concentration in living tissues. In plants, GOT has been linked to protein metabolism in pumpkin cotyledons (Splittstoesser, 1970). Hulme et al. (1967) concluded that oxaloacetate, a reactant of GOT reaction, may be involved in regulation of respiration during maturation of fruits. Yu et al. (1967) proposed that amino acids could serve as the precursors for the production of carbonyl compounds in ripening tomatoes. The formation of carbonyl compounds was attributed to transamination. Yu et al. (1967) also observed high glutamate concentration in ripe tomatoes. It was conceivable GOT was involved in flavor production in ripening tomato fruits.

Few characterizations of GOT in fruit tissues have been published. Romani (1962) studied GOT and alanine aminotransferase in pears and observed higher enzyme activities in the peel than in pulp tissue. Besford and Hobson (1973) observed a decline in GOT activity in tomatoes during ripening period. Rech and Crouzet (1974) purified the alanine transaminase in tomatoes 660-fold and presented kinetics data of the enzyme to be quite different from the same transaminase isolated from animal origin.

This study was carried out to establish the presence of GOT in peaches, to investigate the changes of specific activity of GOT during the maturation period and to characterize the peach GOT. The effect of succinic acid-2,2-dimethylhydrazide (SADH), a chemical used to hasten peach ripening, on GOT activity was also examined.

EXPERIMENTAL

Peaches

Peach trees, cultivars 'Redhaven' and 'Redskin,' were grown at the Simpson Experiment Station near Clemson, S.C. Half of the 'Redskin' trees were sprayed with 2000 ppm SADH just prior to the pit-hardened stage of maturation. Peaches were harvested from the trees at weekly intervals throughout the maturation period.

GOT extraction

For each extraction, 300g of peach pulp tissues were blended with 200 ml of a cold extraction buffer at 4°C. The buffer contained 1M K_2HPO_4, 0.1M KCl, and 16 mM sodium metabisulfite. The slurry was centrifuged at 18,000 × G for 30 min and the supernatant was brought to saturation with ammonium sulfate and left overnight at 0°C. The precipitate was collected by centrifugation at 25,000 × G and frozen at −20°C until assayed for GOT activity. The frozen precipitates retained full GOT activity for several months.

GOT assay

The precipitate was dissolved in 0.1M phosphate buffer and dialyzed vs 10^{-3} M phosphate buffer overnight. The pH was maintained at 7.4. The method of Romani (1962) was followed for the GOT assay. All components in the assay systems were buffered to pH 7.4 to prevent nonspecific destruction of NADH. One milliunit of GOT activity was defined as the amount of enzyme extract that could cause a decline of absorbance at 340 nm of 0.0021 per min which represented the consumption of one nanomole of NADH. Each assay was duplicate measurements of at least two levels of peach extracts. An assay was considered valid only if the absorbance change at 340 nm was proportional to the amount of peach extract added. Except in the kinetics study, the incubation time for GOT assay was 5 hr. Specific activity of GOT was expressed as units GOT per g protein. Protein content was determined by the commonly used Lowry method using bovine serum albumin as standard.

Temperature and pH optima

A partially purified peach GOT preparation, the precipitate of 25–50% ammonium sulfate saturation of the crude extract, was used for these purposes. The procedures were the same as a previous study on peach polyphenol oxidase (Jen and Kahler, 1974).

Kinetics study

Aspartate concentrations of 0.83, 1.67, 3.33, 6.67, 10 and 20 mM were used for GOT assay at two oxoglutarate concentrations, 1.67 and 3.33 mM. The reactions were conducted at room temperature using a Gilford 2400S recording spectrophotometer.

RESULTS & DISCUSSION

FIGURES 1 and 2 show the changes of GOT specific activity during the maturation period of 'Redhaven' and 'Redskin' peaches respectively. In both peach cultivars, the specific ac-

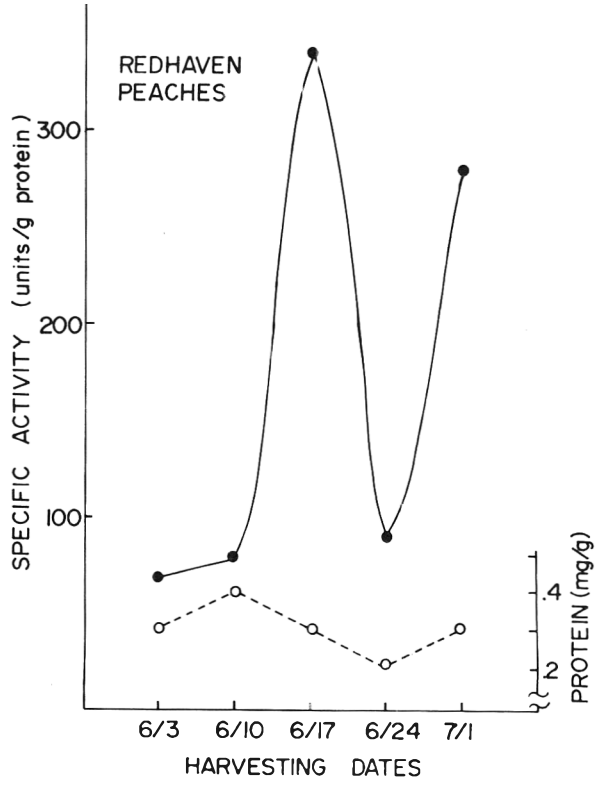


Fig. 1—GOT activity in 'Redhaven' peaches during maturation. Extractable protein content was in mg per g fruit on a fresh weight basis.

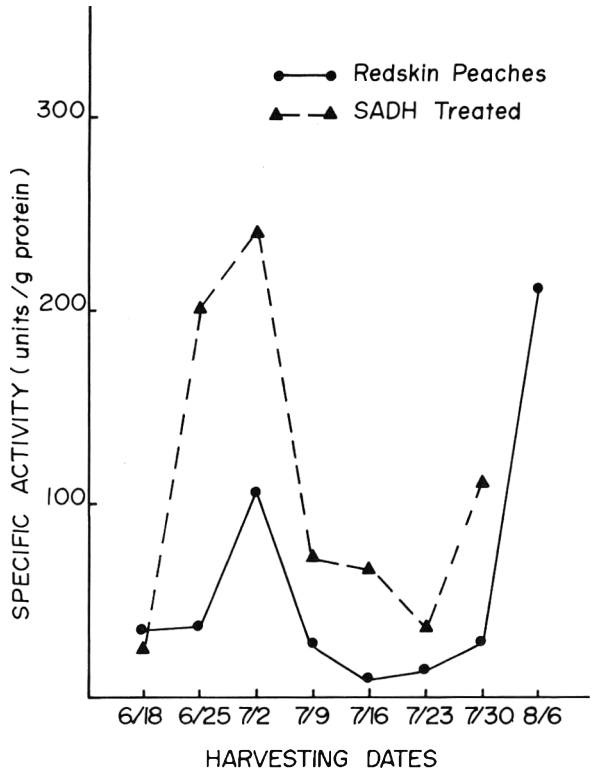


Fig. 2–GOT activity in 'Redskin' peaches during maturation, and the effect of SADH treatment.

tivity of GOT was low at the pit-hardened stage, but rose to a peak in two weeks and declined to a very low level until just before the ripening time when another rise occurred. 'Redskin' trees sprayed with SADH produced fruits with a higher GOT activity, particularly during the 2 wk immediately following the SADH treatment. The fruits were fully ripe about 1 wk ahead of the controls. The extractable protein per g fruit stayed reasonably constant as shown in Figure 1. The upsurge of GOT activity near the end of maturation was, perhaps, the result of accelerated protein biosynthesis to provide ripening enzymes for the fruit. It may also be involved in the production of flavoring compounds as proposed by Yu et al. (1968). The peak of GOT activity during the early maturation period correlated with the observed shuffle of proteinaceous amino acids and the decline in aspartate content reported earlier (Jen et al., 1975a). It is possible that GOT was closely related with protein metabolism in peach tissues during maturation.

The 'Redhaven' peaches possessed higher overall GOT activity than 'Redskin' peaches. Whether this higher activity was due to the shorter growing season of the 'Redhaven' than the 'Redskin' peaches was not known. The protein content of 'Redhaven' peaches is known to be greater than that of 'Redskin' peaches throughout the maturation period (Jen et al., 1975b). In ripening tomatoes, a slight decline of GOT activity was observed (Besford and Hobson, 1973) but that portion of enzyme located in mitochondria reached an activity peak in green-orange fruit. It was interesting to note that in ripe tomatoes lowered GOT activity may correspond to higher glutamate concentration as reported by Yu et al. (1967).

For characterization of the peach GOT, an enzyme extract from the ripe 'Redskin' peaches was partially purified from the crude homogenate by precipitation between 25–50% saturation with ammonium sulfate. This fraction gave a 12-fold purification and 60% yield in comparison with the crude extract. This is similar to the 13-fold purification of D-methionine:pyruvate aminotransferase obtained from peanut seedlings using a 35–50% ammonium sulfate fractionation of the crude extract (Durham et al., 1973).

Figure 3 shows the pH optimum of peach GOT was in the range 7.8–8.1. This value is close to the pH optimum reported by Romani (1962) for pear GOT at pH 8.4, by Splittstoesser (1970) for pumpkin cotyledon GOT at pH 8.0, and by Wong and Cossins (1969) for pea seedling GOT at pH 8.0. *Dolichos lablab* contained GOT with pH optimum in the range 8.2–8.5 (Patwardhan, 1970). Hobson (1974) in his electrophoretic investigation of enzymes from developing tomatoes, showed the GOT was concentrated into four zones at all stages of development. It is possible that isozymes of GOT exist in peaches.

The effect of temperature on peach GOT assay is shown in Figure 4. The reaction rate increased linearly from 10 to 36°C and then levelled off. Assays of peach GOT were not performed at temperatures higher than 46°C because of the spontaneous decarboxylation of oxaloacetate that would alter the coupled malate dehydrogenase reaction. In pears, the temperature optimum for GOT was reported to be at 37°C while it was 50°C for alanine aminotransferase (Romani, 1962). In tomatoes, alanine aminotransferase was inactivated quickly at 50°C (Rech and Crouzet, 1974). It should be noted that in these two references, coupled dehydrogenases were used as the assay method.

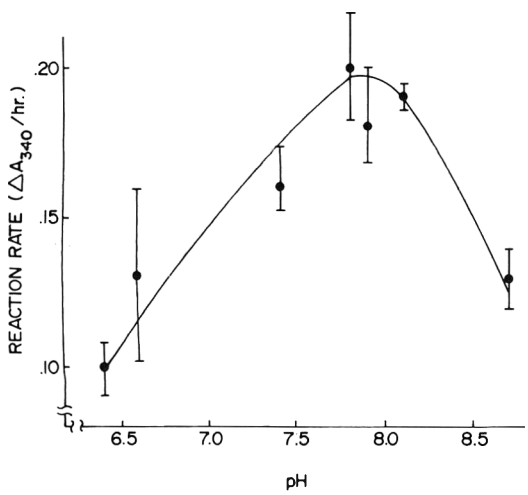


Fig. 3–Effect of pH on peach GOT activity. Vertical bars show the standard deviations.

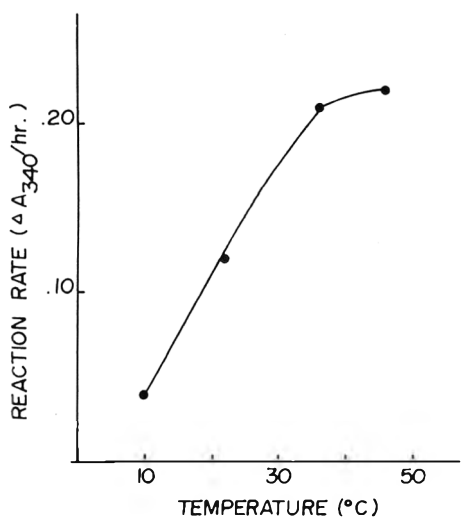


Fig.4–Effect of temperature on peach GOT activity.

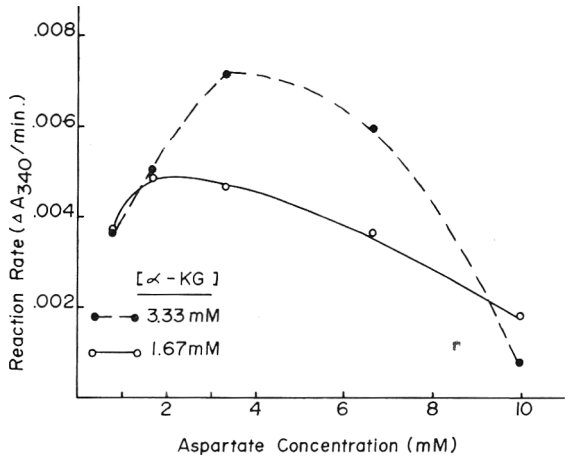


Fig. 5—Effect of substrate concentration on peach GOT activity.

An attempt was made to study the effect of substrate concentration on the peach GOT reaction (Fig. 5). It was interesting to note that at each oxoglutarate concentration, either greater or lesser ratios than the one to one ratio resulted in slower reaction rates. At a higher aspartate concentration, 20 mM, no measurable reaction occurred. This could be attributed either to a large burst of oxaloacetate formation which inhibited the coupled malate dehydrogenase reaction (Besford and Hobson, 1973) or to substrate inhibition of GOT as observed in mammalian cytoplasmic systems (Velick and Vavra, 1962; Henson and Cleland, 1964). In small green peaches at the pit-hardened stage, a high concentration of aspartate existed in the peach proteins (Jen et al., 1975a) which corresponds to low GOT activity found in this study. The surge of GOT activity during early maturation correlated with a sharp decline in aspartate content in peach proteins, indicating GOT was closely related with protein metabolism. Since aspartate occupies over 90% of the free amino acids pool in peaches (unpublished data), it was possible the peach GOT was regulated by substrate inhibition. This proposal received a blow when a less concentrated peach extract was used to repeat the kinetics study. Little or no inhibition was observed by high aspartate concentration. Until a purified peach GOT is available, the reaction mechanism of GOT remains unclear.

It appears that peach GOT has similar characteristics as GOT isolated from other plant sources. The level of peach GOT activity during the maturation period, however, differs to that reported in ripening tomatoes. The changes of peach GOT activity throughout the maturation period indicated that the enzyme probably plays an important role in protein metabolism of fruit tissues.

REFERENCES

Besford, R.T. and Hobson, G.E. 1973. Glutamate oxaloacetate transaminase activity in developing Lycopersicon esculentum. Phytochem. 12: 1255.

Durham, J.I., Morgan, P.W., Prescott, J.M. and Lyman, D.M. 1973. An aminotransferase specific for the D-enantiomorph of methionine. Phytochem. 12: 2123.

Henson, C.P. and Cleland, W.W. 1964. Studies of glutamic oxaloacetic transaminase isozymes. Biochem. 3: 338.

Hobson, G.E. 1974. Electrophoretic investigation of enzymes from developing Lycopersicon esculentum fruit. Phytochem. 13: 1383.

Hulme, A.C., Rhode, M.J.C. and Wooltorton, L.S.C. 1967. The respiration climacteric in apple fruits: some possible regulatory mechanisms. Phytochem. 6: 1343.

Jen, J.J., Graham, C.S. and Skelton, B.J. 1975a. Changes of amino acid composition of peach proteins during maturation. HortScience 10: 129.

Jen, J.J., Ibrahim, I.M., Graham, C.S. and McCollum, W.M. 1975b. Amino acid composition of proteins in peach alcohol insoluble solids. J. Food Sci. 40: 436.

Jen, J.J. and Kahler, K.R. 1974. Characterization of polyphenol oxidase in peaches grown in the southeast. HortScience 9: 590.

Patwardhan, M.V. 1960. Glutamic-aspartic transaminase of Dolichos lablab. Biochem. J. 75: 401.

Rech, J. and Crouzet, J. 1974. Partial purification and initial studies of the tomato L-alanine:2-oxoglutarate aminotransferase. Biochem. Biophys. Acta 350: 392.

Romani, R.J. 1962. Properties of transaminases in tissues of the pear. Plant Physiol. 37: 523.

Splittstoesser, W.E. 1970. Characterization of aspartate aminotransferase in germinating pumpkin cotyledons. Plant & Cell Physiol. 11: 579.

Velick, S.F. and Vavra, J. 1962. A kinetic and equilibrium analysis of the glutamic oxaloacetate transaminase mechanism. J. Biol. Chem. 237: 2109.

Wong, K.F. and Cossins, E.A. 1969. Studies of the particulate and soluble aspartate aminotransferase in germinating pea cotyledons. Phytochem. 8: 1327.

Yu, M., Olson, L.E. and Salunkhe, D.K. 1967. Precursors of volatile components in tomato fruit. 1. Compositional changes during development. Phytochem. 6: 1457.

Yu, M., Olson, L.E. and Salunkhe, D.K. 1968. Precursors of volatile components in tomato fruit. 2. Enzymatic production of carbonyl compounds. Phytochem. 7: 555.

Ms received 2/26/75; revised 4/24/75; accepted 4/28/75.

Presented at the 35th annual meeting of the Institute of Food Technologists in Chicago, June 9—11, 1975.

Technical contribution No. 1243 of the South Carolina Agricultural Experiment Station. Thanks are due to Dr. B.J. Skelton for supply of peaches used in this study.

R. PRESSEY and J. K. AVANTS
USDA Richard B. Russell Agricultural Research Center, ARS, Athens, GA 30604

CUCUMBER POLYGALACTURONASE

INTRODUCTION

FABIAN et al. (1932) first suggested that the pectic substances might be involved in textural changes in cucumbers. This was supported by the observation that the insoluble pectin in cucumbers is converted to a soluble form during the pickling process (Fabian and Johnson, 1938). Furthermore, Bell et al. (1950) observed a good correlation between cucumber firmness and pectolytic activity in curing brines. The possible source of the enzyme(s) may be the molds in the brine (Demain and Phaff, 1957). But cucumbers contain a polygalacturonase (Bell, 1951) and the importance of this enzyme in pectin solubilization in cucumbers remains to be determined.

The only published information on cucumber polygalacturonase was obtained by using the viscometric assay with pectin as the substrate (Bell, 1951). Unusually long incubation periods (6 days) were required, suggesting extremely low activity. A possible reason for the low observed activity is that pectate rather than pectin is the substrate for polygalacturonases (Jansen and MacDonnell, 1945). Furthermore, the viscometric method would be sensitive for random-cleaving enzymes, and it is now known that exopolygalacturonases occur in higher plants. We have therefore prepared concentrated solutions of partially purified cucumber polygalacturonase and re-examined its properties.

EXPERIMENTAL

Materials and methods

The substrates pectate, PGA (polygalacturonate) I, PGA II, reduced PGA II, and PGA III were prepared and purified as described earlier (Pressey and Avants, 1973). The oligogalacturonates were prepared by partially hydrolyzing pectate with fungal pectinase (Sigma Chemical Co.) followed by resolution of the oligomers by column chromatography on DEAE-Sephadex A-50. This was achieved by elution with increasing concentrations of NaCl. The oligogalacturonates were characterized and identified by paper chromatography and AGA (anhydrogalacturonic acid)/reducing group ratios (Pressey and Avants, 1975).

Table 1—Kinetic parameters of the hydrolysis of galacturonans by cucumber polygalacturonase

Substrate	K_m M X 10^6	Maximum velocity
Digalacturonate	570	0.5
Trigalacturonate	240	2.1
Tetragalacturonate	230	2.8
Pentagalacturonate	200	3.9
Hexagalacturonate	170	4.5
PGA III[a]	68	4.2
PGA II[b]	51	4.8
PGA I[c]	22	5.6
Pectate[d]	34	7.2

[a] Assuming D.P. = 13 (D.P. = degree of polymerization)
[b] Assuming D.P. = 20
[c] Assuming D.P. = 79
[d] Assuming D.P. = 201

Polygalacturonase activity was assayed by measuring the release of reducing groups The standard reaction mixture consisted of 0.2 ml 0.1M acetate-Tris buffer, pH 5.5, 0.5 ml 1% PGA I, pH 5.5, 0.1 ml 0.01M $CaCl_2$ and 0.2 ml enzyme solution in 0.15M NaCl. The assay conditions and unit of activity have been reported (Pressey and Avants, 1973).

Extraction and purification of polygalacturonase

Burpee hybrid cucumbers were peeled, and 100g of slices blended with 100 ml 0.15M NaCl in a VirTis homogenizer. Ten homogenates were prepared and combined, and further macerated with a Polytron homogenizer (Brinkmann Instruments). The pH of the slurry was adjusted to 6 with dilute NaOH. The sample was stirred in the cold for 2 hr, then centrifuged at 8000 × G for 30 min. The supernatant solution was concentrated to about 60 ml by ultrafiltration with a PM-10 membrane (Amicon Corp.) and dialyzed against 0.15M NaCl for 16 hr.

The dialyzed solution was clarified by centrifugation and applied to a 5 × 90 cm column of Sephadex G-100 in 0.15M NaCl, pH 6. The column was eluted with 0.15M NaCl. A single peak of polygalacturonase activity was detected by the standard assay. The fractions containing the enzyme were pooled and concentrated to about 10 ml by ultrafiltration; specific activity of the preparation was 39.

RESULTS

Extractability of cucumber polygalacturonase

The pectic enzymes in higher plants usually are associated with the insoluble cell wall material (Kertesz, 1938; McCready et al., 1955). A relatively high concentration of NaCl is normally required to solubilize the enzymes. After the enzymes have been extracted and separated from the cell wall components, they remain soluble in solutions of low ionic strength (Patel and Phaff, 1960). This property permits the removal of the water-soluble constituents prior to extraction of the enzymes and hence facilitates their purification. In contrast to this general behavior of pectic enzymes, we found that cucumber polygalacturonase was readily extracted by water. Single step extractions of cucumber slices with equal weights of cold water, 0.1M NaCl, 0.25M NaCl, and 1.0M NaCl yielded nearly equal amounts of activity. Extraction of 100g slices with water at pH 6 released 63 units of polygalacturonase activity. Re-extraction of the residue with an additional 100 ml water yielded another 2 units of activity. But only 3 units of activity were obtained when the water insoluble residue was extracted with 100 ml of 1.0M NaCl.

Effects of substrate size and concentration

The action of cucumber polygalacturonase was studied on a series of substrates with increasing chain lengths consisting of oligogalacturonates, polygalacturonates and pectate. The kinetic data are presented in Table 1. The enzyme hydrolyzed digalacturonate slowly, but the rate of reaction increased markedly as the number of monomeric units in the substrate increased to about 6. The reaction velocity increased slowly as the substrate size increased further and was maximal for pectate, the largest substrate. K_m decreased with increasing substrate size and was lowest for PGA I.

Mode of action

Comparison of the change in viscosity of a polymeric substrate with the rate of chain cleavage can help identify the

mode of enzyme hydrolysis. Reaction mixtures containing 0.5% pectate, pH 5.5, and cucumber polygalacturonase were therefore monitored for viscosity and reducing groups. At an enzyme level of 0.04 ml/ml, the viscosity dropped 9% after 2 hr while 0.63 μmoles reducing groups were released. This represents cleavage of 2.7% of the glycosidic linkages in the substrate. Higher levels of polygalacturonase increased the rates of viscosity loss and reducing group formation, but the viscosity remained relatively high even after extensive degradation of the substrate based on the levels of reducing groups. This is characteristic of a terminal cleaving enzyme, whereas a random cleaving polygalacturonase would decrease the viscosity markedly with the release of only a few reducing groups (Pressey and Avants, 1973).

If the cucumber enzyme cleaves terminal bonds, questions remain on the nature of the product released and whether the substrate is attacked from the reducing or nonreducing end. The product was a reducing sugar as measured by the arsenomolybdate method, but it was inactive in the thiobarbituric acid and ultraviolet absorption assays (Preiss and Aswell, 1962). The latter assays rule out the possibilities that the product was 4-deoxy-5-ketouronic acid or an unsaturated oligogalacturonate which would be formed if the enzyme were a trans-eliminase rather than a hydrolase. Paper chromatographic analysis using the solvent ethyl acetate-acetic acid-water (10:5:6) of the enzymatic hydrolyzates of polygalacturonate showed a single spot. This component corresponded to galacturonic acid in R_f and its color reaction with diphenylamine-aniline spray reagent. Digalacturonate and higher oligogalacturonates were not visible on the chromatograms.

That galacturonate is the product of cucumber polygalacturonase action was confirmed by analyzing the products of pentagalacturonate hydrolysis. A reaction mixture containing 12.5 mg pentagalacturonate and 0.5 ml polygalacturonase was incubated at 37° for 2 hr. The reaction was stopped by heating the sample in boiling water. The solution was applied to a 2.5 x 40 cm column of DEAE-Sephadex A-50 in water at pH 6. Elution was conducted with a linear gradient of 0.05–0.2M NaCl, and the 10 ml fractions were analyzed for AGA. The elution pattern is presented in Figure 1. Based on elution volumes for known oligogalacturonates on this column, the peaks correspond to galacturonate (2.50 mg), trigalacturonate (1.78 mg), tetragalacturonate (5.50 mg) and pentagalacturonate (1.70 mg). Digalacturonate, which normally eluted at fraction 32, was not detectable. It can be readily calculated that the galacturonate was formed by cleavage of pentagalacturonate to tetragalacturonate followed by hydrolysis of tetragalacturonate to trigalacturonate. From the data of Table 1, one would not expect appreciable hydrolysis of the small amount of trigalacturonate. Degradation, therefore, occurs by stepwise removal of monomer units from the substrate.

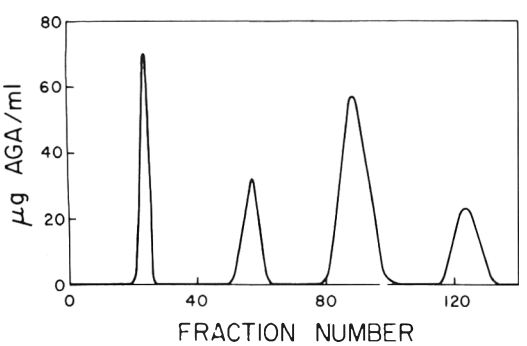


Fig. 1–The elution profile from a DEAE-Sephadex A-50 column of the products of partial hydrolysis of pentagalacturonate by cucumber polygalacturonase. The experimental details are given in the text. The peaks represent galacturonate, trigalacturonate, tetragalacturonate, and pentagalacturonate, in that order from left to right.

The procedure used for peach exopolygalacturonase to establish which end of the substrate molecule is attacked (Pressey and Avants, 1973) was applied to the cucumber enzyme. The rates of cleavage of PGA II and borohydride-reduced PGA II were nearly identical (0.26 and 0.27 μmoles reducing groups/ml/hr). The products of a partial hydrolysis of reduced PGA II were separated on the basis of solubility of their strontium salts in 63% ethanol. The two fractions (soluble and insoluble salts) were deionized with Dowex-50 (H^-) and analyzed for reducing groups. Most of the reducing groups (96%) were in the strontium-soluble fraction. The results indicate that the reduced terminal group in the substrate does not affect the susceptibility of the substrate to attack by cucumber polygalacturonase. Furthermore, the only reducing groups generated by the enzyme are the monomer units released, and the undegraded residue of partially hydrolyzed reduced PGA II remains nonreducing. Therefore, cleavage must proceed from the nonreducing ends of the substrate molecules.

Effects of pH and divalent cations

Cucumber polygalacturonase was active between pH 3 and 8 with an optimum at 5.5. The range of activity and the optimum were independent of substrate size. Ca^{2+} activated the partially purified enzyme 2.5-fold, with maximal activation at 0.4 mM $CaCl_2$. Mg^{2+}, Sr^{2+}, Ba^{2+} and Mn^{2+} at 0.4 mM levels did not affect the activity. As with peach expolygalacturonase (Pressey and Avants, 1973), 0.1M EDTA or citrate reduced the activity to zero.

Molecular weight

The molecular weight of the enzyme was estimated by gel filtration on a 2.5 x 90 cm column of Sephadex G-100 in 0.15M NaCl. From the elution volumes of cytochrome C, ovalbumin, bovine serum albumin (monomer and dimer), the calculated molecular weight of cucumber polygalacturonase is 59,000.

Polygalacturonase activity in pickling cucumbers

The Burpee hybrid cucumbers used for the isolation and characterization of cucumber polygalacturonase are a slicing variety. A pickling variety of cucumber was also examined for activity. Freshly harvested Pixie cucumbers were separated on the basis of size into three groups with average weights of 55, 135 and 230g. Extracts were prepared from 100g of each sample by the standard procedure and concentrated to 10 ml. The levels of enzyme activity in the dialyzed solutions were 0.29, 0.63 and 0.87 units/ml for the small, medium and large cucumbers, respectively. The activity in a comparable extract of mature Burpee hybrid cucumbers was 1.56 units/ml.

DISCUSSION

THE POLYGALACTURONASE in cucumbers is an exo-splitting enzyme. This is the third exopolygalacturonase identified in higher plants, with previous reports of this enzyme in carrots (Hatanaka and Ozawa, 1964) and peaches (Pressey and Avants, 1973). The cucumber enzyme is similar to the peach enzyme in pH optimum, cation activation and molecular weight. It is also similar to the carrot and peach enzymes in the stepwise removal of monomer units from the nonreducing ends of the substrate molecules. It exhibits the highest affinity for large substrate molecules which it cleaves most rapidly.

The physiological function of an exopolygalacturonase in cucumbers is not clear. As in carrots, this appears to be the only pectate hydrolyzing enzyme in cucumbers. Polygalacturonases have been implicated in the softening of various fruits (Hasegawa et al., 1969; Hobson, 1964; Pressey et al., 1971), but these enzymes are endo-splitting. Their action on a

relatively few bonds could markedly alter the pectin molecules and consequently the cell walls. If an exopolygalacturonase participates in the disruption of cell walls associated with softening of cucumbers, the mechanism is not random cleavage of pectin but rather specific hydrolysis of terminal linkages. Current knowledge of plant cell wall structure (Keegstra et al., 1973) does not indicate importance to terminal blocks of galacturonates, nor is there evidence that pectin is highly branched. But it is conceivable that certain linkages susceptible to exopolygalacturonase action might be critical bonds in the cell wall of firm fruit.

REFERENCES

Bell, T.A., Etchells, J.L. and Jones, I.D. 1950. Softening of commercial cucumber salt-stock in relation to polygalacturonase activity. Food Technol. 4: 157.

Bell, T.A. 1951. Pectolytic enzyme activity in various parts of the cucumber plant and fruit. Bot. Gaz. 113: 216.

Demain, A.L. and Phaff, H.J. 1957. Cucumber Curing: Softening of cucumbers during curing. J. Agr. Food Chem. 5: 60.

Fabian, F.W., Bryan, C.S. and Etchells, J.L. 1932. Experimental work on cucumber fermentation. Mich. Agr. Expt. Sta. Tech. Bull. 126.

Fabian, F.W. and Johnson, E.A. 1938. Experimental work on cucumber fermentation. Mich. Agr. Exp. Sta. Tech. Bull. 157.

Hatanaka, C. and Ozawa, J. 1964. Enzymic degradation of pectic acid. Part I. Limited hydrolysis of pectic acids by carrot polygalacturonase. Agr. Biol. Chem. 28: 627.

Hasegawa, S., Maier, V.F., Kaszychi, H.P. and Crawford, J.K. 1969. Polygalacturonase content of dates and its relation to maturity and softness. J. Food Sci. 34: 527.

Hobson, G.E. 1964. Polygalacturonase in normal and abnormal tomato fruit. Biochem. J. 92: 324.

Jansen, E.F. and MacDonnell, L.R. 1945. Influence of methoxyl content of pectic substances on the action of polygalacturonase. Arch. Biochem. 8: 97.

Keegstra, K., Talmadge, K.W., Bauer, W.D. and Albersheim, P. 1973. The structure of plant cell walls. 3. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. Plant Physiol. 51: 188.

Kertesz, A.I. 1938. Pectic enzymes. 2. Pectic enzymes of tomatoes. Food Res. 3: 481.

McCready. R.M., McComb, E.A. and Jansen, E.F. 1955. The action of tomato and avocado polygalacturonase. Food Res. 10: 186.

Patel, D.S. and Phaff, H.J. 1960. Studies on the purification of tomato polygalacturonase. Food Res. 25: 37.

Preiss, J. and Ashwell, G. 1962. Alginic acid metabolism in bacteria. 1. Enzymatic formation of unsaturated oligosaccharides and 4-deoxy-L-erythro-5-hexoselulose uronic acid. J. Biol. Chem. 237: 309.

Pressey, R., Hinton, D.M. and Avants, J.K. 1971. Development of polygalacturonase activity and solubilization of pectin in peaches during ripening. J. Food Sci. 36: 1070.

Pressey, R. and Avants, J.K. 1973. Separation and characterization of endopolygalacturonase and exopolygalacturonase from peaches. Plant Physiol. 52: 252.

Pressey, R. and Avants, J.K. 1975. Modes of action of carrot and peach exopolygalacturonases. Phytochem. Accepted for publication.

Ms received 2/13/75; revised 4/24/75; accepted 4/28/75.

G. S. RANHOTRA and R. J. LOEWE
Nutrition Lab., American Institute of Baking, Chicago, IL 60611

EFFECT OF WHEAT PHYTASE ON DIETARY PHYTIC ACID

INTRODUCTION

IT IS MAINTAINED that possible absence (Anonymous, 1967; Kon et al., 1973) or inhibition (Bitar and Reinhold, 1972; Reinhold et al., 1973) of intestinal phytase activity in man is perhaps responsible for the high incidence of mineral-deficiency diseases in population groups consuming high-cereal and thus high-phytate diets. The presence of undegraded phytates as in the "natural" foods (Berlyne et al., 1973; McBeans and Speckmann, 1974) or of partially-degraded phytates as in bread (Reinhold et al., 1973; Berlyne et al., 1973; Ranhotra, 1972; Ranhotra et al., 1974a) thus remains a potential source capable of inducing deficiencies of iron, magnesium, zinc, calcium and possibly other elements. In animal species where the presence of active intestinal phytase and resultant phytate cleavage has been shown, phytate interference constitutes much less serious a problem (Anonymous, 1967; Pileggi et al., 1955; Maddaiah et al., 1963; Nelson et al., 1971; Ranhotra et al., 1974b). Although the ability of man to hydrolyze phytates remains a controversial subject, some hydrolysis in the digestive tract does invariably occur probably due to microbial phytases or nonenzymatic cleavage (Nicolaysen and Njaa, 1951; Hegsted et al., 1954; Subrahmanyan et al., 1955). Whether plant phytases are also involved in this hydrolysis is not understood. Present work, using wheat phytase, was thus undertaken to examine this.

EXPERIMENTAL

FOUR EXPERIMENTS were conducted. In experiment 1, wheat protein concentrate (WPC) prepared from fibrous mill-fractions was obtained commercially (Dixie-Portland Mills). In all other experiments, WPC used represented a mixture (1:1) of finely ground bran and shorts; other materials tested included whole wheat flour, high-fat soy flour, corn starch, and sodium phytate (General Biochemicals).

All studies were conducted using male Sprague-Dawley rats fed non-phytate diets for 4–6 wk and weighing, on the day of test-feeding, about 150–250g. Rats were starved overnight before test diets were fed and, 3 hr after feeding, all rats were sacrificed and ingesta collected from their stomach and intestines. Pooled ingesta (5–8 rats per diet) were thoroughly homogenized and a portion freeze dried for the determination of phytic acid content; determinations were made after the dried ingesta and test diets were exposed to room-air to equalize in moisture content. All results are thus expressed on equal moisture basis.

Thermal inactivation of phytase in wheat (WPC and flour) was carried out by heating test samples, in a closed container, for 3 hr at 100°C (Table 1). Isolated wheat phytase required adequate moistening with water before partial inactivation could be attained as above; samples were freeze dried following such an inactivation, finely ground and then used.

Phytase activity in wheat was measured (triplicate determinations) by the method of Peers (1953). Using Michaelis barbital sodium-acetate buffer (pH, 2.6–9.4), phytase activity was measured at 55°C and also at 37°C and at three pH (2.6, 5.2 and 8.0) to simulate gastrointestinal conditions. No phytase activity was measurable in the soy preparation tested (method of Gibbins and Norris, 1973). Unit of activity (U) is defined as the amount of enzyme releasing 1 mg of inorganic phosphorus from 1.6×10^{-3} M phytic acid at pH 5.15 in 1 hr (Ranhotra, 1973). Phytic acid in the diet and in pooled ingesta was determined by the method of Makower (1970) as described earlier (Ranhotra, 1972, 1973). A crude preparation of wheat phytase was prepared by the method of Peers (1953) using finely ground bran and shorts; final steps of precipitation and dialysis did not appear, under our laboratory conditions, to increase enzyme activity appreciably and hence were not undertaken.

RESULTS & DISCUSSION

ALTHOUGH SOME EVIDENCE to the contrary has been reported recently (Bitar and Reinhold, 1972), it is still maintained that man lacks intestinal phytase and thus cannot hydrolyze dietary phytates. Hydrolysis of dietary phytates in the digestive tract is, however, suggested by balance studies (Nicolaysen and Njaa, 1951; Hegsted et al., 1954; Subrahmanyan et al., 1955) but it is not known if plant phytases are involved in this hydrolysis.

Results in experiment 1 (Table 2) indicate that while wheat phytase activity decreased greatly after WPC was ingested, appreciable activity still remained in the stomach ingesta of rats. Also, more than half of the ingested phytate was hydrolyzed. Because no phytase is reported to be secreted by the stomach, the phytate hydrolysis was thus brought about by wheat phytase and/or nonenzymatically e.g., through gastric acidity. These results were examined in greater depth in the succeeding experiments. Thus in experiment 2 (Table 3), when animals were fed corn starch (no phytase present) containing added phytate, considerable phytate hydrolysis occurred in

Table 1—Thermal (100°C) inactivation of wheat phytase

Time, hr	Phytase activity[a] U/100g	Loss, %
0.0	1014	0.0
1.5	761	25.0
3.0	122	88.0
4.5	88	91.3
6.0	69	93.2
7.5	60	94.1

[a] Method of Peers (1953)

Table 2—Phytate hydrolysis in stomach (Experiment 1)

	Phytate phosphorus[a] mg/100g	Phytase activity[b] U/100g
WPC (diet)	1033	395
WPC (ingesta)	492	142

[a] Air-dried basis
[b] Method of Peers (1953)

Table 3—Phytate hydrolysis during digestion (Experiment 2)

Diet	Added (A) Native (N)	Phytase activity[a] at 37°C				Phytate phosphorus		
		Diet	pH 2.6	pH 5.2	pH 8.0	Diet	Stomach ingesta[b]	Small intestine ingesta[b]
		U/100g				mg/100g		
A_1 Starch + phytate	–	0	0	0	0	456	66 (86)	316 (31)
A_2 Starch + phytate	A	224	589	338	142	494	6 (99)	337 (32)
B_1 Soy	A	203	623	432	34	684	574 (16)	125 (82)
B_2 Soy	A[c]	149	98	23	0	677	692 (0)	98 (86)
C_1 Whole wheat flour	N	193	187	169	2	295	48 (84)	52 (82)
C_2 Whole wheat flour	N[c]	23	0	0	0	277	267 (4)	91 (67)
D_1 WPC	A[c],N	349	867	347	0	1390	770 (45)	242 (83)
D_2 WPC	A[c],N[c]	153	103	0	0	1312	959 (27)	292 (78)

[a] Determined by the method of Peers (1953) but using Michaelis buffer. Activities of diets, where isolated phytase was also added differed somewhat from the calculated contributed values. Phytase activity of isolated enzyme: 890 U/100g (uninactivated), and 409 U/100g (partially inactivated).
[b] Values within parentheses indicate percent dietary phytate hydrolyzed
[c] Phytase inactivated

the stomach, apparently nonenzymatically. More phytate was hydrolyzed, however, when isolated wheat phytase was also added to the above diet (A_1 vs A_2). The significance of wheat phytase was again apparent when rats were fed soy (contains phytate but no phytase activity); the addition of phytase to soy brought about gastric hydrolysis of dietary phytate while no hydrolysis occurred when the added phytase was inactivated (diets B_1 vs B_2). In fact some phosphorylation to phytate in the stomach was even observed. Evidence for an important role of wheat phytase was most convincing, however, when whole wheat flour was fed to the animals; in the absence of thermal inactivation of wheat phytase, 84% of the dietary phytate was hydrolyzed in the stomach whereas thermal inactivation of phytase (Table 1) resulted in almost complete lack of phytate hydrolysis (diets C_1 vs C_2). Further evidence suggesting the role of wheat phytase is provided by diets D_1 and D_2; rats fed phytase-inactivated WPC hydrolyzed, in the stomach, appreciably less phytate when the added phytase was also inactivated. Although replicate studies were not conducted, results with different materials tested (starch, soy, WPC, wheat flour) all point to the contribution—to varying degrees—of wheat phytase to phytate hydrolysis in the stomach. No appreciable difference in diet intake or stomach ingesta of each rat per diet was observed; this permitted pooling of ingesta which was necessary to yield adequate samples for analytical purposes.

It was observed that gastric hydrolysis of phytates in WPC varied greatly from experiment to experiment (Tables 2–4)

Table 4—Phytate hydrolysis during digestion (Experiments 3 and 4)

Diet	Phytase activity[a] at 37°C				Phytate phosphorus			
					Ingesta[b]			
							Small intestine	
	Diet	pH 2.6	pH 5.2	pH 8.0	Diet	Stomach	Upper	Lower
	U/100g				mg/100g			
				Experiment 3				
WPC	942	–	–	–	1182	1112 (6)	742 (37)[d]	
WPC[c]	92	–	–	–	1180	1155 (2)	913 (23)[d]	
				Experiment 4				
WPC	376	799	405	96	1550	1388 (12)	514 (67)	1302 (16)
WPC[c]	41	0	0	0	1421	1412 (1)	628 (56)	1341 (6)

[a] Determined by the method of Peers (1953) but using Michaelis buffer in Experiment 4
[b] Values within parentheses indicate percent dietary phytate hydrolyzed
[c] Phytase inactivated
[d] Represents ingesta collected from the entire small intestine

Table 5—Activity of isolated wheat phytase

Method		37°C		
Peers	Peers[a]	pH 2.6	pH 5.2	pH 8.0
U/100g			U/100g	
1300	890	1435	1279	1047

[a] Using Michaelis buffer

apparently depending on a number of factors: e.g., source and particle size of WPC, rate of ingestion, transit time and others. The effect of prior thermal inactivation of phytase in WPC was, however, still quite apparent in each case.

When wheat phytase was measured under simulated gastrointestinal conditions, its activity increased or only slightly decreased (Tables 3 and 4) at gastric pH (2.6). Activity then decreased at the pH (5.2) the ingesta would normally encounter when leaving the stomach and sharply so at a higher pH (8.0) typifying conditions in the intestine. This viewed in conjunction with phytate hydrolysis that occurred in the stomach of rats suggests that wheat phytase probably exerted its effect most profoundly in the stomach. Isolated wheat phytase also exhibited a sharp increase in its activity at simulated gastric conditions (37°C; pH 2.6) followed by gradual, though less pronounced, decreases at simulated intestinal conditions (Table 5).

Results in Tables 2–4 are also suggestive, though only weakly, of continued phytate cleavage in the intestine. Since intestinal phytase is secreted in the rat (Ranhotra et al., 1974b), assessment of the contribution of wheat phytase, if any, towards this cleavage is difficult to make. This is further complicated by the fact that active and probably variable absorption of digested nutrients occurs in the intestine resulting in a lack of consistency between comparable diets (Table 3). Although diet B did not conform to the pattern, inactivation of native and added phytase (diets C_1 vs C_2, and D_1 vs D_2) caused some reduction in the amount of phytate hydrolyzed in the intestine (Table 3). Also, when phytate hydrolyzed in the stomach was negligible (Table 4), effect of phytase inactivation became more apparent in the intestine; inactivation causing a reduction in the amount of phytate hydrolyzed. To obtain a better evidence of this, another experiment was conducted (Table 4). Here the ingesta was collected separately from the upper small intestine (mainly duodenum) and lower small intestine. It was now observed (experiment 4) that appreciably less phytate was hydrolyzed in the upper small intestine when dietary WPC-phytase was inactivated. Also, under simulated gastrointestinal conditions, no phytase activity in inactivated WPC was observed and that measured by the method of Peers (1953) was only slight.

Thus although results do not indicate the precise contribution of wheat phytase to the phytate hydrolysis in the digestive tract, they do indicate that wheat phytase is involved, more profoundly so in the stomach. This is of particular significance in humans because of the inhibitory effect of undegraded phytates on the availability of some essential minerals.

REFERENCES

Anonymous. 1967. Effect of phytate on iron absorption. Nutr. Rev. 25: 218.

Berlyne, G.M., Ari, J.B., Nord, E. and Shainkin, R. 1973. Bedouin osteomalacia due to calcium deprivation caused by high phytic acid content of unleavened bread. Am. J. Clin. Nutr. 26: 910.

Bitar, K. and Reinhold, J.G. 1972. Phytase and alkaline phosphatase activities in intestinal mucosa of rat, chicken, calf and man. Biochim. Biophy. Acta 268: 442.

Gibbins, L.N. and Norris, F.W. 1973. Phytase and acid phosphatase in dwarf beans, Phaseolus vulgaris. Biochem. J. 86: 67.

Hegsted, D.M., Trulson, M.F. and Stare, F.J. 1954. Role of wheat and wheat products in human nutrition. Physiol. Rev. 34: 221.

Kon, S., Olson, A.C., Frederick, D.P., Eggling, S.B. and Wager, J.R. 1973. Effect of different treatments on phytate and soluble sugars in California small white beans (Phaseolus vulgaris). J. Food Sci. 38: 215.

Makower, R.U. 1970. Extraction and determination of phytic acid in beans (Phaseolus vulgaris). Cereal Chem. 47: 288.

Maddaiah, V.T., Hulett, B.J., Kurnick, A.A. and Reid, B.L. 1963. Availability of calcium phytate, sodium phytate and dicalcium phosphate in chickens, hens and rats. Poultry Sci. 42: 1286.

McBeans, L.D. and Speckmann, E.A. 1974. A recognition of the interrelationship of calcium with various dietary components. Am. J. Clin. Nutr. 27: 603.

Nicolaysen, R. and Njaa, L.R. 1951. Investigations on the effect of phytic acid on the absorption of calcium in rats, pigs and man. Acta Physiol. Scand. 22: 246.

Nelson, T.S., Shieh, T.R., Wodzinski, R.J. and Ware, J.H. 1971. Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. J. Nutr. 101: 1289.

Peers, F.G. 1953. The phytase of wheat. Biochem. J. 53: 102.

Pileggi, V.J., Deluca, H.F. and Steenbock, H. 1955. The role of vitamin D and intestinal phytase in the prevention of rickets in rats on cereal diets. Arch. Biochem. Biophys. 58: 194.

Ranhotra, G.S. 1972. Hydrolysis during breadmaking of phytic acid in wheat protein concentrate. J. Food Sci. 37: 12.

Ranhotra, G.S. 1973. Factors affecting hydrolysis during breadmaking of phytic acid in wheat protein concentrate. Cereal Chem. 50: 353.

Ranhotra, G.S., Loewe, R.J. and Puyat, L.V. 1974a. Phytic acid in soy and its hydrolysis during breadmaking. J. Food Sci. 39: 1023.

Ranhotra, G.S., Loewe, R.J. and Puyat, L.V. 1974b. Effect of dietary phytic acid on the availability of iron and phosphorus. Cereal Chem. 51: 323.

Reinhold, J.G., Lahimgarzadeh, A., Nasr, K. and Hadayati, H. 1973. Effect of purified phytate and phytate-rich bread upon metabolism of zinc, calcium, phosphorus, and nitrogen in man. The Lancet 1: 283.

Subrahmanyan, V., Narayanarao, M., Ramarao, G. and Swaminathan, M. 1955. The metabolism of nitrogen, calcium and phosphorus in human adults on a poor vegetarian diet containing Ragi (Eleusine coracana). Brit. J. Nutr. 9: 350.

Ms received 3/6/75; revised 4/25/75; accepted 4/28/75.

S. P. NENE, U. K. VAKIL and A. SREENIVASAN
Biochemistry & Food Technology Div., Bhabha Atomic Research Centre, Bombay-400 085 India

EFFECT OF GAMMA RADIATION ON PHYSICO-CHEMICAL CHARACTERISTICS OF RED GRAM (Cajanus cajan) STARCH

INTRODUCTION

IT IS KNOWN that gamma irradiation alters, though to subtle degrees, the physico-chemical properties of macronutrients in foods. Several studies on the effects of ionizing radiation on starch in wheat (Lai et al., 1959; Milner, 1961) and barley (Faust and Massey, 1966) endosperm have been reported. The ultimate products of starch breakdown by irradiation have been characterized and these include low molecular weight dextrins (Ananthaswamy et al., 1970b) and deoxycompounds (Scherz, 1968). These changes, while of no major significance from the point of view of nutritional quality, may affect the physical and rheological properties of irradiated foods, resulting in increased solubility of starch in water (Deschreider, 1959) and decrease in swelling power (Tollier and Guilbot, 1970) and in relative viscosity (Vakil et al., 1973) of starch paste.

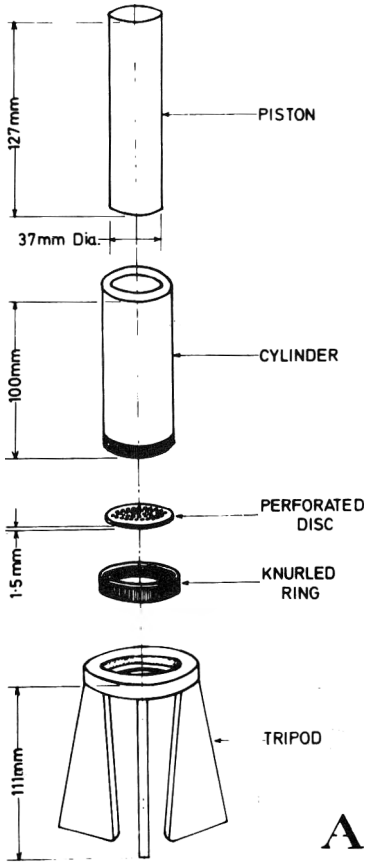
We have observed that radiation processing (1–3 Mrad) of pulses, particularly of red gram (*Cajanus cajan*), brings about significant reduction in cooking time and improves the textural quality. Red gram samples, irradiated at one Mrad were highly acceptable as judged by sensory evaluation using a nine-point hedonic scale. However, excessive browning and off-flavors developed on cooking the samples irradiated at two or three Mrad, making them unacceptable (unpublished data). Because of the practical importance of these observations, the present studies were undertaken to determine the compositional changes in irradiated red gram starch with special reference to the characterization of the radiolytic breakdown products and its in vitro susceptibility to alpha-amylase action. Rheological properties, such as gelatinization viscosity, swelling power and solubility of irradiated starch were also determined.

MATERIALS & METHODS

RED GRAM flour was purchased from a local market. Alpha-amylase (bacterial) was an E. Merck product; sucrose (Analar) and raffinose were purchased from the British Drug House Laboratory Chemicals Div. of Glaxo Laboratories, India.

Irradiation procedure

100-g lots of the pulse were packed in polythene bags and exposed



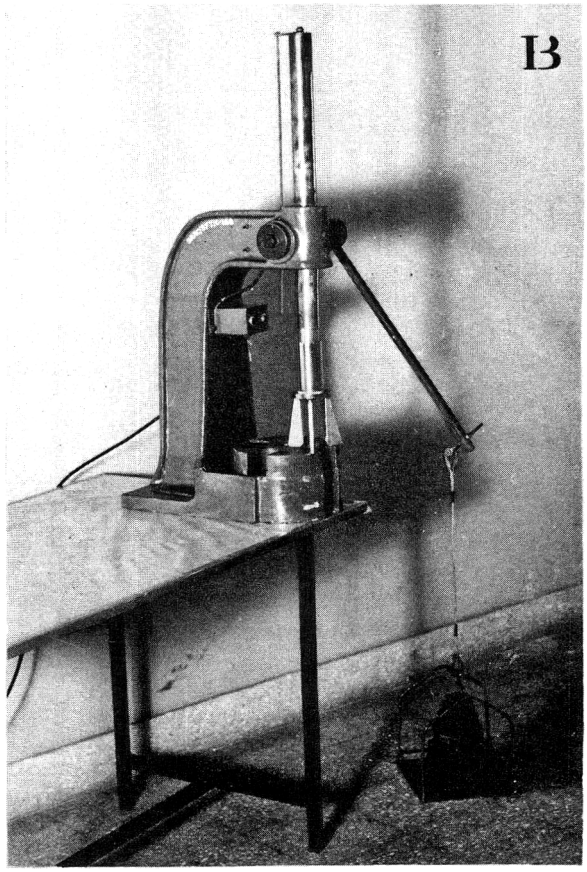


Fig. 1—Texturometer. Details for the sketch (A) and actual photograph (B) of arbor press and texturometer, are described in the text.

at ambient temperature (25°C) to a cobalt-60 source of gamma radiation (Gamma Cell 220, Atomic Energy of Canada Ltd.) having a flux of 15 Krad/min., at dose level of 1 Mrad. Absorption of radiation was checked with ferrous sulphate and ceric sulphate dosimetry (Weiss, 1952).

Isolation of starch

Red gram flour was suspended in 0.1M NaCl and centrifuged at 2000 × G. The upper protein layer was scraped out and the process repeated three times. The sediment was then washed four times with toluene (0.1 Vol) to remove the last traces of proteins and dried at 50°C under reduced pressure. Moisture content of starch was 13%.

Determination of the cooking time

Red gram (5g) samples were cooked for 2–30 min in 100 ml of boiling water. The extent of softening during cooking was measured by a texturometer (Fig. 1A) designed in our laboratory. This consisted of a stainless steel cylindrical chamber, fitted with a removable perforated disc at the bottom and a movable piston operating from the top. To measure the cooking times, test material cooked for various time intervals was put between them and to extrude it, pressure was applied on the piston by the arbor press, attached to a pan. The pulse was taken as cooked, when the weight added to the pan remained almost constant as for samples cooked for longer times. This was monitored by an electromechanical device, actuating an indicator lamp and recorded (Fig. 1B).

Estimation of sugars

Total reducing sugars were estimated colorimetrically using glucose as reference standard (Nelson, 1944). Nonreducing sugars were hydrolyzed with 6N HCl for 24 hr at room temperature and estimated as reducing sugars.

Separation of sugars by paper chromatography

Red gram flour from control and irradiated (1 Mrad) samples was dispersed in water (1g/10 ml), cooked for 25 and 16 min, respectively, at 100°C with constant stirring and cooled to room temperature (25°C). Uncooked and cooked samples were then extracted with 75% ethanol, centrifuged and the supernatants made to volume. An aliquot was applied on Whatman No. 3 filter paper. Descending chromatography, using butanol:acetic acid:water (12:3:5 v/v) as solvent system was carried out for 90 hr. Sucrose and raffinose were used as standards. Chromatograms were dried and sprayed with aniline (1% in acetone) diphenylamine phosphate reagent to detect the sugars (Smith, 1960). For quantitative analysis, areas corresponding to different spots were cut out from the undeveloped chromatogram using the developed one as marker. Sugars were eluted with distilled water and estimated colorimetrically, using phenol and H_2SO_4 reagents according to Duboise et al. (1956). Sucrose was used as a standard.

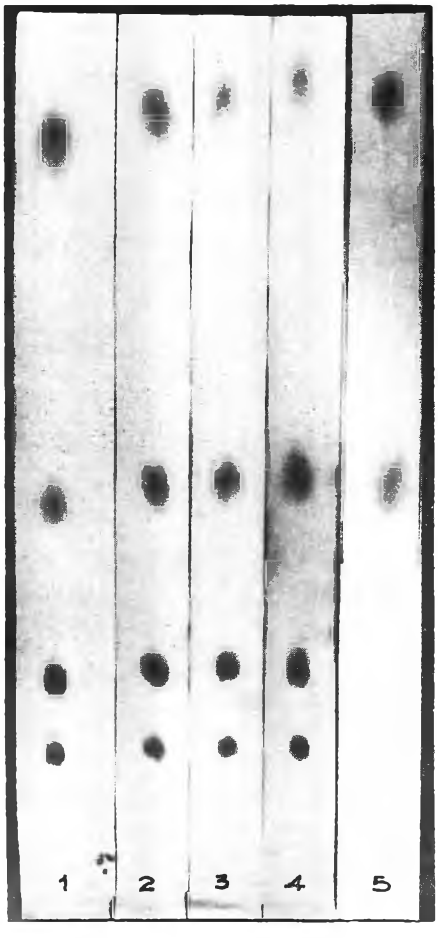


Fig. 2—Chromatographic separation of nonreducsugars: Total sugars were extracted with 70% ethanol from red gram samples, and aliquots were applied on Whatman No. 3 filter paper. Descending chromatography, using butanol:acetic acid: water (12:3:5 v/v) as the solvent system, was carried out. (1) control, cooked; (2) irradiated, cooked; (3) control, raw; (4) irradiated, raw; (5) sucrose (Rf. 1.0) and Raffinose (Rf. 0.4).

Table 1—Radiation effect on red gram sugars[a]

Sample	Initial sugars	
	Reducing (mg/g)	Nonreducing (mg/g)
Control	16.4 ± 1.2	85.7 ± 3.1
Irradiated (1 Mrad)	17.0 ± 0.8	83.5 ± 2.7

[a] Values are averages of three experiments. Aqueous extracts of red gram flour were analyzed for reducing and nonreducing sugars as described in the text.

In vitro alpha-amylolysis

Samples (1g) of unirradiated and irradiated (1 Mrad) red gram flour were cooked for 25 and 16 min, respectively. These were allowed to cool and suspended in 20 ml of 0.02M glycerophosphate buffer pH 5.9; 100 mg of alpha-amylase was added and the mixture incubated under toluene at 37°C with frequent shaking. Necessary blanks were carried out to correct for sugars present initially in the uncooked samples. At stated time intervals, an aliquot from the incubating mixture was removed and centrifuged. Reducing sugars were estimated in the supernatant and expressed in terms of maltose released. Rate of hydrolysis was measured up to 5 hr. Alpha-amylolysis of isolated starch (uncooked) was also carried out under similar experimental conditions.

Measurement of viscosity changes

Red gram flour (65g, 60 mesh) or 40g of isolated starch was mixed with distilled water (final slurry, 500 ml) to form a homogenous lump-free suspension. This was heated in the Brabender Amylograph with a constant temperature rise of 1.5°C/min. The maximal gelatinization viscosity, expressed as Amylogram Units (A.U.), temperature at which it took place and the time required were recorded. Pasting temperature, at which the viscosity of the slurry began to rise was also noted for the evaluation of the amylogram.

Solubility of red gram starch

Isolated red gram starch (5g) was dispersed in 100 ml of water and heated for 30 min in a water bath, maintained at the indicated temperatures with gentle stirring. The suspension was centrifuged and the supernatant dried under vacuum. Solubility was determined by weighing the residual dissolved starch (Schoch, 1964).

Swelling power of starch

Starch samples were cooked as described above and centrifuged. Swelling power was measured by determining the water retention capacity of undissolved starch, after making appropriate corrections for the dissolved starch. The swelling power of starch was calculated according to the following equation (Schoch, 1964).

$$\text{Swelling power (corrected)} = \frac{\text{wt of sedimented paste} \times 100}{\text{wt of sample} \times (100 - \%\ \text{solubles})\ \text{(on dry basis)}}$$

RESULTS

Cooking time

It was observed that the sensitivity and efficacy of the texturometer, fabricated by us, were comparable to those of the Instron Universal testing machine. Typical curves obtained with red gram showed exponential and logarithmic relationships of cooking time with the softening of the pulse as measured by the texturometer. These closely resembled the stress-strain curve obtained with Instron; the area under the curve

was measured by a planimeter and plotted against time (Nene et al., 1974). The time taken for cooking in terms of softening was reduced by 38.5% in irradiated (1 Mrad) sample (16 min) compared to the unirradiated one (26 min).

Initial sugars in irradiated red gram

Results on the effect of irradiation on red gram sugars are presented in Table 1. Initial water-soluble reducing and nonreducing sugars were not appreciably affected by radiation treatment at 1 Mrad. It can be noted that red gram contains about five times more nonreducing than reducing sugars.

Characterization of nonreducing sugars

Changes in the distribution pattern of nonreducing sugars, on cooking, were determined in control and irradiated samples. Figure 2 shows the chromatographic separation of sugars, extracted from different samples. Positions of sucrose and raffinose were established from the Rf values of authentic samples. Stachyose and verbascose were identified from the reported values obtained, using a similar solvent system (Nigam and Giri, 1961). Results on quantitative analyses of sugars are shown in Table 2. In unirradiated raw sample, these were composed of (%) sucrose (27), raffinose (16), stachyose (28) and verbascose (28). On irradiation (1 Mrad), slightly more raffinose was liberated, other sugars remaining almost unchanged. On cooking, significantly more sugars were liberated from irradiated sample (71.0 mg/g in 16 min) compared to control one (62.5 mg/g in 25 min). However, a significant decrease in raffinose + stachyose content in irradiated sample on cooking (40%) as compared to corresponding control (43%) was observed.

Susceptibility of red gram starch to alpha-amylase action

Results on the alpha-amylolysis of control and irradiated red gram flour (cooked or uncooked) as well as isolated starch (uncooked) are plotted in Figure 3. It can be seen that increasingly more maltose was liberated with progression of time in all the samples. Starch was more susceptible to alpha-amylase action in irradiated compared to unirradiated samples. At 5 hr, comparatively more maltose was liberated from uncooked irradiated starch (130 mg/g) than from flour (100 mg/g). Cooking further activated the susceptibility of red gram starch (flour) to alpha-amylase action. But the effect of cooking was much more pronounced with irradiated samples.

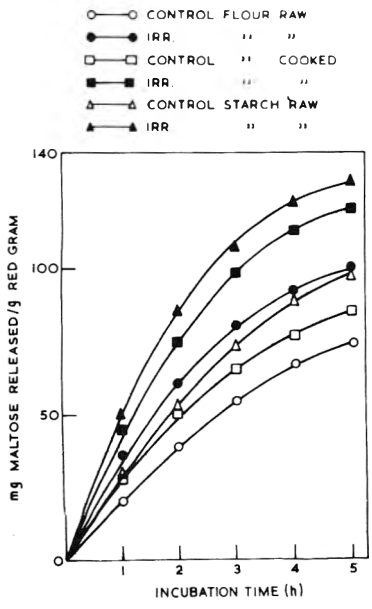


Fig. 3—Alpha-amylolysis of irradiated red gram starch: Action pattern of alpha-amylolysis was followed as described in the text. Reducing sugars, liberated into the incubation mixture, were expressed in terms of maltose. Each point represents the average of two individual experiments, carried out in triplicate.

Table 2—Effect of irradiation and cooking on nonreducing sugars of red gram[a]

Sugars	Distribution of sugars			
	Control		Irradiated	
	Raw (%)	Cooked (%)	Raw (%)	Cooked (%)
Sucrose	27 ± 0.5	30 ± 0.52	25 ± 2.73	30 ± 0.27
Raffinose + stachyose	44 ± 0.86	43 ± 0.24	51 ± 1.34	40* ± 0.64
Verbascose	28 ± 0.1	26 ± 0.1	25 ± 1.4	28 ± 2.4

[a] Values are averages of triplicate results of two independent experiments. Control and irradiated (1 Mrad) red gram samples were cooked for 25 and 16 min, respectively. Spots corresponding to each nonreducing sugar were cut from the undeveloped chromatogram (Fig. 2) and eluted with distilled water. Sugars were estimated quantitatively as described in the text.
* $P < 0.05$.

Rheological properties of irradiated starch

Amylograms presented in Figure 4 show the differences in gelatinization viscosity of red gram flour and of isolated starch on irradiation. Irradiated (1 Mrad) flour exhibited a very low

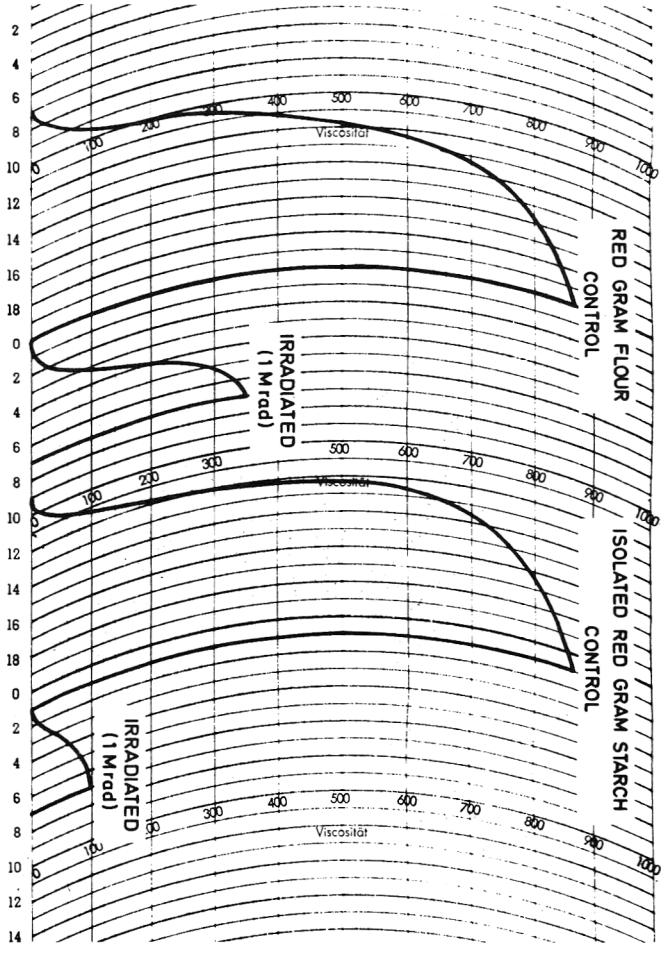


Fig. 4—Amylograms of irradiated red gram: To determine the gelatinization viscosity, water-flour or water-starch slurry was heated in Brabender Amylograph revolving container with a steady rise in temperature of 1.5°C/min until complete gelatinization occurred.

maximal gelatinization viscosity (350 A.U.) compared to the control (860 A.U.). However, in isolated starch, this dropped from 860 A.U. in the control to 100 A.U. in irradiated samples. Results of further analyses of the amylograms, compiled in Table 3, revealed that the pasting temperatures at which the viscosity began to rise from the base line on the recording paper, were comparable in all the samples, varying between 79–81°C. However, peak viscosity was reached at a much lower temperature (at 90°C) in irradiated samples compared to unirradiated ones (95°C). The time taken to reach the amylogram peak was 46 and 43 min for unirradiated and irradiated samples, respectively.

Solubility and swelling power of red gram starch

Changes in the swelling power and solubility of starch isolated from control and irradiated red gram are shown in Figures 5 and 6, respectively. These were determined at intervals of 5° over a temperature range from 70–95°C. It can be seen that swelling power of starch from irradiated sample was decreased (Fig. 5) with concomitant increase in solubility (Fig. 6).

Table 3–Assessment of amylograms[a]

Sample	Gelatinization viscosity (A.U.)	Pasting temp (°C)	Peak viscosity temp (°C)	Gelatinization time (min)
Whole flour:				
Control	860	81	95	47
Irradiated (1 Mrad)	350	80.5	90	43
Isolated starch:				
Control	860	80	95	47
Irradiated (1 Mrad)	100	79	90	43

[a] Values are averages of three experiments. Individual experimental factors were evaluated from amylograms obtained in Figure 3.

DISCUSSION

IT IS KNOWN that in cereals and legumes, starch is mainly responsible for their textural quality, especially for changes during cooking (Osman, 1967). It was observed that this attribute, measured in terms of softening, was improved on irradiation (1 Mrad) in cooked red gram (Nene et al., 1974). The total reducing sugars of irradiated pulse showed no significant increase over the control (Table 1). Potato (Mishina and Nikuni, 1960) and wheat (Ananthaswamy et al., 1970b) starch, however, exhibited rapid breakdown on irradiation at moderate dose levels releasing more reducing sugars. This suggests that the breakdown of starch in red gram was probably limited to higher maltodextrins. Though the quantity of total nonreducing sugars was not affected by irradiation, on cooking, significant ($P < 0.05$) decrease in raffinose and stachyose contents together was observed (Table 2). This observation is of practical interest, since beans are known to possess flatulence factors causing intestinal disturbances and gas formation in humans following their ingestion (Steggerda and Dimmick, 1966). These factors have limited the acceptability and consumption of beans in spite of their high protein content. Rackis et al. (1970) have shown that the oligosaccharides-raffinose and stachyose are associated with the gas-producing factor when incubated in thioglycollate media with anaerobic bacteria of the intestinal tract of dogs; this property has been observed with soybean, cottonseed and peanuts. Hasegawa and Moy (1973), while examining the feasibility of reducing the flatulence factors in soybean, observed that both these sugars were utilized more rapidly in metabolic processes during germination in gamma-irradiated soybean. Recently, a method has been developed to remove these galacto-oligosaccharides by treatment of soybean with an enzyme preparation from *Aspergillus saitoi* (Sugimoto and Van Buren, 1970). Thus, the acceleration by irradiation in the degradation of these oligosaccharides to monosaccharides such as glucose and fructose which are easily digested and absorbed in the gastro-intestinal tract, may be beneficial in promoting the use of legumes. Further work is however necessary to ascertain more precisely the effects of irradiation on the flatulence-causing saccharides in legumes.

Further evidence for the radiosensitivity of red gram starch was obtained by subjecting it to alpha-amylase action. Relatively more maltose was liberated from irradiated, uncooked

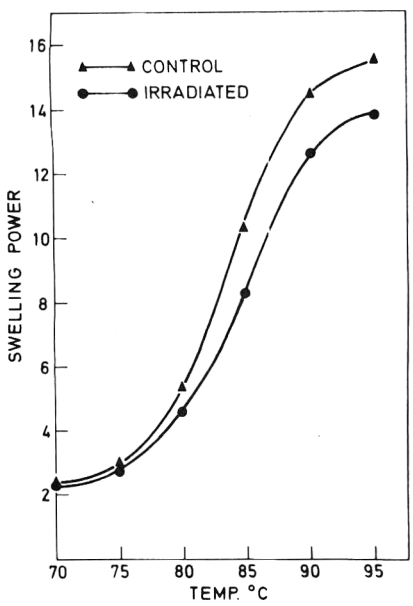


Fig. 5–Swelling power of red gram starch: Isolated red gram starch (5g) was dispersed in water (100 ml) and heated with gentle stirring for 30 min in a water bath maintained at the indicated temperatures. The swelling capacity was measured as described in the text.

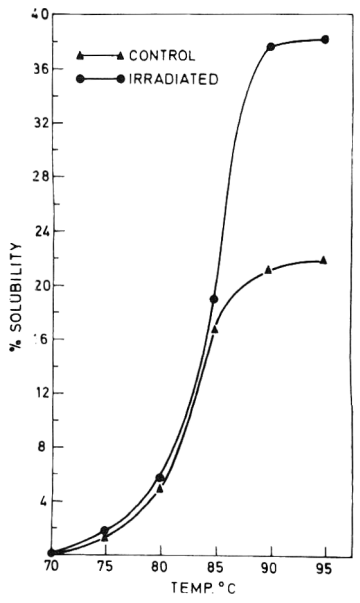


Fig. 6–Solubility of isolated red gram starch: 5g of starch was dispersed in water and heated as described in Figure 5. The sample was then centrifuged and solubility determined after drying the supernatant and weighing the residual dissolved starch.

and cooked samples, compared to their respective controls (Fig. 3). Increased susceptibility to enzymic hydrolysis and higher 'maltose value' of irradiated wheat flour have been attributed to the depolymerization and degradation of starch (Ananthaswamy et al., 1970a).

Degradation of red gram starch has also been observed in terms of a decrease in gelatinization viscosity (Fig. 4) and an increase in solubility (Fig. 6) on heating. At one Mrad, gelatinization viscosity is almost completely lost in isolated red gram starch (Table 3). A similar decrease in Amylogram units of irradiated wheat starch (Ananthaswamy et al., 1971) and increase in amylopectin solubility (Deschreider, 1966) have been reported. Deschreider (1960) has attributed these changes to shortening of polysaccharide chains, depending upon the radiation dose. Shift in the iodine complex towards shorter wave lengths with irradiated amylose (Whistler and Ingle, 1967) also supports this view. Fall in the peak viscosity results in smaller differences in pasting and peak viscosity temperatures (Table 3) in irradiated red gram samples.

It is known that visco-elastic properties of wheat flour influence and govern the inherent bread-making quality of wheat. In fact, bread prepared from irradiated (20–200 Krad) wheat shows improvement in terms of loaf volume and crumb structure (Rao et al., unpublished data). This is due to an increase in amylase-susceptible starch degradation products in irradiated wheat. Thus, the decrease in swelling power in irradiated red gram (Fig. 5) can be a beneficial attribute, improving the textural quality on cooking, since the bursting of starch and the subsequent rupture of the legume could be prevented. Radiation processing of red gram can, therefore, result in improvement of the quality of the final product.

REFERENCES

Ananthaswamy, H.N., Vakil, U.K. and Sreenivasan, A. 1970a. Susceptibility to amylolysis of gamma irradiated wheat. J. Food Sci. 35: 792.

Ananthaswamy, H.N., Vakil, U.K. and Sreenivasan, A. 1970b. Effect of gamma radiation on wheat starch and its components. J. Food Sci. 35: 795.

Ananthaswamy, H.N., Vakil, U.K. and Sreenivasan, A. 1971. Some physico-chemical changes in gamma-irradiated wheat. In "Proceedings of Symposium on Basic Mechanisms in Radiation Biology and Medicine," New Delhi, Feb. 11—13, p. 347.

Deschreider, A.R. 1959. Systematic study of flour treated with gamma rays. 1. Action on polysaccharides. Fermentatio 1: 31.

Deschreider, A.R. 1960. Changes in starch and its degradation products on irradiating wheat flour with gamma rays. Starke 12: 197.

Deschreider, A.R. 1966. Action of the gamma rays on the constituent elements of the wheat flour. IAEA/SM-73/4, Karlsruhe, Germany, 6—10 June.

Duboise, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350.

Faust, M. and Massey, L.M. Jr. 1966. The effect of ionizing radiation on starch breakdown in barley endosperm. Rad. Research 29: 33.

Hasegawa, Y. and Moy, J.H. 1973. Reducing oligosaccharides in soybeans by gamma-radiation controlled germination. "Proceedings of a Symposium on Radiation Preservation of Food." IAEA-SM-166/39, p. 89.

Lai, S.P., Finney, K.F. and Milner, M. 1959. Treatment of wheat with ionizing radiations. 4. Oxidative, physical and biochemical changes. Cereal Chem. 36: 401.

Milner, M. 1961. Technological effects of gamma irradiation of wheat. In "Proceedings of the Fifth International Congress of Biochemistry, Moscow, 1961," Vol 8, p. 108. Pergamon Press Ltd.

Mishina, S. and Nikuni, Z. 1960. The effect of gamma rays on potato starch. Mem. Inst. Sci. and Ind. Res., Osaka Univ. 17: 216. Cited in Nuclear Sc. Abstr. (1962) 16: 22237.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 373.

Nene, S.P., Vakil, U.K. and Sreenivasan, A. 1974. Improvement in the textural qualities of irradiated legumes. Acta Alimentaria (In press).

Nigam, V.N. and Giri, G.V. 1961. Sugars in pulses. Can J. Biochem. & Physiol. 39: 1847.

Osman, E.N. 1967. Starch in food industry. In "Starch: Chemistry and Technology," Ed. Whistler, R.L. and Paschall, E.F. Vol 2, p. 163. Academic Press.

Rackis, J.J., Sessa, D.J., Steggerda, F.E., Shimizu, T., Anderson, J. and Pearl, S.L. 1970. Soybean factors relating to gas production by intestinal bacteria. J. Food Sci. 35: 634.

Rao, S.R., Vakil, U.K. and Sreenivasan, A. (Unpublished data.)

Scherz, H. 1968. Presence of deoxy compounds in irradiated carbohydrates. Nature 219: 611.

Schoch, T.J. 1964. Swelling power and solubility of granular starches. In "Methods in Carbohydrate Chemistry," Ed. Whistler, R.L. Vol 4, p. 106. Academic Press.

Smith, I. 1960. Sugars. In "Chromatographic and Electrophoretic Techniques," Vol. 4. Chromatography, Ed. Smith, I., p. 246. Interscience Publishers, Inc.

Steggerda, F.R. and Dimmick, J.F. 1966. Effect of bean diets on concentration of carbon dioxide in flatus. Am. J. Clin. Nutr. 19: 120.

Sugimoto, H. and Van Buren, J.P. 1970. Removal of oligosaccharides from soy milk by an enzyme from Aspergillus saitoi. J. Food Sci. 35: 655.

Tollier, M.T. and Guilbot, A. 1970. Development of certain physicochemical properties of the starch granule as a function of irradiation conditions. Starke 2: 296.

Vakil, U.K., Aravindakshan, M., Srinivas, H., Chauhan, P.S. and Sreenivasan, A. 1973. Nutritional and wholesomeness studies with irradiated foods: India's program. IAEA, SM-166/12, Vienna, p. 673.

Weiss, J. 1952. Chemical dosimetry using ferrous and ceric sulfates. Nucleonics 10: 28.

Whistler, R.L. and Ingle, T.R. 1967. Radiation of starch. In "Starch: Chemistry and Technology," Ed. Whistler, R.L. and Paschall, E.F. Vol 1, p. 409. Academic Press.

Ms received 7/22/74; revised 10/10/74; accepted 10/14/74.

ROBERT J. PETERSON, H. J. IZZO,[1] ERIC JUNGERMANN[1]
and STEPHEN S. CHANG
Dept. of Food Science, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

CHANGES IN VOLATILE FLAVOR COMPOUNDS DURING THE RETORTING OF CANNED BEEF STEW

INTRODUCTION

CANNED BEEF STEW and other canned meat products play an important role in the U.S. meat industry. The flavor of canned meat products often differs from the flavor of the analogous noncanned product familiar to the housewife. The flavor note in the canned meat products which leads to this difference in flavor has been termed "retort flavor."

There is no information available in the literature on the volatile flavor compounds of canned beef stew per se. However, a number of articles have appeared which discuss the volatile flavor compounds of canned or pressure-cooked beef. The role of these compounds in both the "retort flavor" and the overall flavor of the canned beef has also been discussed (Olson et al., 1959; Luh et al., 1964a, b; Brennan and Bernhard, 1964; Ziemba and Mälkki, 1971; Mussinan et al., 1973; Persson and von Sydow, 1973; Wilson et al., 1973).

The earlier work on canned beef flavor was primarily concerned with the identification of NH_3 and the lower molecular weight sulfur compounds such as H_2S, mercaptans and the mono- and disulfides (Olson et al., 1959; Luh et al., 1964a, b; Brennan and Bernhard, 1964). The authors associated these compounds in varying degrees with the "retort flavor," but indicated the possibility of as yet unidentified compounds also contributing to the "retort flavor."

Ziemba and Mälkki (1971) colorimetrically determined the content of H_2S, methyl mercaptan and methyl sulfide in canned beef processed at 121°C for various times. The content of H_2S increased rapidly at first, passed through a maximum and then decreased to a steady level at longer process times. The content of methyl mercaptan and methyl sulfide increased moderately at first and then showed a steady slight increase with longer processing times. The content of methyl mercaptan and methyl sulfide was approximately the same at all process times and significantly less than the content of H_2S. Through the use of an olfactometric technique in sensory evaluation of the sample aromas, the authors concluded that H_2S, both directly and as a possible precursor of volatile condensation products, plays a positive role in the canned beef flavor except where it is out of balance with the other odor components present.

Mussinan et al. (1973) and Wilson et al. (1973) studied the pyrazine and sulfur-containing compounds, respectively, in the volatiles of pressure-cooked beef. A total of 33 pyrazines and 46 sulfur compounds, including thiophenes and thiazoles were identified, indicating the complex nature of the volatiles' pattern of canned beef.

The most comprehensive study of the volatiles of canned beef has recently been reported by Persson and von Sydow (1973). They used GC-MS analysis to examine a headspace sample from a concentrated solution of canned beef flavor volatiles. 95 compounds were identified, among these were 21 sulfur compounds, 12 aldehydes, 16 ketones, 11 furans, 8 alcohols, and 15 alkyl benzenes. The predominant odor descriptions used in assessing the odor of the chromatographic effluent of the canned beef volatiles were burnt, green, sickly and sulfurous.

In further work on the "retort flavor" of canned beef (Persson et al., 1973), significant correlations between "retort flavor" and the concentrations of H_2S and methyl mercaptan were found by a taste panel. Other correlations between "retort flavor" and other combinations of compounds were found, including methyl sulfide, ethanal, and 2-methylbutanal in various combinations with H_2S and/or methyl mercaptan. The authors also found that the branched chain aldehydes arising from the Strecker degradation, i.e., 2-methylpropanal, etc., were highly intercorrelated and correlated with methyl mercaptan and H_2S and, therefore, with the "retort flavor."

Since the canned beef stew used in the present work also contained carrots, a review of the literature on the volatiles of canned carrots showed only two major studies. Hrdlicka et al. (1968) investigated the carbonyls of canned carrots and found a number of aldehydes and 2-enals; Heatherbell et al. (1971) studied fresh and canned carrots and found mainly quantitative differences between the samples. Ethyl mercaptan, methyl sulfide and dimethylstyrene were the only compounds found in canned carrots but not in the fresh carrots.

No literature on the volatile components of canned potatoes, which is another major constituent of the beef stew, has been reported. However, a number of papers have appeared concerning the volatiles of boiled potatoes (Self et al., 1963; Gumbmann and Burr, 1964; Buttery et al., 1970; Nursten and Sheen, 1974).

The present paper reports (1) the existence of quantitative and qualitative differences between the flavor compounds of canned beef stew and the analogous noncanned product; and (2) the identification of the volatile components of the canned beef stew.

EXPERIMENTAL

Materials used

The canned and fresh stews employed in this research were supplied by Armour-Dial, Inc's Research Department. The canned beef stew sample was a modified commercial product containing 16.7% raw beef chuck, 14.2% dehydrated potato cubes, 6.3% dehydrated carrots and 62.8% gravy. The gravy consisted of 82.6% water, 13.4% ground beef plate, 2.8% starch, 1.0% wheat flour and 0.2% caramel coloring. The canned stew was processed at 121°C for 75 min in 404 × 309 tinplate cans coated with an all-purpose epoxyphenolic liner. The cans were supplied by American Can Company (New York, N.Y.).

The raw materials and their percent composition in the fresh beef stew were identical to those of the canned stew. The beef chuck was sautéed in Wesson oil (Hunt-Wesson Foods, Fullerton, Calif.) until the surface of the meat was "roasted brown." The meat was then added to the gravy and simmered for approximately 2 hr. The rehydrated carrots and potatoes were then added and simmering was continued for about 45 min. The stew was then frozen in aluminum trays holding 6 lb of stew. Both samples were stored at −31°C for a maximum of 6 months.

Isolation of volatile flavor compounds

The volatile constituents of the fresh and canned stew samples were isolated, extracted and concentrated in an identical manner. A fine

[1] Present address: Armour-Dial, Inc., Greyhound Tower, Phoenix, AZ 85077

sample slurry was prepared with distilled water using a Waring Blendor (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn.). The volatile flavor compounds were then isolated from the slurry with the use of the apparatus described by Herz and Chang (1966), utilizing the principles of flash vaporization and vaporization from a thin, heated sample film under vacuum. The sample slurry was maintained at 70–75°C in the reservoir flask, and was continuously metered into the vaporizer section under a vacuum of 0.01 Torr. The flow rate was sufficient to maximize stripping of the volatiles, but prevented caking of stripped material in the vaporizers. The walls of the vaporizers were heated to 105°C by circulating heated glycerine through the outer jackets. Volatile flavor compounds plus water were flash evaporated from the sample slurry as it was metered from atmospheric pressure into a vacuum. As the slurry flowed down the vaporizer walls in a thin film, vaporization continued.

Volatiles were collected in a series of cold traps cooled with dry ice. The condensate collected in the traps was combined, saturated with reagent grade sodium chloride and extracted with reagent grade, anhydrous ethyl ether.

Two replicate samples of canned stew were treated in the manner described above. The amount of replicate I was 4.675 kg and that of replicate II was 4.699 kg. An additional 22.7 kg of canned stew was isolated, concentrated and subsequently combined with replicates I and II. 29.5 kg of fresh stew was treated in like manner.

Preliminary concentration of the ether extracts was carried out using a 30-plate Oldershaw distillation column. All of the extracts were concentrated to a volume of 50 ml. Final concentration was accomplished with a spinning band distillation apparatus (Kontes Glass Co., Vineland, N.J.). Replicates I and II were concentrated to a final volume of 2.48 ml. After the chromatographic study of the reproducibility, the two replicate solutions were combined with the remaining canned stew volatiles solution and concentrated to a final volume of 2.11 ml. The final volume of the fresh stew sample solution was adjusted to 1.95 ml in order that the concentrations of the canned and fresh sample solutions would be equal based on initial weight of samples.

Gas chromatography

A Beckman GC-55 (Beckman Instruments, Inc., Fullerton, Calif.) equipped with a flame ionization detector was used for the quantitative GC analyses. This chromatograph is equipped with a 9:1 column effluent splitter installed in the packed column system. A 6 ft × 1/8 in. o.d. stainless steel column, packed with 5% OV-101 on 80/100 mesh Chromosorb W-HP, was used for the analyses. The helium flow rate was 33 ml/min and the temperature was programmed from 50°C (held for 3.2 min) at a rate of 5°C/min to 215°C and held there. The chromatograms were obtained employing the column saturation method of Blumenthal and Chang (1973). Sample injection size was 10.0 μl for the two replicates and 5.0 μl for the fresh and canned stew samples. An odor profile was obtained for both samples by a panel of four experienced persons by sniffing the chromatographic effluent.

Preparative chromatography of the canned sample was carried out with a Beckman GC-5 equipped with a thermal conductivity detector. The canned stew volatiles were separated into 24 broad fractions using a 10 ft × 1/8 in. o.d. stainless steel column packed with 10% SE-30 on 70/80 mesh Chromosorb W-AW, DMCS. The helium flow rate was 30 ml/min and the temperature was programmed from 50°C (held for 3.2 min) at a rate of 5°C/min to 210°C and held there. The gas chromatography was repeated until the sample was exhausted. Each fraction was accumulatively collected in a hairpin cold trap according to the method of Thompson (1968). The fractions 3–24 thus collected were chromatographed a second time using a 10 ft × 1/8 in. o.d. stainless steel column packed with 10% Carbowax 20M on 60/80 mesh Chromosorb W-AW, DMCS. Helium flow rate was 30 ml/min and the temperature was programmed in a manner to secure optimum resolution of each fraction. For fractions 3–24, collection of appropriate sized subfractions was carried out in two sets of hairpin cold traps. Approximately 25% of a subfraction was collected in one trap to be used in GC-MS analysis while the remaining 75% was collected in the second trap for IR analysis. When the amount of a subfraction was insufficient, all of it was collected in one trap for mass spectral analysis or further purification. The fractions 1 and 2 and those subfractions requiring a third chromatography were chromatographed on a 10 ft × 1/8 in. o.d. stainless steel column packed with 10% OV-17 on 60/80 mesh Chromosorb W-AW, DMCS. Helium flow rate was 30 ml/min and the temperature was programmed in a manner to secure optimum resolution of the sample.

Identification of the gas chromatographic fractions

The pure components obtained from the repeated chromatography were analyzed by infrared and mass spectrometry. For the mass spectrometry a du Pont mass spectrometer (Monrovia, Calif.) model 21-490, with a jet separator, was interfaced with a Varian Moduline 2700 gas chromatograph (Varian Aerograph, Walnut Creek, Calif.) with an FID detector. A 6 ft × 1/8 in. o.d. stainless steel column packed with 10% OV-101 on 60/80 mesh Chromosorb W-AW, DMCS was used. The helium flow rate was 30 ml/min and the temperature was programmed as the sample characteristics required. The column effluent was split 1:1 and the mass spectra were recorded at 70 ev. The jet separator temperature was 250°C and the ion source temperature was 270°C. Infrared spectra were recorded on a Beckman IR-8 according to the procedure reported by Kawada et al. (1966). Compounds were identified by comparing their spectra with published spectra and agreement of retention time with the authentic compound. An identification was considered tentative, if only mass spectral data were available.

RESULTS & DISCUSSION

Both the canned and fresh stew flavor isolates had the typical aromas associated with their respective original product. The chromatograms of the two replicate samples matched peak for peak, except for two peaks which differed by more than 5%. Since the replicate chromatograms were qualitatively identical and the quantitative differences were minor, the experimental methodology was considered to be reproducible.

The packed column chromatograms of the fresh and canned stew volatiles are presented in Figure 1. It should be noted that the fresh stew chromatogram was recorded at one-half the sensitivity of the canned stew chromatogram. These two curves show distinct qualitative, as well as quantitative differences, indicating that the volatiles in the fresh stew differ from those in the canned stew, both in chemical composition and amounts.

The qualitative differences between the samples were substantiated by the results of the odor profile. There were no peaks found which could be considered to possess the exact canned stew or fresh stew aroma. The predominant odor descriptions used to characterize the peaks of the canned stew chromatograms were: dirty green, raw green, roasted grain and heated rubber or plastic. These were all considered to be objectionable, heavy odors. The fresh stew volatiles lacked these objectionable notes and were characterized by earthy, nutty, sweet green and carrot-like odors which were all pleasant and clean odors.

Total eluate trapped from the gas chromatograph for both samples had odors identical to the original sample isolate. This indicated that all volatile components important to the aroma of the sample could be eluted. Figure 2 shows the initial preparative chromatogram of the canned stew volatiles.

A total of 102 compounds was either positively or tentatively identified in the volatiles of the canned stew. These are presented in Tables 1 and 2. The compounds identified consisted of 21 saturated aliphatic hydrocarbons, 2 unsaturated aliphatic hydrocarbons, 7 cyclic hydrocarbons, 12 aromatic hydrocarbons, 13 alcohols, 9 aldehydes, 11 ketones, 1 keto-aldehyde, 5 esters, 1 lactone, 1 acid, 6 furan compounds, 10 nitrogen/sulfur-containing ring compounds, and 3 miscellaneous compounds.

The saturated and mono-unsaturated hydrocarbons do not play a significant role in the flavor of the canned stew, since they possess only weak odors. These compounds have been identified previously in many foods and probably arose as a result of thermal oxidative decomposition of the beef fat and other lipids present (Yamato et al., 1970; Krishnamurthy and Chang, 1967; Reddy et al., 1968).

The identified hydrocarbons contained a series of methyl-branched compounds with the branch occurring at the 2, 3, or 4 position of the main carbon chain. This series of hydrocarbons has not been previously identified as thermal oxidative

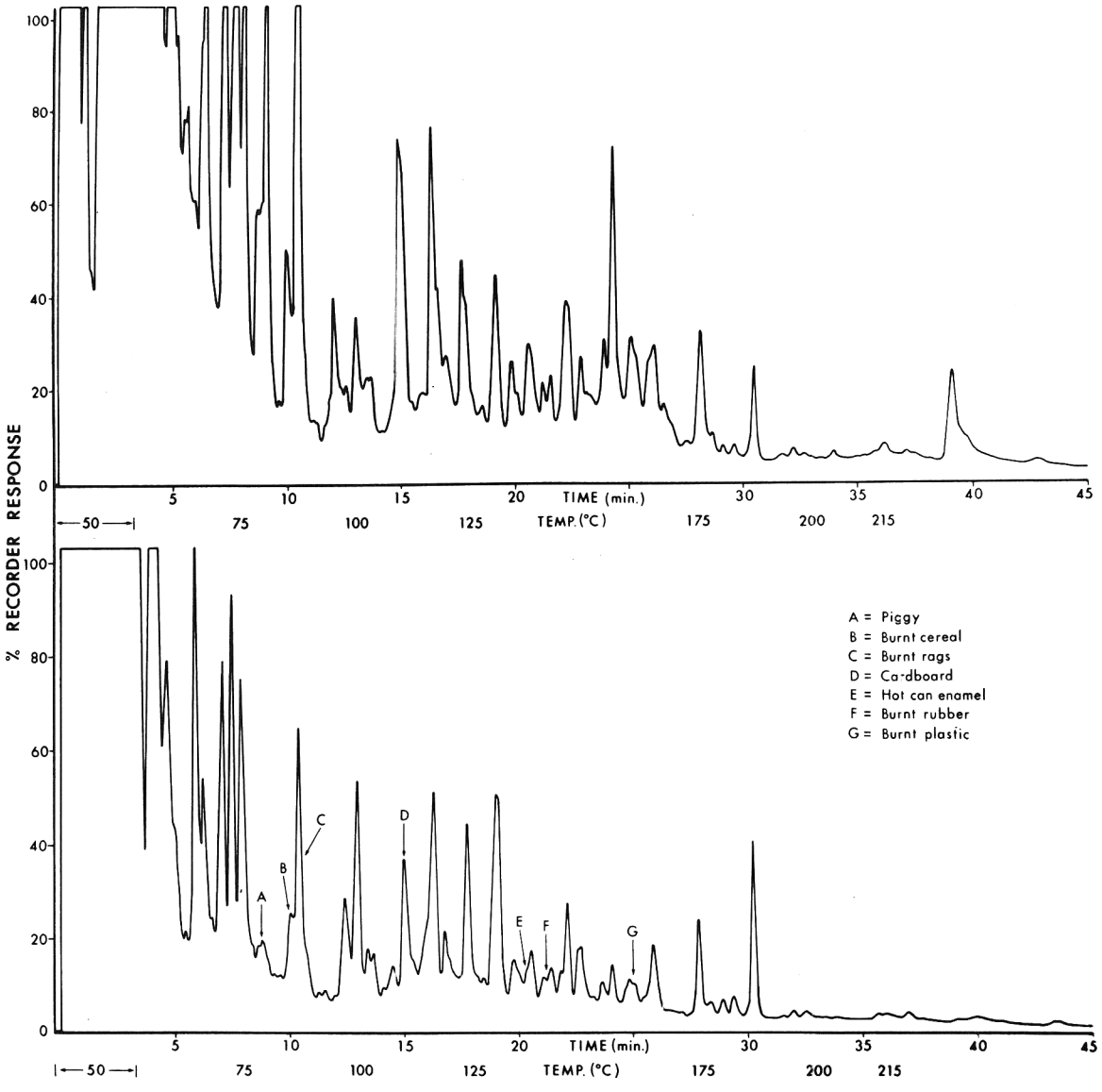


Fig. 1—Gas chromatograms of the fresh and canned beef stew volatile compounds. (Top—fresh beef stew; Bottom—canned beef stew)

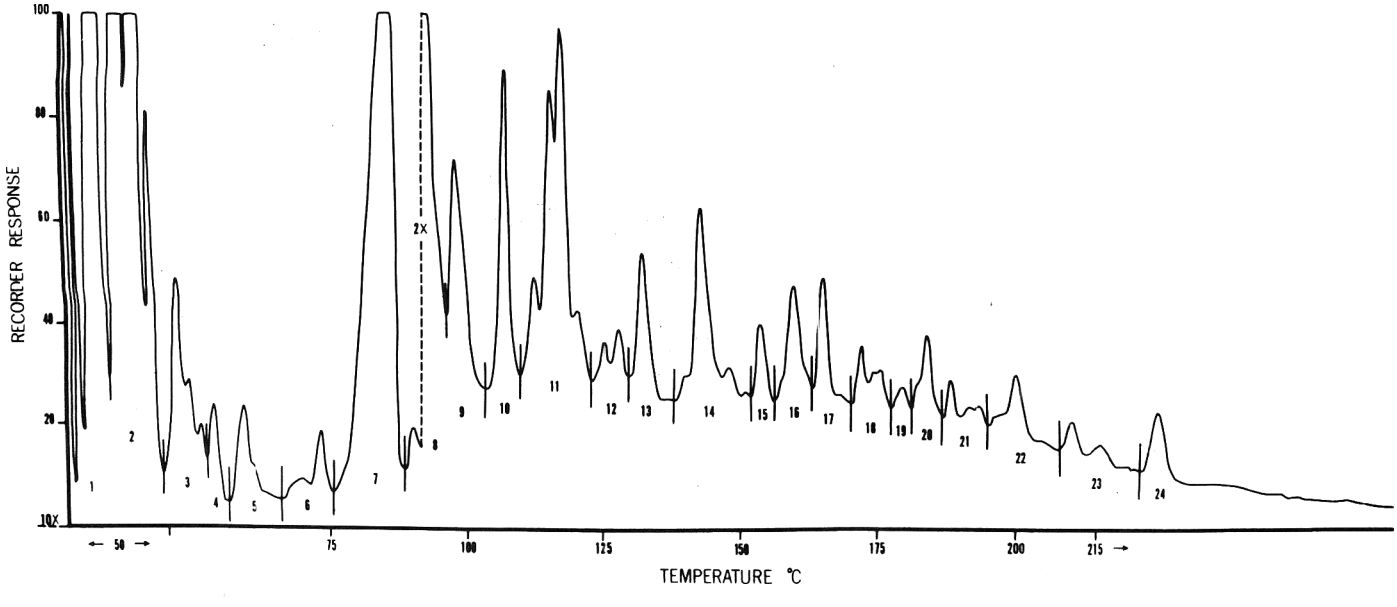


Fig. 2—Gas chromatogram of the canned beef stew volatiles used to collect the board fractions.

decomposition products of fats or fatty acids with the exception of a series of 2-methyl alkanes found in the irradiation products of butterfat (Merritt et al., 1967). If the precursor of these hydrocarbons is fatty material then a rearrangement of the straight fatty acid chains is required.

A family of alkylcyclohexanes was also found which probably play only a minor role in the canned stew flavor. This type of compound has been previously identified in cooked chicken (Hobson-Frohock, 1970) and in certain fruits and fruit products, especially those derived from grapes (van Straten and de Vrijer, 1973).

The mono- and sesquiterpenes found have been identified

Table 1–Compounds identified in the volatiles isolated from canned beef stew

Fraction no.[a]	Identified as	Peak size[b]
A. Saturated aliphatic hydrocarbons		
2-2-1	2-Methylpentane	M
4-1	Heptane	M
7-1	Octane	S
7-2	2-Methyloctane	S
14-2	Dodecane	M
15-2	2-Methyldodecane	S
16-3	Tridecane	M
17-3	2-Methyltridecane	S
18-2	Tetradecane	S
20-3	Pentadecane	M
21-3	2-Methylpentadecane	S
21-4	Hexadecane	S
22-3	Heptadecane	S
23-3	Octadecane	S
B. Unsaturated aliphatic hydrocarbons		
16-4	1-Tridecene	S
22-3	1-Heptadecene	XS
C. Alicyclic hydrocarbons		
3-1	Cyclohexane	M
5-1	Methylcyclohexane	L
7-2	Ethylcyclohexane	S
14-7	α-Phellandrene	M
18-5	β-Caryophyllene	M
D. Aromatic hydrocarbons		
3-3	Benzene	L
6-2	Methylbenzene	S
7-6	Ethylbenzene	M
7-7	1,3-Dimethylbenzene	M
10-3	3-Ethylmethylbenzene	S
11-4-2	4-Isopropylmethylbenzene	S
14-9	Naphthalene	M
E. Alcohols		
1-4	Ethanol	L
3-5	1-Butanol	S
6-5, 7-8	1-Pentanol	L
8-7	1-Hexanol	L
8-6	4-Methyl-2-hexanol	M
12-2	6-Methyl-1-heptanol	M
11-6	1-Octene-3-ol	M
11-16	Benzyl alcohol	XS
11-13	Phenol	S
9-4	2-Butoxyethanol	M
F. Aldehydes		
2-8-2	3-Methylbutanal	S
3-4	Butanal	M
6-3, 7-5, 8-3	Hexanal	L
6-6, 7-11	2-Ethylbutanal	M
9-2, 10-2	Heptanal	M
13-2	Nonanal	L
23-5	Pentadecanal	M
10-4	Benzaldehyde	L

Table 1–continued

Fraction no.[a]	Identified as	Peak size[b]
G. Ketones		
1-5, 1-6-1	Acetone	L
7-5	3-Methyl-2-hexanone	M
7-6	5-Methyl-2-hexanone	M
11-4-1	2-Octanone	M
14-5	2-Decanone	M
22-5	2-Pentadecanone	S
4-3	3-Hydroxy-2-butanone	S
7-12	2,3-Butanedione	S
19-4	β-Ionone	S
H. Esters and lactones		
1-4	Ethyl formate	L
*2-6-3, 2-7, 2-8-1, 3-2, 4-2, 6-1, 7-2, 7-3	Ethyl acetate	L
23-6, 24-2	Dibutyl phthalate	M
11-11-2	γ-Caprolactone	S
I. Acid		
2-8-3	Acetic acid	M
J. Furan compounds		
11-3	2-Pentylfuran	S
8-8	Furfuryl alcohol	M
7-13	2-Furaldehyde	L
10-5	5-Methyl-2-furaldehyde	M
7-14, 9-5	2-Acetylfuran	L
11-9	5-Methyl-2-acetylfuran	S
K. Nitrogen compounds		
5-4, 6-4, 7-8	Pyridine	M
7-11	Methylpyrazine	M
9-3	2,5-Dimethylpyrazine	S
11-14	2-Formylpyrrole	M
12-4	2-Acetylpyrrole	M
7-6	2,4,5-Trimethyl-Δ^3-oxazoline	L
8-4	2,4,5-Trimethyloxazole	M
L. Sulfur compounds		
11-10	2-Acetylthiazole	M
14-11	Benzothiazole	S
11-11-1	Thiophene-2-carboxaldehyde	M
M. Miscellaneous compounds		
5-2	1,1-Diethoxyethane	L
2-9	Trichloromethane	S
11-8	1,4-Dichlorobenzene	XS

[a] The first, second and third numerals indicate the gas chromatographic fractions collected during the first, second and third chromatographies, respectively.

[b] XS = Extra small; S = Small; M = Medium; L = Large.

* Possible contaminent due to solvent

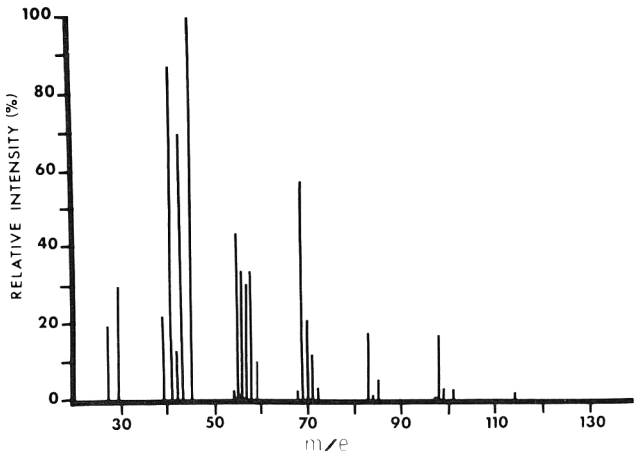


Fig. 3–Mass spectrum of the fraction identified as 4-methyl-2-hexanol.

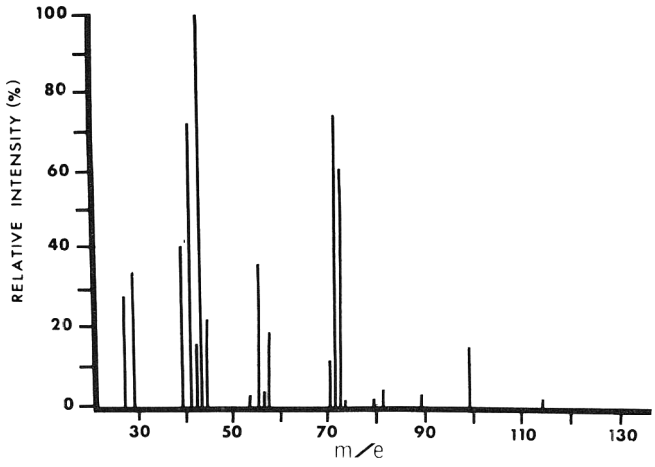


Fig. 5–Mass spectrum of the fraction identified as 3-methyl-2-hexanone.

by Heatherbell et al. (1971) in canned carrots. These compounds probably play a role in the overall canned stew flavor contributing to the greenish and green-carrot notes.

The 12 aromatic hydrocarbons found are felt to play a minor role in the canned stew aroma. Persson and von Sydow (1973) found a series of aromatic hydrocarbons in canned beef. These authors used unlacquered cans, hence the aromatic hydrocarbons found in the present investigation might not have completely arisen from either the thermal breakdown of the epoxyphenolic can liner or as a residual liner solvent. Johnson et al. (1969) have reviewed the formation of volatile aromatic hydrocarbons in foods.

The primary straight-chain alcohols found have a fusel oil odor with a greenish note and probably play a role in the overall canned stew flavor. Both the unsaturated alcohols found have characteristic odors and might play a significant role in the "retort flavor," especially the 1-octen-3-ol which might be important in the heavy, metallic, fungal notes. The above alcohols have been identified by Persson and von Sydow (1973) in canned beef. The two branched alcohols, 4-methyl-2-hexanol and 6-methyl-1-heptanol (Fig. 3 and 4), have not been previously reported in the literature. Phenol might have arisen as a result of the thermal decomposition of the epoxyphenolic can liner, although phenol has been found in many noncanned, high-temperature processed foods.

The two ether-alcohols found are perhaps residues of the solvent system used in applying the liner to the cans. The 1-chloro-2-propanol tentatively identified may also be a residue of this solvent system.

The straight chain aldehydes probably contribute to the overall aroma of the canned stew, especially to the green character of the odor. Both the 3-methylbutanal and benzaldehyde found contribute to the canned stew flavor, the 3-methylbutanal having a burnt-roasted odor and the benzaldehyde a sweet, almond-like odor.

A series of 2-alkanones was identified and most of these were previously found in canned beef by Persson and von Sydow (1973). Both 3-hydroxy-2-butanone (acetoin) and its oxidation product, 2,3-butanedione (diacetyl) were identified, and these buttery odor compounds have been cited as contributing to the flavor of boiled beef (Hirai et al., 1973).

Another series of branched compounds found in the canned stew volatiles consisted of 2-ethylbutanal, 3-methyl-2-hexanone (Fig. 5 and 6), 5-methyl-2-hexanone and 6-methyl-2-heptanone. Neither the 2-ethylbutanal nor the 3-methyl-2-hexanone have been previously reported in the literature.

β-Ionone has been associated with the thermal breakdown of β-carotene and has a violet-like odor and may play a role in the overall flavor of the canned stew.

2-Pentylfuran has been found in a number of foods and is

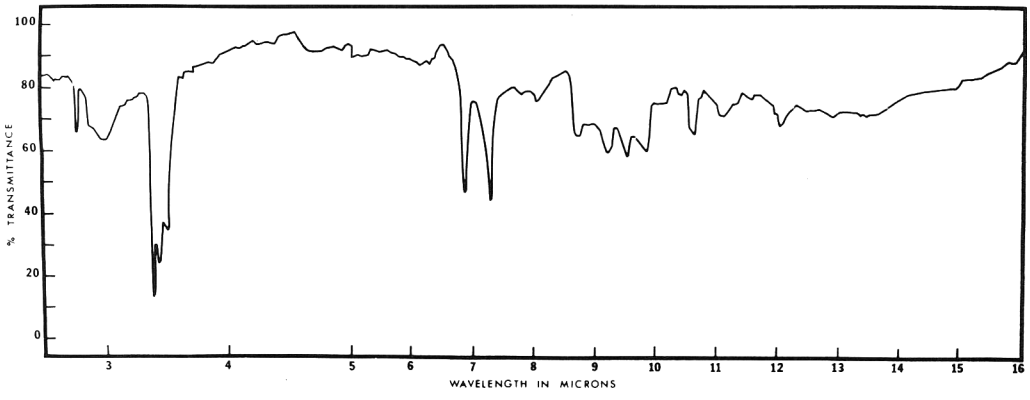


Fig. 4–Infrared spectrum of the fraction identified as 4-methyl-2-hexanol.

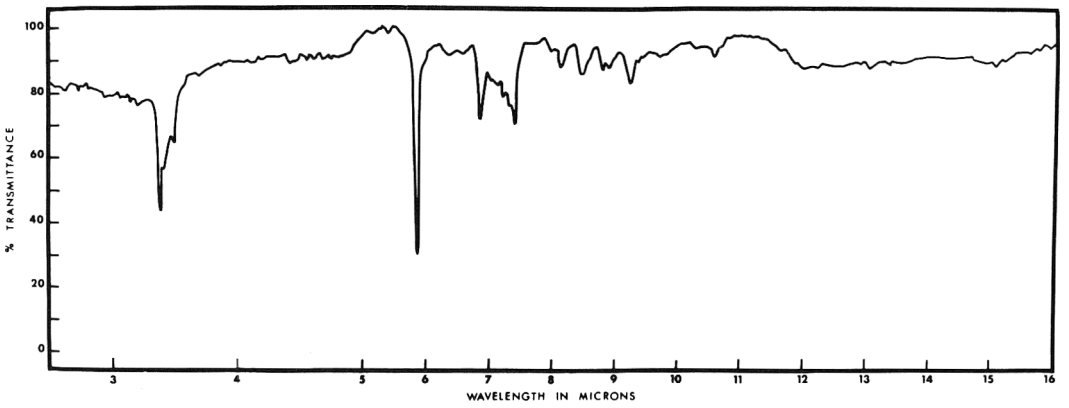


Fig. 6–Infrared spectrum of the fraction identified as 3-methyl-2-hexanone.

believed to be the primary cause of reversion flavor in soybean oil (Smouse and Chang, 1967). The other furan compounds identified are all oxygenated derivatives, probably arising from the thermal decomposition of carbohydrates. All possess considerable odor properties and contribute significantly to the sweet, roasted grain notes of the "retort flavor." These furan compounds have been previously reported in the flavor isolates of many thermally processed foods.

The two acylpyrrole compounds found in the canned stew volatiles have been shown to be products of heated sugar-amino acid or protein mixtures (Langner and Tobias, 1967; Ferretti et al., 1970; Shigematsu et al., 1972). Both of the acylpyrroles found had rather unpleasant, heated plastic or antiseptic odors when evaluated as GC effluents. These compounds may, therefore, play a role in the objectionable "retort flavor" of the stew.

Many interesting and significant ring compounds containing nitrogen and/or sulfur as part of the ring system were identified. Pyridine has a vile odor when concentrated, but when diluted, it has a somewhat bitter, pleasant, roasted note and may play an important role in the more pleasing canned stew flavor notes. Similarly, the two pyrazines found which have toasted, earthy, somewhat potato-like odors probably contribute in a positive manner to the canned stew flavor.

The 2,4,5-trimethyl-Δ^3-oxazoline identified has been identified previously as a volatile constituent of boiled beef by Chang et al. (1968). The completely unsaturated analogue of this compound, 2,4,5-trimethyloxazole, was also found. This was the first time that this compound was identified as a component of the volatile flavor compounds of food. The infrared and mass spectra of this compound are shown in Figures 7 and 8.

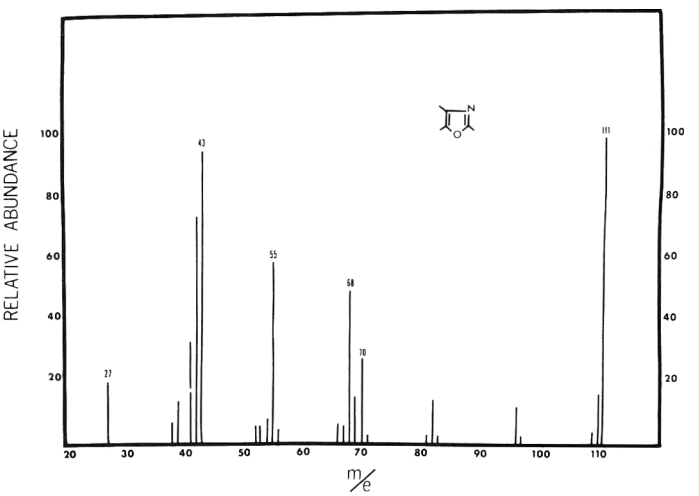


Fig. 7–Mass spectrum of the fraction identified as 2,4,5-trimethyloxazole.

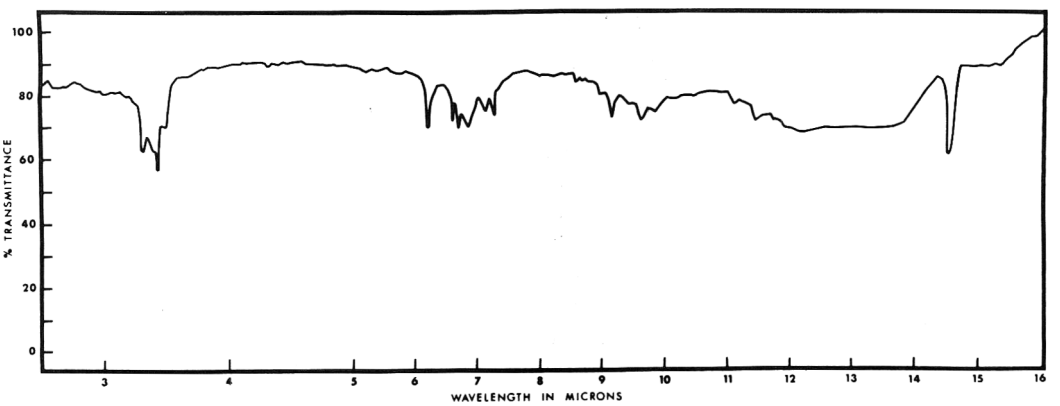


Fig. 8–Infrared spectrum of the fraction identified as 2,4,5-trimethyloxazole.

Table 2—Compounds tentatively identified in the volatiles isolated from canned beef stew

Fraction no.[a]	Identified as	Peak size[b]
A. Saturated aliphatic hydrocarbons		
2-2-2, 2-3-2	3-Methylpentane	L
7-1	3-Methyloctane	S
11-1	3-Methylnonane	XS
14-2	3-Methylundecane	S
17-3	4-Methyltridecane	XS
17-3	3-Methyltridecane	S
19-2	4-Ethyltetradecane	XS
B. Alicyclic hydrocarbons		
7-2	Trimethylcyclohexane	S
14-8	γ-Terpinene	S
C. Aromatic hydrocarbons		
11-4-1	Trimethylbenzene	S
14-4	Dimethylstyrene	S
14-4	C_5-Alkylbenzene	S
14-4	C_6-Alkylbenzene	S
14-5	C_4-Alkylbenzene	XS
D. Alcohols		
3-6	1-Penten-3-ol	M
5-5	2-Ethoxyethanol	L
3-8	1-Chloro-2-propanol	S
E. Aldehyde		
14-8	Ethylbenzaldehyde	S
F. Ketones		
10-3	6-Methyl-2-heptanone	S
14-10	Methylacetophenone	S
G. Keto-aldehyde		
7-4	3-Methyl-4-oxopentanal	M
H. Esters		
16-8	Terpinyl acetate	S
21-14	Diethyl phthalate	XS

[a] The first, second and third numerals indicate the gas chromatographic fractions collected during the first, second and third gas chromatographies, respectively.
[b] XS = Extra small; S = Small; M = Medium; L = Large.

The two thiazole compounds found have been tentatively identified previously in pressure cooked beef by Wilson et al. (1973). Their presence was confirmed by this investigation. Benzothiazole has an odor which can be described as heated rubber-like or heavy and dirty. The 2-acetylthiazole had an aroma described as somewhat popcorn-like with a strong nutty-roasted character. The thiophene-2-carboxaldehyde has a sharp, sweet-nutty, somewhat roasted grain-like odor and may contribute to the sweet, heavy, grain-like notes of the "retort flavor."

The present investigation concludes that there are significant quantitative and qualitative differences in the volatile components of canned and fresh beef stew. The "retort flavor" is not due to a single constituent, but is probably due to a relatively complex mixture of a number of components. The production of volatile flavor compounds in the canned stew is due primarily to thermal oxidative fat decomposition, thermal decomposition of amino acids and carbohydrates, and the browning reactions characteristic of roasting or baking processes.

REFERENCES

Blumenthal, M.M. and Chang, S.S. 1973. A method for obtaining reproducible quantitative gas chromatograms of volatiles isolated from foods. J. Agr. Food Chem. 21: 1123.

Brennan, M.J. and Bernhard, R.A. 1964. Headspace constituents of canned beef. Food Technol. 18: 743.

Buttery, R.G., Seifert, R.M. and Ling, L.C. 1970. Characterization of some volatile potato components. J. Agr. Food Chem. 18: 538.

Chang, S.S., Hirai, C., Reddy, B.R., Herz, K.O., Kato, A. and Sipma, G. 1968. Isolation and identification of 2,4,5-trimethyl-Δ^3-oxazoline and 3,5-dimethyl-1,2,4-trithiolane in volatile flavor compounds of boiled beef. Chem. and Ind. 1639.

Ferretti, A., Flanagan, V.P. and Ruth, J.M. 1970. Nonenzymatic browning in a lactose-casein model system. J. Agr. Food Chem. 18: 13.

Gumbmann, M.R. and Burr, H.K. 1964. Volatile sulfur compounds in potatoes. J. Agr. Food Chem. 12: 404.

Heatherbell, D.A., Wrolstad, R.E. and Libbey, L.M. 1971. Carrot volatiles. 1. Characterization and effects of canning and freeze-drying. J. Food Sci. 36: 219.

Herz, K.O. and Chang, S.S. 1966. Apparatus for the isolation of volatile compounds from foods. J. Food Sci. 31: 937.

Hirai, C., Herz, K.O., Pokorny, J. and Chang, S.S. 1973. Isolation and identification of volatile flavor compounds in boiled beef. J. Food Sci. 38: 393.

Hobson-Frohock, A. 1970. Use of molecular sieve 5A in the analysis of the flavor volatiles of cooked chicken. J. Sci. Food Agr. 21: 152.

Hrdlicka, J., Curda, D. and Pavelka, J. 1968. Changes during thermal and hydrothermal processes. 9. Volatile carbonyl compounds in carrot processing, in Chem. Abstracts 68: 38330Z.

Johnson, A.E., Nursten, H.E. and Self, R. 1969. Aromatic hydrocarbons in foodstuffs and related materials. Chem. and Ind. 10.

Kawada, T., Mookherjee, B.D. and Chang, S.S. 1966. Chemical reactions involved in the catalytic hydrogenation of oils. 3. Further identification of volatile by-products. J. Am. Oil Chem. Soc. 43: 237.

Krishnamurthy, R.G. and Chang, S.S. 1967. Chemical reactions involved in the deep-fat frying of foods. 3. Identification of nonacidic volatile decomposition products of corn oil. J. Am. Oil Chem. Soc. 44: 136.

Langner, E.H. and Tobias, J. 1967. Isolation and characterization of ether soluble sugar-amino acid interaction products. J. Food Sci. 32: 495.

Luh, B.S., Gonzalez-Acuna, C.G., Leonard, S., Simone, M. and Everson, G.J. 1964a. Aseptic canning of foods. 5. Chemical and flavor changes in strained beef. Food Technol. 18: 212.

Luh, B.S., Gonzalez-Acuna, C.G., Leonard, S. and Simone, M. 1964b. Aseptic canning of foods. 6. Hematin and volatile sulfur compounds in strained beef. Food Technol. 18: 216.

Merritt, C., Forss, D.A., Angelini, P. and Bazinet, M.L. 1967. Volatile compounds produced by irradiation of butterfat. J. Am. Oil Chem. Soc. 44: 144.

Mussinan, C.Y., Wilson, R.A. and Katz, I. 1973. Isolation and identification of pyrazines present in pressure-cooked beef. J. Agr. Food Chem. 21: 871.

Nursten, H.E. and Sheen, M.R. 1974. Volatile flavor components of cooked potato. J. Sci. Food Agr. 25: 643.

Olson, L.E., Greenwood, D.A., Nielsen, H.M. and Wilcox, E.G. 1959. Some techniques for evaluating the flavor of processed meat. Food Res. 24: 696.

Persson, T. and von Sydow, E. 1973. Aroma of canned beef: Gas chromatographic and mass spectrometric analysis of the volatiles. J. Food Sci. 38: 377.

Persson, T., von Sydow, E. and Akesson, C. 1973. Aroma of canned beef: Sensory properties. J. Food Sci. 38: 386.

Reddy, B.R., Yasuda, K., Krishnamurthy, R.G. and Chang, S.S. 1968. Chemical reactions involved in the deep-fat frying of foods. 5. Identification of nonacidic volatile decomposition products of hydrogenated cottonseed oil. J. Am. Oil Chem. Soc. 45: 629.

Self, R., Casey, J.C. and Swain, Y. 1963. The low boiling volatiles of cooked foods. Chem. and Ind. 863.

Shigematsu, H., Kurata, T., Kato, H. and Fujimaki, M. 1972. Volatile compounds formed on roasting DL-α-alanine with D-glucose. Agr. Biol. Chem. 36: 1631.

Smouse, T.H. and Chang, S.S. 1967. A systematic characterization of the reversion flavor of soybean oil. J. Am. Oil Chem. Soc. 44: 509.

Thompson, J.A. 1968. Ph.D. thesis, Dept. of Food Science, Rutgers, The State University of New Jersey, New Brunswick, N.J.

van Straten, S. and de Vrijer, F. 1973. "Lists of Volatile Compounds in Food," 3rd ed. Central Institute for Nutrition and Food Research, Zeist, Netherlands.

Wilson, R.A., Mussinan, C.J., Katz, I. and Sanderson, A. 1973. Isolation and identification of some sulfur chemicals present in pressure-cooked beef. J. Agr. Food Chem. 21: 873.

Yamato, T., Kurata, T., Kato, H. and Fujimaki, M. 1970. Volatile carbonyl compounds from heated beef fat. Agr. Biol. Chem. 34: 88.

Ziemba, Z. and Mälkki, Y. 1971. Changes in odor components of canned beef due to processing. Lebensm.-Wiss. u. Technol. 4: 118.

Ms received 2/16/75; revised 4/23/75; accepted 4/28/75.

Presented at the 34th Annual Meeting of the Institute of Food Technologists, New Orleans, La., May 12—15, 1974.

Paper of the Journal Series, New Jersey Agricultural Experiment Station, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

This investigation was supported by a grant-in-aid from Armour-Dial, Inc.

A. O. OKUBANJO[1] and J. R. STOUFFER
Dept. of Animal Science, N.Y. State College of Agriculture & Life Sciences
Cornell University, Ithaca, NY 14853

POSTMORTEM GLYCOLYSIS AND ISOMETRIC THAW TENSION DEVELOPMENT AND DECLINE IN BOVINE SKELETAL MUSCLE UNDERGOING THAW RIGOR

INTRODUCTION

VARIOUS STUDIES have established that postmortem shortening occurs by a sliding of the thick and thin filaments such as occurs during physiological contraction (Stromer et al., 1967; Stromer and Goll, 1967) and that this shortening is the primary cause of carcass stiffening during the development of rigor. The coincidence between the onset of rigor mortis and appreciable increase in the modulus of elasticity was reported by Bate-Smith (1939) who noted that new crossbonds were formed between the muscle contractile units following the loss of ATP. These physical changes have been quantified during normal rigor development through the measurement of the isometric tension developed (Jungk et al., 1967; Schmidt et al., 1968; Chrystall et al., 1970 and Busch et al., 1972).

Similar but more drastic changes have been associated with the incidence of thaw rigor. Szent-Gyorgyi (1949) in his studies with frog sartorius and rabbit psoas muscles observed that tension developed more slowly during thaw rigor but that a sudden change in temperature was sufficient to induce a fast contraction resulting in an early development of isometric tension. Bendall (1960) in studies with rabbit psoas muscle observed that rate of thaw contracture and rigor were dependent largely on the rate of thawing and the chemical pattern was similar but more rapid than normal rigor. Studies by Jungk et al. (1967), however, indicate that the rapid development of isometric tension in bovine skeletal muscle is an intrinsic characteristic of thaw rigor. In a previous paper, Okubanjo et al. (1975) suggested that thaw rigor muscle strips after rapidly developing tension, will suffer an accelerated decline in tension due to the rupturing of actin-myosin cross-linkages within the muscle fibers.

In the present paper, we have attempted to extend the above study and previous ones by Jungk et al. (1967) and Scopes and Newbold (1968) on postmortem glycolysis and isometric tension development during thaw rigor in bovine skeletal muscle at various temperatures and gaseous environments.

EXPERIMENTAL

BOVINE STERNOMANDIBULARIS muscles were obtained as previously described by Okubanjo et al. (1975). The muscles were split into strips approximately 0.1–0.3 cm^2 in cross section with the long axis oriented in the fiber direction. Strips possessing any visually damaged fibers were rejected. Intact strips were tied at rest length by both ends to steel frames and rapidly frozen at -29°C in a gravity air flow sharp freezer within 30 min of exsanguination. Subsequent incubation of short strips of the frozen muscle were carried out at 3°, 25° and 37°C in an atmosphere of moist nitrogen.

The extraction procedure outlined by Newbold and Scopes (1967) was modified to include a fast freezing step in liquid nitrogen following incubation of the meat samples for the different periods. The frozen samples were homogenized with 15 volumes of ice cold 0.6N perchloric acid at 3°C. The resulting slurry was filtered and immediately neutralized with 1N potassium hydroxide solution to phenolphthalein end point. After standing in the cold for a few hours, the precipitated potassium perchlorate was removed by centrifugation for 10 min at 4000 rpm followed by filtration.

The ultraviolet estimation of ATP was carried out using phosphoglyceratekinase as described in Sigma Technical Bulletin No. 366-UV (Sigma Chemical Co., 1967). Optical density measurement was made at 340 nm on a Beckman DU-2 Spectrophotometer using water as a reference.

Inorganic phosphate was determined on the perchloric acid extract of the muscles following the procedure of Taussky and Shorr (1953). For pH measurement, meat samples were homogenized rapidly in 3 volumes of deionized distilled water following the procedure of Buck and Black (1967). The pH of the resulting slurry was determined immediately with the glass electrode of a Sargent pH meter.

The development and release of isometric thaw tension was followed on a Grass Polygraph Model 7, equipped with D.C. driver amplifiers, low level D.C. preamplifiers, an ink writing oscillograph with four channels and force transducers capable of measuring to the nearest 1g force. Measurements were carried out at 3°, 25° and 37°C in an environment of oxygen, carbon dioxide or nitrogen. To maintain a constant temperature and moist atmosphere, incubation chambers were constructed of plexiglass (Fig. 1) which allowed the flushing gases to

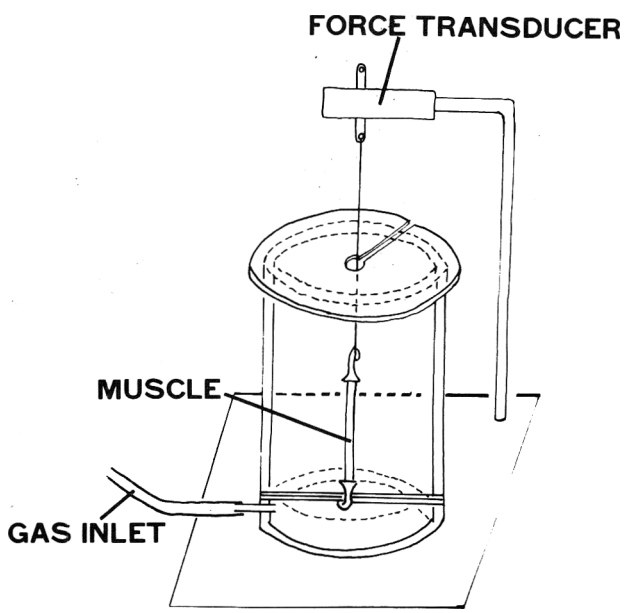


Fig. 1–A schematic diagram of the incubation chamber for isometric thaw tension measurements.

[1] Present address: Dept. of Animal Science, University of Ibadan, Ibadan, Nigeria, Africa

bubble through a solution of 10^{-3}M sodium azide before coming in contact with the suspended muscle strips. The sodium azide was included to inhibit bacterial growth especially at the higher temperatures. The chambers were further lined with moist filter paper to enhance a saturated humid environment.

Each frozen muscle strip 0.1–0.3 cm^2 in cross section and 9 cm long was rapidly weighed and clamped 1 cm from each end with small clips. Following a 3-min tempering at 3°C, strips were attached to the force transducers at one end and at the other, to a horizontal support rod approximately 2 cm from the base of the incubation chamber.

Limited phase contrast microscopic observations were carried out on muscle strips at the end of isometric thaw tension measurements following the procedure outlined in a previous paper (Okubanjo et al., 1975).

Analysis of variance, coefficients of simple correlation and Scheffe's test for differences between means were performed by methods of Snedecor and Cochran (1973) to study the relationship of measured parameters.

RESULTS & DISCUSSION

THE RELATIONSHIP between ATP degradation, pH decline and the accumulation of inorganic phosphate during the incubation of muscle strips in an atmosphere of moist nitrogen are illustrated in Figures 2 to 4. At the three temperatures, the pattern of biochemical changes was similar except for the time scale. The initial mean values of ATP at the onset of thawing were 7.45, 7.28 and 7.36 μmoles/g of fresh tissue at 3°, 25° and 37°C, respectively. These values are slightly higher than previous observations on bovine sternomandibularis by Scopes and Newbold (1968) but are lower than was observed in bovine semitendinosus or psoas muscle by Busch et al. (1967). These differences in ATP may be due to the different procedures used for the estimation of ATP or to the rapidity with which the muscles were removed from the animal and prepared for analysis.

The initial mean values for inorganic phosphate were 16.71, 16.21 and 15.86 μmoles/g at 3°, 25° and 37°C, respectively. The pH values were 6.95, 6.93 and 6.86 for 3°, 25° and 37°C, respectively. These values are lower than the characteristic resting muscle pH of 7.3–7.5 previously determined for muscle (Marsh and Leet, 1966) possibly due to slight glycolytic changes which may have occurred in the muscle strips prior to and during pre-rigor freezing, or to the procedure used for estimation of pH since the procedure of Buck and Black (1967) may have less sensitivity than the iodoacetate technique of Marsh and Leet (1966).

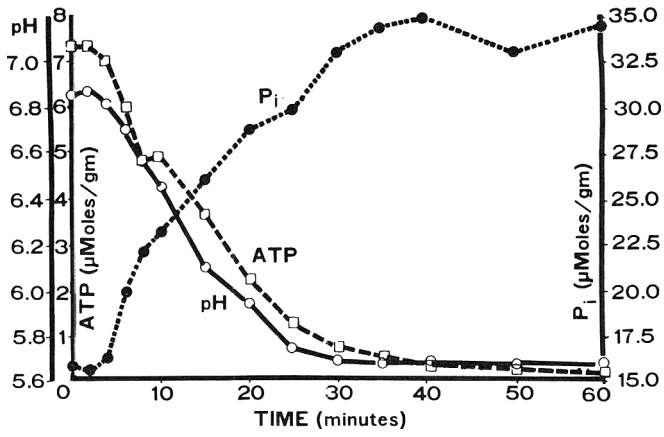


Fig. 2—The time course of changes in the levels of ATP, inorganic phosphate and pH in bovine sternomandibularis undergoing thaw rigor at 3° C.

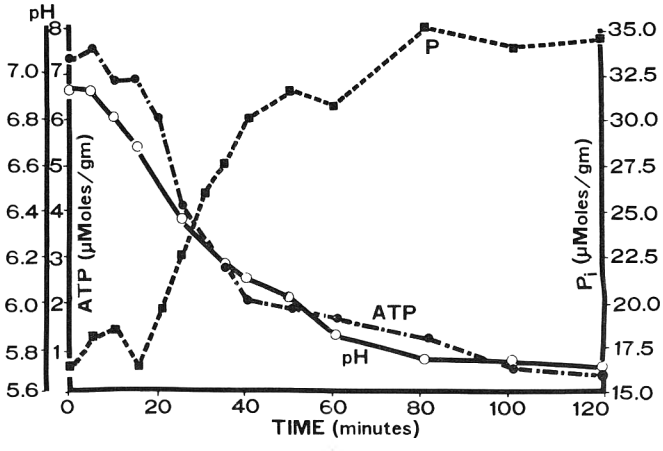


Fig. 3—The time course of changes in the levels of ATP, inorganic phosphate and pH in bovine sternomandibularis undergoing thaw rigor at 25° C.

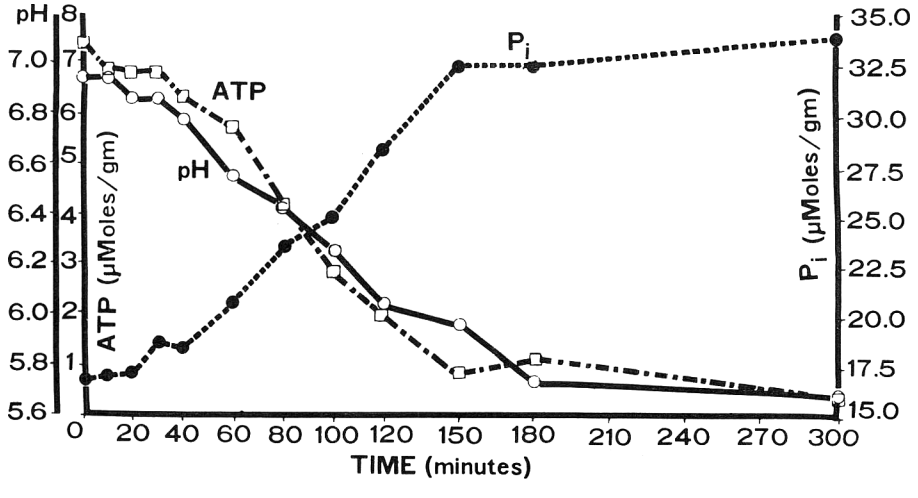


Fig. 4—The time course of changes in the levels of ATP, inorganic phosphate and pH in bovine sternomandibularis undergoing thaw rigor at 37° C.

A short latent period which decreased with rise in incubation temperatures preceded any drastic change in the rates of ATP degradation, pH decline and accumulation of inorganic phosphate. The rates of change were inversely related to temperature. At 3°C, both the concentration of ATP and the pH value reached very low levels within 300 min while the concentration of inorganic phosphate had almost reached the maximum. At 25° and 37°C, these time periods were 120 and 60 min, respectively. Bendall (1960) reported faster changes in thaw rigor at 17°C with ultimate pH and ATP loss in 30 min. These periods exhibit sharp contrasts to similar changes during normal rigor in which a total loss of ATP and the ultimate pH were obtained at 2°C in 48–72 hr (Busch et al., 1967), at 25°C in 24 hr (Cassens and Newbold, 1967a, b) and at 37°C in 8 hr (Cassens and Newbold, 1966).

Correlation coefficients were calculated for the various combinations of the different parameters for each temperature. Results substantiated the negative relationship between ATP and Pi levels with correlations of r = −0.98, −0.93 and −0.94 at 3°, 25° and 37°C, respectively. Level of ATP was highly correlated with pH, r = 0.96, 0.95 and 0.95 at 3°, 25° and 37°C, respectively. Further, the concentration of inorganic phosphate at the various stages of thaw rigor was highly, but negatively, correlated with pH, r = −0.97, −0.94 and −0.96 at 3°, 25° and 37°C, respectively.

The foregoing investigation was made anaerobically under moist nitrogen, a procedure that precluded the more efficient resynthesis of ATP through the oxidative glycolytic pathway. Similar studies carried out in air at room temperature (Scopes and Newbold, 1968) indicate no essential difference from the present observations. In a previous study (Okubanjo et al., 1975), it was shown that during thaw rigor, muscle mitochondria suffer substantial in vivo destruction resulting in various degrees of swelling and shrinkage. Such physical damage may cause severe conformational changes in the membrane system and interfere with the spatial ordering of the mitochondrial enzymes and cofactors. Thereby, the uncoupling of the rephosphorylation of ADP from the electron transport system may favor a higher rate of degradation of the residual ATP.

In the present paper, the tension developed in isometrically held, pre-rigor frozen, muscle strips when subsequently exposed to temperatures above freezing will be referred to as "isometric thaw tension" or just plain "thaw tension" in difference to "isometric tension" developed during normal rigor.

The isometric thaw tension developed in pre-rigor frozen strips exposed to 3°, 25° and 37°C under the influence of three gaseous environments are shown in Figures 5 to 7. The values are presented in g/cm^2 and have been calculated on the assumption that the specific gravity of bovine muscle is 1.06.

The results indicate that, as in normal rigor, pre-rigor frozen bovine muscle held isometrically developed thaw tension very rapidly on exposure to temperatures above freezing. Subsequent to the attainment of maximum thaw tension, there ensued a loss of ability of the muscle strips to maintain the thaw tension. The pattern of thaw tension development at the three temperatures appeared to be similar in the three gaseous environments. In contrast to the development of isometric tension during normal rigor, there was an absence of a recognizable delay phase. The reason for the rapid onset of tension development in thaw rigor may be due to inactivation of the sarcoplasmic reticulum by freezing and thawing. Bendall (1960) discussed similar findings with the conclusions that at

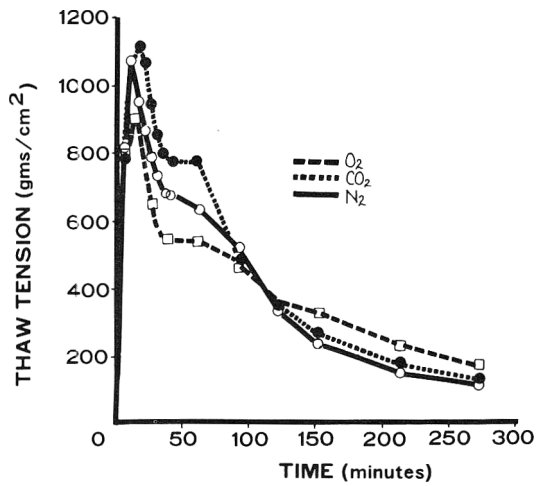


Fig. 6—The effect of various gaseous environments on the development of isometric thaw tension in pre-rigor frozen bovine sternomandibularis thawed at 25°C.

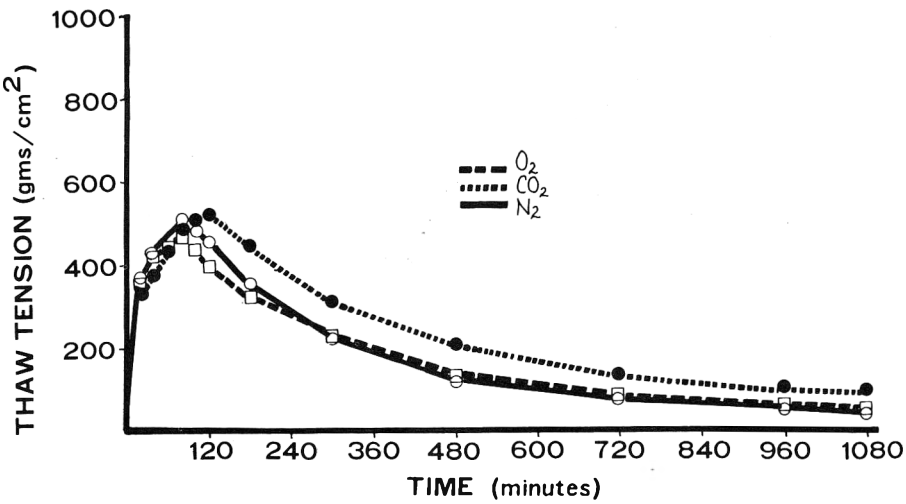


Fig. 5—The effect of various gaseous atmospheres on the development of isometric thaw tension in pre-rigor frozen bovine sternomandibularis thawed at 3°C.

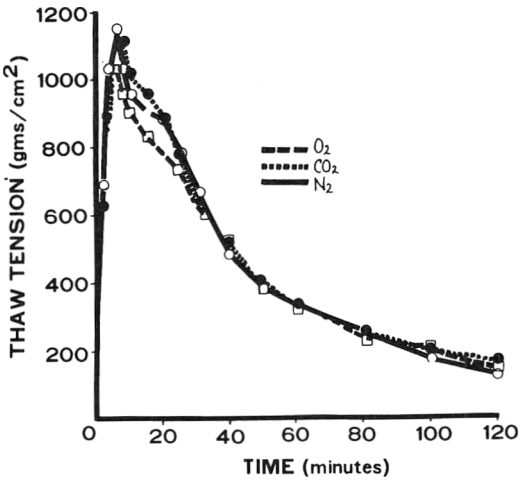


Fig. 7—The effect of various gaseous atmospheres on the development of isometric thaw tension in pre-rigor frozen bovine sternomandibularis thawed at 37°C.

thawing Ca^{2+} is released inhibiting the relaxing factor and stimulating ATPase activity, thus starting immediate contraction. He further stated that contraction ceased when the salt balance was equilibrated allowing the relaxing factor to take control again.

The rate and extent of thaw tension development were more strongly affected by the temperature than by the gaseous environments to which the muscle strips were exposed. Over twice as much thaw tension was developed at 25°C or 37°C as at 3°C (Table 1). The time to attain peak thaw tension decreased sharply at the two higher temperatures when compared with those at 3°C. Also at all three temperatures, tension was reached significantly faster in O_2 than in CO_2 or N_2 atmosphere.

The peak tension measured in this study was appreciably higher than that previously reported on bovine semitendinosus (Jungk et al., 1967). Much of the difference may have been due to improved sensitivity of the instrument used (±1g vs ±5g) or to differences in the procedure used to initiate thaw rigor. In contrast with the present observation, Busch et al. (1967) indicated that in normal rigor, isometric tension development in bovine semitendinosus did not start until about 6 hr after death at 16°C, and at about 3 hr at 37°C although it was almost immediate at 2°C probably due to the cold shortening effect. Since the maximum strength of tetanic contraction under normal condition is of the order of 3 kg/cm^2, the maximum thaw tension observed at 25°C and 37°C for the three gaseous environments was approximately one-third the strength of normal tetanic contraction. While at 3°C, it was approximately one-sixth.

Post thawing decline in isometric thaw tension was most rapid at 37°C, decreased somewhat at 25°C and was least at 3°C. As shown in Figures 5 to 7, the muscle strips have almost lost all thaw tension after 120 min at 37°C, 270 min at 25°C and 1080 min at 3°C. This contrasts sharply with the 48–312 hr period necessary for loss of isometric tension in normal rigor as observed by Goll et al. (1970) in bovine semitendinosus muscle. The initial rate of decline of thaw tension was again similar for each temperature in the three flushing gases. A secondary plateau was developed in the decline curve at 25°C, and to a lesser extent at 37°C. Szent-Gyorgyi (1949) attributed similar biphasic response in pre-rigor frozen frog sartorius and rabbit psoas muscles to the sudden introduction of the frozen muscle strips to elevated temperatures. While this may be causative, it seems more likely that the sudden onset of tension in some sarcomeres results in rupture of the attachment of myosin cross-bridges to actin filaments in other sarcomeres in the same myofibril and caused the fast initial decline. Further, sarcomeres developing the least cross-bonds were more susceptible to the severe disruption resulting in physical pulling out of the actin filaments completely from the A band as described in previous work (Okubanjo et al., 1975). Apparently then, the secondary plateau was coincident with reduced disruption of the attachment of myosin cross-bridges to actin filaments.

Limited phase contrast microscopic studies on muscle strips held isometrically in the different gaseous environments and at all temperatures showed this to be the case. The structural damage was more severe in strips incubated at 25°C and 37°C than in those at 3°C although structural differences due to the gaseous environments were not as severe. It is therefore possible that the actual maximum tension attained by muscle strips during thaw rigor is a net balance between (1) continuing thaw tension development in the intact fibers as a result of increased numbers of actin-myosin rigor cross-links being formed and (2) increasing loss of the ability to maintain this thaw tension at any particular period due to the breaking of actin-myosin bonds and a subsequent loss of thaw tension. On an intact fast frozen carcass therefore, the attempted shortening at the height of thaw rigor by opposing muscles on the same bones would result in stiffness more drastic than in normal rigor.

Table 1—Relative tension and time at peak of isometric thaw tension development in pre-rigor frozen bovine sternomandibularis at various temperatures and in various gaseous environments[a]

	Temperature and environment								
	3°C			25°C			37°C		
Parameter	O_2	CO_2	N_2	O_2	CO_2	N_2	O_2	CO_2	N_2
Tension, g	494.98	545.22	551.05	1,107.58	1,225.38	1,199.33	1,190.68	1,235.35	1,210.97
	(91.50)[b]	(87.03)	(93.71)	(226.56)	(204.56)	(237.16)	(129.76)	(143.00)	(197.34)
Time, min	77.33[c]	110.95[d]	84.07	9.95[c]	13.64[d]	10.78	5.03[e]	6.89[d]	5.40
	(21.89)	(20.48)	(12.01)	(1.54)	(2.43)	(1.39)	(1.20)	(2.36)	(1.55)

[a] Mean of determinations on 12 muscles
[b] Standard deviation from the mean
[c,d] Value with a superscript is significantly different ($P < 0.01$) from other two values at the same temperature.
[e] Value with superscript is significantly different ($P < 0.05$) from other two values at the same temperature.

However, unlike normal rigor, the loss of ability to maintain tension would occur more rapidly due to the rupture of actin-myosin cross-linkages. The latter may be compared to rigor-stretching observed by Hegarty et al. (1973) in rigor-stretch turkey with the difference that the force necessary for the slippage was generated within the muscle strip rather than being imposed externally. It is not clear whether the loss of isometric thaw tension observed in this study could be regarded as a true resolution of thaw rigor since stretching of sarcomeres are localized and not uniformly spread throughout the fiber as would be expected with true resolution of rigor.

Another feature of this study was the relationship between the pattern of ATP degradation and thaw tension development. Bate-Smith (1939) observed that during normal rigor development, new cross bonds were formed between the muscle contractile units following the loss of ATP. Kushmerick and Davies (1968) as well as Schmidt and Briskey (1968) have also shown that an appreciable degradation of both ATP and ADP is essential for the development of muscle inextensibility. Conversely, postmortem shortening requires ATP as an energy source. A comparison of Figures 2 to 4 with Figures 5 to 7 shows that the development of thaw tension occurred at all thawing temperatures before the decrease in level of ATP.

Goll et al. (1970) have stated that the reason that rigor mortis does not occur until just before the complete loss of ATP is that the decline in ATP concentration lowers the ability of the sarcoplasmic reticulum to accumulate Ca^{2+} against a concentration gradient, and this release of Ca^{2+} is required to initiate tension development. As clearly demonstrated in this study of thaw rigor, the ability of the sarcoplasmic reticulum to accumulate Ca^{2+} is destroyed so that Ca^{2+} is released in the presence of ATP and the clear demarcation between rigor mortis and ATP concentration becomes evident.

REFERENCES

Bate-Smith, E.C. 1939. Changes in elasticity of mammalian muscle undergoing rigor mortis. J. Physiol. 96: 176.

Bendall, J.R. 1960. Postmortem changes in muscle. In "Structure and Function of Muscle," Ed. Bourne, G.H., Vol 3, p. 261. Acad. Press, New York and London.

Buck, E.M. and Black D.L. 1967. The effect of stretch-tension during rigor on certain physical characteristics of bovine muscle. J. Food Sci. 32: 539.

Busch, W.A., Parrish, F.C. and Goll, D.E. 1967. Molecular properties of postmortem muscle. 4. Effect of temperature on adenosine triphosphate degradation, isometric tension parameters and shear resistance of bovine muscle. J. Food Sci. 32: 390.

Busch, W.A., Goll, D.E. and Parrish, F.C. Jr. 1972. Molecular properties of postmortem muscle. Isometric tension development and decline in bovine, porcine and rabbit muscle. J. Food Sci. 37: 289.

Cassens, R.G. and Newbold, R.P. 1966. Effect of temperature on post mortem metabolism in beef muscle. J. Sci. Food Agric. 17: 254.

Cassens, R.G. and Newbold, R.P. 1967a. Temperature dependence of pH changes in ox muscle post mortem. J. Food Sci. 32: 13.

Cassens, R.G. and Newbold, R.P. 1967b. The effect of temperature on the time course of rigor mortis in ox muscle. J. Food Sci. 32: 269.

Chrystall, B.B., Cook, C.F. and Bailey, M.E. 1970. A simple apparatus for muscle extensibility measurement. J. Food Sci. 35: 499.

Goll, D.E., Arakawa, N., Stromer, M.H., Busch, W.A. and Robson, R.M. 1970. Chemistry of muscle proteins as a food. In "Physiology and Biochemistry of Muscle as a Food," Ed. Briskey, E.J., Cassens, R.G. and Marsh, B.B. The University of Wisconsin Press, Madison, Wisc.

Hegarty, P.V.J., Dahlin, K.J., Benson, E.S. and Allen, C.E. 1973. Ultrastructural and light microscope studies on rigor-extended sarcomeres of avian and porcine skeletal muscle. J. Anat. 115: 203.

Jungk, R.A., Snyder, H.E., Goll, D.E. and McConnell, K.G. 1967. Isometric tension changes and shortening in muscle strips during postmortem aging. J. Food Sci. 32: 158.

Kushmerick, M.J. and Davies, R.E. 1968. The role of phosphate compounds in thaw contraction and the mechanisms of thaw rigor. Biochem. Biophys. Acta. 153: 279.

Marsh, B.B. and Leet, N.G. 1966. Studies on meat tenderness. 3. The effects of cold shortening on tenderness. J. Food Sci. 31: 450.

Newbold, R.P. and Scopes, R.K. 1967. Postmortem glycolysis in ox skeletal muscle. Effect of temperature on the concentration of glycolytic intermediates and cofactors. Biochem. J. 105: 127.

Okubanjo, A.O., Stouffer, J.R., Bensadoun, A. and Sack, W.O. 1975. Ultrastructure of thaw rigor bovine muscle. J. Food Sci. 40: In press.

Schmidt, G.R. and Briskey, E.J. 1968. Observations on the development of rigor mortis in porcine muscle under controlled environmental conditions. J. Anim. Sci. 27: 1764.

Schmidt, G.R., Cassens, R.G. and Briskey, E. 1968. Development of an isotonic isometric rigorometer. J. Food Sci. 33: 239.

Scopes, R.K. and Newbold, R.P. 1968. Postmortem glycolysis in ox skeletal muscle. Effect of pre-rigor freezing and thawing on the intermediary metabolism. Biochem. J. 109: 197.

Sigma Chemical Company. 1967. The ultraviolot determination of ATP. Sigma Technical Bulletin No. 366-UV, rev. Aug. 1967.

Snedecor, G.W. and Cochran, W.G. 1973. "Statistical Methods." Iowa State University Press, Ames, Iowa.

Stromer, M.H. and Goll, D.E. 1967. Molecular properties of postmortem muscle. 3. Electron microscopy of myofibrils. J. Food Sci. 32: 386.

Stromer, M.H., Goll, D.E. and Roth, L.E. 1967. Morphology of rigor-shortened bovine muscle and the effect of trypsin on pre- and post-rigor myofibrils. J. Cell Biol. 34: 431.

Szent-Gyorgyi, A. 1949. Free energy relationships and contraction of actomyosin. Biol. Bull. 96: 140.

Taussky, H.H. and Shorr, E. 1953. A microcolorimetric method of the determination of inorganic phosphorus. J. Biol. Chem. 202: 675.

Ms received 1/16/75; revised 4/23/75; accepted 4/28/75.

R. C. WHITING and J. F. RICHARDS
Dept. of Food Science, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada

THAW RIGOR INDUCED ISOMETRIC TENSION AND SHORTENING IN BROILER-TYPE CHICKEN MUSCLES

INTRODUCTION

THE SEVERE SHORTENING that a muscle undergoes when frozen and thawed prerigor and the resulting loss of tenderness has been reviewed by Luyet (1966), Bendall (1971), Newbold and Harris (1972) and Marsh (1972). The observed effects of the rate of freezing and thawing on thaw rigor have not always been consistent. The freezing rate has usually had a nonsignificant effect on tenderness (Marsh et al., 1968; Jakobsson and Bengtsson, 1973; Streeter and Spencer, 1973), while a slower thawing rate has usually produced more tender meat (Marsh et al., 1968; Korslund and Essary, 1971). Marsh et al. (1968) and Behnke et al. (1973a, b) found that the length of time that muscle is exposed to −3°C has a marked influence on subsequent shortening and tenderness.

Prerigor aging affected the severity of thaw rigor when measured by shear press, panel scores or shortening on lamb (Marsh and Thompson, 1958; Marsh et al., 1968; McCrae et al., 1971), beef (Jakobsson and Bengtsson, 1973) and chicken (Dawson et al., 1956). The toughness was maximum at slaughter and decreased after sufficient aging and/or slow thawing.

The postmortem isometric tension pattern of poultry white muscle has been determined by Jungk and Marion (1970) and Wood and Richards (1974). In this study, the strength and duration of the isometric tension and muscle shortening were measured at varying times postmortem in red and white muscles to further characterize the thaw rigor phenomena.

EXPERIMENTAL

COMMERCIAL BROILERS of both sexes from 8–14 wk old were exsanguinated in a restraining cone and muscle strips parallel to the fiber direction were immediately excised and prepared for isometric tension measurement on an E & M Physiograph (Narco BioSystems Inc., Houston, Texas) by a procedure similar to that of Wood and Richards (1974). Up to six muscle strips, each 5.0 cm were excised from Pectoralis major (white) and Biceps femoris (red). The strips were weighed, placed in ambient temperature phosphate buffer (pH 7.2) and attached to the physiograph within 25 min postmortem. The weight of each strip, averaging 0.71g for P. major and 0.42g for B. femoris, was used to calculate cross-sectional area.

The strips were frozen while attached to the physiograph by draining the buffer from the chambers and carefully flowing Genetron-12 (dichlorodifluoromethane, Allied Chemical Co., Morristown, N.J., b.p. −29.8°C) over the strips for 10–15 sec. After approximately 2 min, the buffer was returned to the chambers to rapidly thaw the muscles unless a slower rate of thaw in air was desired.

Muscle strips for shortening determinations were excised simultaneously with the strips for isometric tension measurements and 5.0 cm lengths marked with pins. These strips were aged in phosphate buffer at ambient temperature, frozen by immersion in Genetron-12, and thawed in buffer for a minimum of 10 min before being remeasured to determine shortening.

In the experiment to determine the shortening response of muscle held at −1°C, the strips were cut to 5.0 cm lengths and heat sealed in a single layer of vinyl film. The excised strips were immediately frozen in air (−36°C), coded and held from 1–3 days before use. A total of 144 muscle strips from five birds were pooled and randomized before assignment to treatments. The frozen muscle strips were thawed at −1°C for 0, 1, 3 or 5 hr, then were held at 5°C for 1 hr or 21°C for 30 min. A 4% ethanol solution at −1 ± 0.5°C (Blue M cooling unit, Blue M Electric Co., Blue Island, Ill.) and water at 5° and 21°C were used to quickly bring the muscles to the desired temperatures. After each temperature treatment the lengths of the strips were remeasured (± 0.1 cm).

RESULTS & DISCUSSION

THE ISOMETRIC TENSION of control strips in phosphate buffer at 22°C peaked at an average of 4 hr postmortem for P. major and 5.5 hr for B. femoris (Fig. 1). The difference was nonsignificant ($p > 0.05$); however, a similar tendency for maximum tension to be reached earlier in white than red muscle was observed by Schmidt et al. (1970) in porcine muscle. The time and maximum tension for P. major

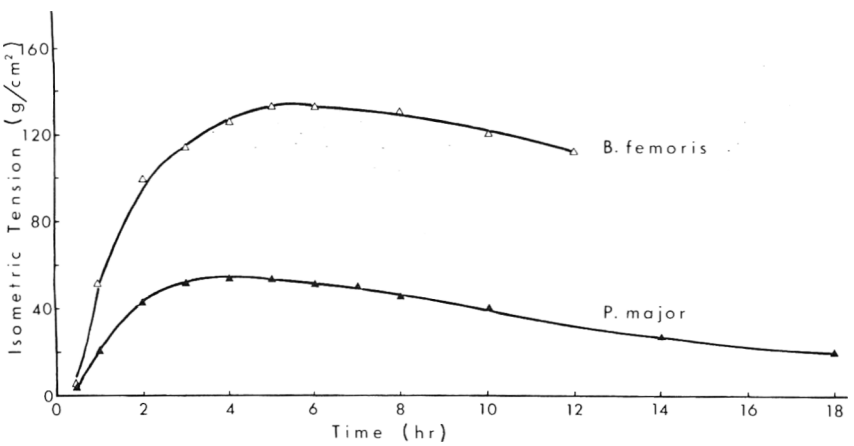


Fig. 1—Isometric tension pattern of chicken muscle strips at 22°C. (Each point for P. major is an average of two strips from three birds, and for B. femoris, an average of one strip from six birds.)

(55g/cm^2) agree well with the results of Wood and Richards (1974). The significantly greater ($p < 0.01$) maximum tension of B. femoris compared to P. major (133 vs 55g/cm^2) parallels the findings of Busch et al. (1972) on sections of red and white fibers from porcine semitendinosus at 2°C.

The magnitude of thaw rigor tension in muscles frozen and thawed 30 min postmortem was also greater in B. femoris (Table 1), although statistically significant ($p < 0.01$) only for the strips thawed in buffer. The greater time necessary to achieve maximum tension with thawing in air relative to buffer (19 vs 1 min respectively) was associated with a significantly lower ($p < 0.05$) tension for both muscles, perhaps because the strips thawed in air were exposed for a greater length of time to −3°C where glycolytic reactions deplete the muscles energy reserves while the residual ice crystals impede shortening (Marsh et al., 1968; Behnke et al., 1973a, b).

The rapid onset of the thaw rigor required approximately 1 min in buffer to achieve maximum tension in both muscles, but declined rapidly in P. major and relatively slowly in B. femoris (Fig. 2). The initial tension rise was due to buoyancy loss upon removal of the buffer, weight of the refrigerant and probably a slight cold shortening. White muscle showed the ability to contract after a second freezing whereas red muscle showed a sharp tension decline with repetitive freeze-thawings which may reflect extensive structural damage from the freezings and strong contractions.

P. major, in addition to the rapid tension peak upon thawing, showed a second gradual increase before declining. This second increase was extremely variable in time and intensity, but generally it slightly exceeded the normal rigor mortis tension and peaked approximately 1 hr after thawing in muscles frozen immediately post-slaughter. Frog muscle fibers given a slow freeze-slow thaw treatment by Luyet et al. (1965) underwent a four step transformation in shortening: (1) a sudden contraction; (2) partial relaxation lasting a minute or two; (3) a rest stage of long duration and then (4) an irreversible shortening to 45–50% of the before-freezing length. The freezing and thawing rates used in our work were similar to the slow freeze-slow thaw treatment, and produced a tension pattern in P. major corresponding to the four step shortening pattern. B. femoris, however, showed no evidence of this pattern.

Bendall (1971) attributed thaw rigor to a rapid flux of Ca^{+2} ions out of the Ca-pump of the sarcotubular system which stimulated the contractile ATPase. The rapid relaxation was attributed to the Ca-pump regaining control and removing the Ca^{+2} ions, provided that ATP was still available. White muscle has a more highly developed sarcoplasmic reticulum than red muscle which if translated into a more rapid recapture of Ca^{+2} could explain the shapes of the initial peaks observed here (Lawrie, 1968; Franzini-Armstrong, 1973).

Muscle strips frozen on the physiograph and companion strips frozen unrestrained both declined in intensity of thaw rigor with aging. P. major (Table 2) maintained its ability to produce tension for 1-1/2 hr before declining to approximately half of the initial tension increase at rigor onset (4–6 hr postmortem) and losing the remaining contractibility soon after. Similarly, the unrestrained strips frozen prerigor exhibited a greater degree of shortening than those frozen when near or in rigor.

Table 1–Maximum isometric tension of P. major and B. femoris strips frozen and thawed 30 min postmortem at 22°C in buffer and air[a]

Muscle	Maximum isometric tension	
	Buffer 22°C (g/cm^2)	Air 22°C (g/cm^2)
B. femoris	476 ± 98	327 ± 141
P. major	244 ± 77	201 ± 78

[a] Means and standard deviations of nine birds with two strips each for B. femoris and eight birds with one strip each for P. major

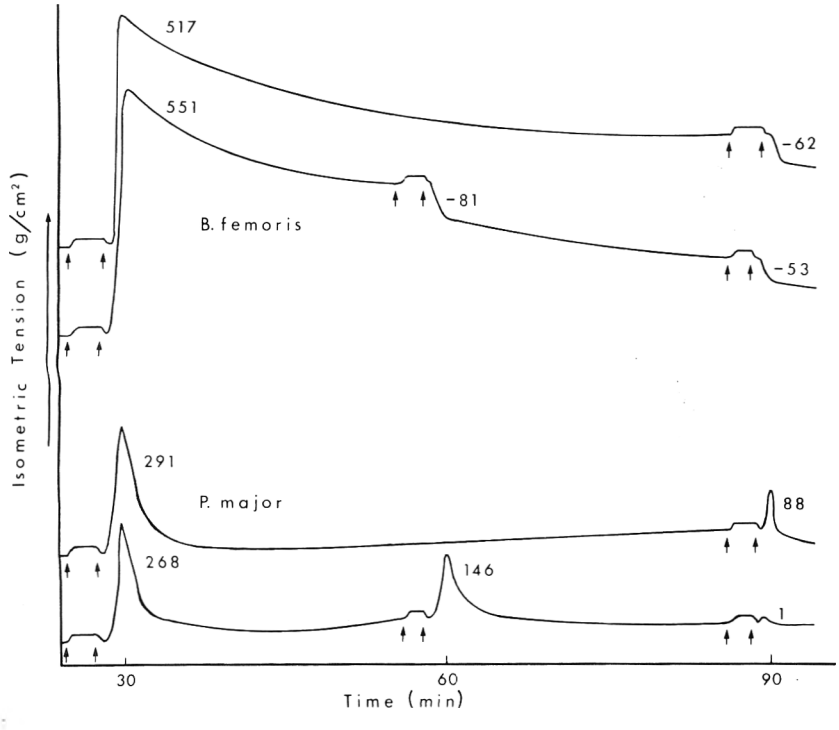


Fig. 2–Isometric tension of P. major and B. femoris strips subjected to repetitive freezing and thawing. The first arrow of each pair indicates the withdrawal of buffer and addition of refrigerant and the second arrow indicates the return of the buffer and initiation of thawing. The values represent average net tension change from prefreeze to the immediate post thaw peak or decline based on six birds (one strip for each bird for the double freeze and two strips for each bird for the triple freeze).

B. femoris behaved similarly to P. major in thaw rigor intensity with aging (Table 3). The increase in shortening after freezing and thawing was virtually nonexistent at rigor mortis and despite the greater isometric tension in B. femoris the initial shortening was again about 60%. Thaw rigor isometric tensions of B. femoris appeared to decline more rapidly than P. major, but the data came from different birds making this comparison uncertain. Variation in isometric tension was particularly high for B. femoris because of difficulties in cutting the small strips uniformly and probable greater intramuscular variation in fiber type. The maximum thaw rigor tension for both muscles was approximately five times as great as its normal rigor tension, but this was still considerably less than the estimated muscle capability of about 4 kg/cm^2 (Bendall, 1973).

The declining ability to produce tension with the onset of rigor mortis corresponds very well to data on the consequences of tension (shortening and loss of tenderness) studied by Dawson et al. (1956), Marsh and Thompson (1958), Marsh et al. (1968), McCrae et al. (1971), and Jakobsson and Bengtsson (1973). With decreasing capability to produce tension, a muscle would be less able to shorten on the carcass to the 20–40% range where tenderness is greatly reduced (Newbold and Harris, 1972; Behnke et al., 1973b).

To ascertain the relative importance of the rate of thawing and the temperature of thawing, the muscle strips frozen prerigor were thawed and aged at −1°C for varying times and then raised to 5° or 21°C. This pattern was chosen to simulate the temperature pattern to which thawing meat might be exposed in practice with small strips providing a reasonably precise temperature control.

The B. femoris strips shortened from 5.0 cm to an average of 3.86 cm during freezing while P. major shortened only to 4.41 cm (22.8 and 11.8% shortening, respectively). Shortening was also consistently greater ($p < 0.05$) for B. femoris than the comparable P. major strips after the subsequent thaw treatments with the exception of the direct thaw at 21°C where the maximum shortening of 58% was attained by both muscles (Table 4). The relative behavior of red and white muscle may be species dependent, as Lawrie (1968) reported that white muscle of rabbit shortened more than red during thaw rigor.

Shortening of each muscle consistently was greater after holding at 21°C than at 5°C regardless of the time at −1°C. Increasing the temperature directly from the frozen state to −1° (1 hr), 5° or 21°C resulted in average contractions of 16.0%, 24.6% and 58.2%, respectively for P. major and 24.4%, 38.8% and 58.0% for B. femoris. No evidence of an enhancement of shortening at temperatures near 0°C (i.e., cold shortening) was evident in the thaw rigor patterns.

During incubation at −1°C, the muscles shortened predominantly within the first hour, then they contracted further when the temperature was raised to either 5° or 21°C. The shortening from the frozen state to 5° or 21°C was significant ($p < 0.05$) for every muscle and treatment combination. Muscles held at −1° and then at 5°C, underwent little further contraction when subsequently held at 21°C. Those muscles exposed only to 5°C prior to being held at 21°C exhibited additional shortening of several percentage points. More importantly, increasing the time at −1°C reduced the subsequent shortening of muscles at either 5°C or 21°C. The muscle strips not exposed to treatment at −1°C exhibited the most shortening and with the exception of B. femoris strips held at 5°C, shortening of strips not held at −1°C was significantly greater than that of strips held for 3 or 5 hr at −1°C ($p < 0.05$).

Marsh et al. (1968) and Behnke et al. (1973a, b) holding muscle at −3°C found an acceleration of biochemical changes while shortening was prevented by ice crystals. Our work indicates that temperatures immediately above the freezing point minimize contraction while these biochemical changes con-

Table 2—Isometric tension and shortening of P. major frozen and thawed at varying times postmortem

Time of freezing (hr)	No. of birds	Isometric tension[a]		Shortening[a]	
		Prefreeze (g/cm^2)	Post thaw[b] (g/cm^2)	Prefreeze (%)	Post thaw (%)
0.5	7	7 ± 6	256 ± 32	5 ± 1	58 ± 5
1.0	4	14 ± 12	253 ± 38[c]	14 ± 7	60 ± 4
1.5	4	24 ± 14	300 ± 95	20 ± 11	60 ± 7
2.5	4	39 ± 24	184 ± 84	25 ± 4	60 ± 7
4	4	49 ± 20	155 ± 49	26 ± 2	50 ± 9
6	7	42 ± 16	72 ± 57	28 ± 4	43 ± 7
18	3	20 ± 9	-3 ± 5	31 ± 5	29 ± 4

[a] Means and standard deviations
[b] Tension increase from the prefreeze tension
[c] Mean and standard deviation of three birds

Table 3—Isometric tension and shortening of B. femoris frozen and thawed at varying times postmortem

Time of freezing (hr)	Isometric tension[a]		Shortening[a]	
	Prefreeze (g/cm^2)	Post thaw[b] (g/cm^2)	Prefreeze (%)	Post thaw (%)
0.5	7 ± 3	612 ± 192	12 ± 3	61 ± 13
1	40 ± 21	401 ± 216		
2	92 ± 33	111 ± 66	30 ± 4	43 ± 6
4	87 ± 21	38 ± 34		
6	111 ± 28	-1 ± 10	37 ± 5	41 ± 4
12	115 ± 21	-28 ± 13		

[a] Means and standard deviations, 0.5 to 4 hr from six birds and 6 and 12 hr from five birds
[b] Tension increase from the prefreeze tension

Table 4—Effect of holding at −1°C on the shortening of P. major and B. femoris[a]

Muscle	Time at −1°C (hr)	Shortening after freezing (%)	Shortening after holding at −1°C (%)	Shortening after holding at 5°C (%)[b]	Shortening after holding at 21°C (%)[b]
P. major	0	9.4		24.6a	30.0
	1	10.4	16.2	22.2ab	22.2
	3	10.4	14.4	15.6b	16.2
	5	14.0	17.2	17.6b	18.8
B. femoris	0	18.8		38.8a	48.4
	1	19.8	21.6	32.4a	34.0
	3	26.0	31.2	33.6a	34.8
	5	25.2	31.8	33.2a	34.0
P. major	0	10.0			58.2a
	1	12.4	15.8		30.0b
	3	13.2	19.4		27.8b
	5	14.0	20.0		23.2b
B. femoris	0	20.2			58.0a
	1	24.2	27.2		57.6a
	3	24.0	29.2		39.0b
	5	23.6	31.0		34.2b

[a] Means of 10 strips for P. major and eight strips of B. femoris
[b] Values within each muscle group with different letters are significantly different ($p < 0.05$).

tinue. This minimal shortening both above and below the freezing point undoubtedly accounts for the general finding that meat frozen prerigor is more tender when slowly thawed than when rapidly thawed.

REFERENCES

Behnke, J.R., Fennema, O. and Cassens, R.G. 1973a. Quality changes in prerigor beef muscle at −3°C. J. Food Sci. 38: 539.

Behnke, J.R., Fennema, O. and Haller, R.W. 1973b. Quality changes in prerigor poultry at −3°C. J. Food Sci. 38: 275.

Bendall, J.R. 1971. Structural and biochemical changes during the cooling and freezing of meat. IFST Proceedings, Vol 4, No. 3 (Part 1), 124.

Bendall, J.R. 1973. Postmortem changes in muscle. In "The Structure and Function of Muscle," 2nd ed, Vol 2, p. 243, Ed. Bourne, G.H. Academic Press, N.Y.

Busch, W.A., Goll, D.E. and Parrish, F.C. Jr. 1972. Molecular properties of postmortem muscle. Isometric tension development and decline in bovine, porcine and rabbit muscle. J. Food Sci. 37: 289.

Dawson, L.E., Davidson, J.A., Frang, M. and Walters, S. 1956. The effects of time interval between slaughter and freezing on toughness of fryers. Poultry Sci. 35: 1140.

Franzini-Armstrong, C. 1973. Membranous systems in muscle fibers. In "The Structure and Function of Muscle," 2nd ed, Vol 2, p. 531, Ed. Bourne, G.H. Academic Press, N.Y.

Jakobsson, B. and Bengtsson, N. 1973. Freezing of raw beef: Influence of aging, freezing rate and cooking method on quality and yield. J. Food Sci. 38: 560.

Jungk, R.A. and Marion, W.W. 1970. Postmortem isometric tension changes and shortening in turkey muscle strips held at various temperatures. J. Food Sci. 35: 143.

Korslund, M.K. and Essary, E.O. 1971. Methods of defrosting turkeys. Poultry Sci. 50: 1790.

Lawrie, R.A. 1968. 'Thaw-rigor' and 'cold-shortening' in rabbit muscle. J. Food Technol. 3: 203.

Luyet, B.J. 1966. Behavior of muscle subjected to freezing and thawing. In "The Physiology and Biochemistry of Muscle as a Food," Ed. Briskey, E.J., Cassens, R.G. and Trautman, J.C., p. 353. The University of Wisconsin Press, Madison.

Luyet, B.J., Rapatz, G.L. and Gehenio, P.M. 1965. Observations on the sequence of events encountered in the passage of muscle fibers into "thaw rigor." Biodynamica 9: 283.

Marsh, B.B. 1972. Post mortem muscle shortening and meat tenderness. Proceedings of the Meat Industry Research Conference, American Meat Institute Foundation, p. 109.

Marsh, B.B. and Thompson, J.F. 1958. Rigor mortis and thaw rigor in lamb. J. Sci. Food Agric. 9: 417.

Marsh, B.B., Woodhams, P.R. and Leet, N.G. 1968. Studies in meat tenderness. 5. The effects on tenderness of carcass cooling and freezing before the completion of rigor mortis. J. Food Sci. 33: 12.

McCrae, S.E., Seccombe, C.G., Marsh, B.B. and Carse, W.A. 1971. Studies in meat tenderness. 9. The tenderness of various lamb muscles in relation to their skeletal restraint and delay before freezing. J. Food Sci. 36: 566.

Newbold, R.P. and Harris, P.V. 1972. The effect of pre-rigor changes on meat tenderness. A review. J. Food Sci. 37: 337.

Schmidt, G.R., Cassens, R.G. and Briskey, E.J. 1970. Changes in tension and certain metabolites during the development of rigor mortis in selected red and white skeletal muscles. J. Food Sci. 35: 571.

Streeter, E.M. and Spencer, J.V. 1973. Cryogenic and conventional freezing of chicken. Poultry Sci. 52: 317.

Wood, D.F. and Richards, J.F. 1974. Isometric tension studies on chicken pectoralis major muscle. J. Food Sci. 39: 525.

Ms received 1/23/75; revised 3/26/75; accepted 3/30/75.

Y. B. LEE, G. L. HARGUS, J. A. KIRKPATRICK, D. L. BERNER and R. H. FORSYTHE
Basic Research Dept., Campbell Institute for Food Research, Camden, NJ 08101

MECHANISM OF LIPID OXIDATION IN MECHANICALLY DEBONED CHICKEN MEAT

INTRODUCTION

MECHANICALLY DEBONED poultry meat (MDPM) has received increased interest in recent years for use in emulsified and other processed food products. Flavor instability during storage, however, has limited its usage and lipid oxidation has been considered to be a major cause of quality deterioration in MDPM (Dimick et al., 1972; Froning and Johnson, 1973; Johnson et al., 1974). Recently Moerck and Ball (1974) reported that highly unsaturated fatty acids in the phospholipid fraction appeared to be responsible for the development of oxidative rancidity. Although Froning and Johnson (1973) suggested that heme pigments from bone marrow increased the instability of MDPM and products containing MDPM during frozen storage, it is still not certain that heme is the only oxidative catalyst in MDPM.

It is known that both hemoprotein and nonheme iron function as catalysts in rat tissues (Wills, 1966). Evidence was also presented that both heme and nonheme iron are active catalysts of lipid oxidation in beef (Liu and Watts, 1970). These two types of catalysts could be differentiated by their relative activities at different pH values and in the presence of chelating agents, ascorbic acid, thiol compounds and selective inhibitors (Wills, 1965, 1966; Liu, 1970a). The identification of the main type of catalytic action is important from the practical standpoint of MDPM handling, because the effects of treatment or additives on lipid oxidation may vary both in degree and direction with the two types of catalysts. The possible involvement of enzymatic catalysts in lipid oxidation of MDPM is eliminated, because animal tissues lack lipoxidase, the only known enzyme which catalyzes the direct reaction of unsaturated fats with oxygen (Tappel, 1953a).

The objective of the present study was to characterize the mechanism of lipid oxidation in mechanically deboned chicken meat (MDCM) and to determine the relative importance of hemoprotein and nonheme iron as catalysts of lipid oxidation.

EXPERIMENTAL

Materials

Mechanically deboned chicken meat (MDCM) in this experiment was prepared from upper torso portion of broiler carcass consisting of upper back, ribs and neck with a Bibun mechanical deboner. The deboned material was immediately frozen and transported to the laboratory. A 20% (w/v) homogenate in 0.1M cold phosphate buffer, pH 7.0, was prepared using a Tissumizer (Tekmar Co., Cincinnati, Ohio), then filtered through four layers of cheese cloth. The filtrate was collected and used in the subsequent series of experiments.

Linoleic acid emulsion

Emulsions of linoleic acid were used as substrates for the oxygen uptake study and were always prepared immediately before use. Linoleic acid (1.5g) was added to 6 ml of 0.1M phosphate buffer (pH 7.0) containing 2.5g Tween 20. The emulsification was accomplished by blending with the Tissumizer for 30 sec at 25°C.

Oxidation studies

A modified form of the rapid oxygen uptake method described by Berner et al. (1974) was employed. In a typical experiment, 1.5g of linoleic acid emulsion was added to 46 ml of 0.1M phosphate buffer (held at 25°C in a water bath) in a 50 ml Erlenmeyer flask. The contents were thoroughly stirred until a homogeneous solution was obtained. To the buffered emulsion were added 2 ml of test additive solution and 0.5 ml of MDCM tissue homogenate (20% w/v), giving a final linoleic concentration of 225 mg/50 ml. Immediately after the addition of tissue homogenate, the rate of oxygen uptake was recorded, using the Beckman Oxygen Analyzer model 777 coupled to a Sargent recorder model SR with a chart speed of 0.5 inch/min. The rates were linear after an initial lag period and the oxygen uptake was calculated from the recording by measuring the linear slope over a 3-min period and expressing the values as mm O_2/min. All the oxygen uptake studies were carried out at 25°C and all the values reported are means of duplicate determinations.

Determination of iron

Total iron content of MDCM was determined according to the procedure described by Cameron (1965). Total heme pigments and myoglobin concentration were determined by the method described by Rickansrud and Henrickson (1967). The difference between total heme pigments and myoglobin was expressed as hemoglobin concentration. Nonheme iron was calculated by subtracting total heme iron from total iron content. All the values reported are means of triplicate determinations.

Fatty acid analyses

Fatty acid composition of MDCM fat was analyzed to determine the content of polyunsaturated fatty acids. The method of Bligh and Dyer (1959) was used to extract and purify the lipids.

A modified form of the DeMan method (1964) was used for the preparation of fatty acid methyl esters from the purified lipid extract. The concentration of the interesterification reagent was changed to 0.5% anhydrous sodium methylate dissolved in anhydrous methanol (w/w).

The methyl esters of the fatty acids were separated and identified using gas liquid chromatography on a 30.5m × 0.5 mm SCOT-DEGS column (Perkin-Elmer Co., Norwalk, Conn.). The initial column temperature was 165°C followed by temperature programming from 165°C to 190°C at 2°C/min. The injector temperature was 190°C and the flame ionization detector temperature was 220°C. The flow rate of helium was 10 ml/min. The voltage output of the Perkin-Elmer 900 amplifier was fed to an Infotronics CRS-100 digital integrator and to a 1 millivolt recorder. Integrated GLC peak areas were corrected for response factors obtained from a typical reference mixture, RM-3 (Supelco Inc., Bellefonte, Pa.).

RESULTS & DISCUSSION

Effect of homogenate concentration

At pH 7.0 in phosphate buffer, homogenates of MDCM catalyzed a rapid rate of oxidation of the linoleic acid emulsion. The rate of oxidation increased linearly with homogenate concentration to 0.5% (w/v) suspensions and thereafter at a reduced rate (Fig. 1). The lipid oxidation rate was much faster with MDCM than with the beef homogenate reported by Liu (1970b).

Effect of pH

The rate of oxygen uptake increased with increasing pH (Table 1). The initial lag period was also affected by pH, decreasing with increasing pH. The oxygen uptake rate at pH 7.5

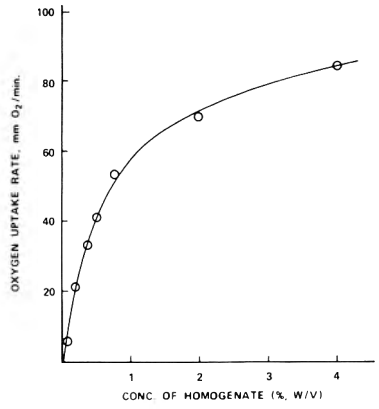


Fig. 1–Relation between MDCM homogenate concentration and oxygen uptake rate at pH 7.0.

was approximately two times greater than the rate below pH 7.0. No lag period was observed above pH 7.0. At alkaline pH, as the oxidation progressed, oxygen uptake rate gradually decreased, presumably because of the oxidative degradation of the heme catalysts (Tappel, 1953b).

It has been well established by other investigators (Wills, 1966; Liu, 1970a) that nonheme iron catalyzed oxidation is pH-sensitive and most active at acidic pH with an optimum pH of 5.5. No catalysis was observed above pH 6.4. On the other hand, hemoprotein catalyzed oxidation is most active at alkaline pH. The results of the present study indicated that the pattern of MDCM homogenate catalyzed oxidation over a pH range 5.0–8.0 was similar to that of hemoprotein catalysis.

Effects of ascorbic acid and chelating agent

Ascorbic acid and chelating agents were reported to have different effects on hemoprotein versus nonheme iron catalysis (Wills, 1965, 1966; Liu, 1970a). The rate of nonheme iron catalyzed oxidation of linoleic acid emulsions was strongly inhibited by EDTA and strongly accelerated by ascorbic acid. On the other hand, hemoprotein catalyzed oxidation was not affected by EDTA but inhibited by ascorbic acid.

As shown in Table 2, ascorbic acid inhibited homogenate catalysis. The extent of inhibition was greater at higher pH. The addition of EDTA did not affect the oxygen uptake rate over a pH range 5.5–7.0. The combination of ascorbic acid and EDTA strongly inhibited the oxygen uptake rate, indicating a synergistic inhibition of lipid oxidation. The results of this experiment further verified that MDCM homogenate catalysis followed the typical pattern of hemoprotein catalysis.

Effect of thiol and cyanide compounds

It was demonstrated in model systems that thiol compounds accelerated nonheme iron catalysis but inhibited hemoprotein catalysis (Liu, 1970a). It was also reported that cyanide is a selective inhibitor for hematin catalysis and at 2×10^{-2} M concentration inhibited lipid oxidation in pork adipose tissue (Tappel, 1962).

As shown in Table 3, addition of cyanide to MDCM homogenate completely inhibited lipid oxidation and mercaptoethylamine also markedly decreased the oxygen uptake rate. This pattern followed the findings in a model system of hemoprotein catalysis.

Effect of destruction of hemoproteins

Treatment of tissues with warm H_2O_2 destroys hemoproteins. Any catalytic function of hemoprotein should therefore be completely destroyed by a prior treatment with H_2O_2. The liberated nonheme iron is a very weak catalyst as compared to an equivalent quantity of heme iron (Wills, 1965).

Treatment of MDCM homogenates with H_2O_2 for 5 min at 50°C markedly decreased the catalytic activity to less than 10% of the original activity (Table 4). Addition of ascorbic acid to the H_2O_2-treated homogenate caused a rapid rate of oxidation owing to the function of ascorbic acid as an inorganic iron activator. The oxidation rate of the H_2O_2-treated homogenate was greater at acidic pH, which is the opposite to the result with intact homogenate shown in Table 2. This would be expected, because the nonheme iron catalysis is most active at acidic pH as discussed previously, and activation by ascorbic acid is also greater at acidic pH as reported for beef homogenate (Liu, 1970b). Increases in the rate of oxygen uptake by the addition of ascorbic acid were almost completely abolished by EDTA, indicating that nonheme iron is a predominant catalyst in the H_2O_2-treated homogenate.

Effect of added hemoprotein

Heme compounds are known to inhibit rather than accelerate lipid oxidation when they are present in high concentrations relative to the unsaturated fatty acids (Lewis and Wills, 1963). These researchers reported that hemoglobin and

Table 1–Effect of pH on the oxygen uptake rate catalyzed by MDCM homogenate[a]

pH	Lag period, min	Oxygen uptake rate mm O_2/min
5.0	3.5 ± 0.2	20.3 ± 1.8
5.5	3.0 ± 0.3	21.8 ± 1.1
6.0	2.3 ± 0.1	23.2 ± 2.0
6.5	2.0 ± 0.2	23.2 ± 1.9
7.0	0.5 ± 0.1	24.7 ± 2.1
7.5	none	40.6 ± 3.2
8.0	none	96.2 ± 5.6

[a] Mean ± S.E., n = 2

Table 2–Effect of ascorbic acid and chelating agent on the oxygen uptake rate catalyzed by MDCM homogenate[a]

	Rate of oxygen uptake, mm O_2/min		
	pH		
Additives	5.5	6.0	7.0
Control	21.4 ± 1.9	21.8 ± 2.2	22.3 ± 1.6
Ascorbic acid, 1 mM	15.4 ± 1.2	13.9 ± 0.8	9.6 ± 0.7
EDTA, 0.6 mM	20.5 ± 1.1	20.8 ± 0.6	19.8 ± 1.3
Ascorbic acid (1 mM) + EDTA (0.6 mM)	3.5 ± 0.3	5.5 ± 0.2	5.7 ± 0.3

[a] Mean ± S.E., n = 2

Table 3–Effect of thiol compound and cyanide on the oxygen uptake catalyzed by MDCM homogenate[a]

	Rate of oxygen uptake, mm O_2/min	
	pH	
Additives	6.0	7.0
Control	20.4 ± 1.8	23.2 ± 1.3
β-mercaptoethylamine, 0.6 mM	4.6 ± 0.2	3.2 ± 0.3
Cyanide, 2×10^{-2} M	0.2 ± 0.03	0.3 ± 0.05

[a] Mean ± S.E., n = 2

other hemoproteins were active catalysts of lipid peroxide formation in solutions more dilute than 10 μM, but inhibited peroxide formation in more concentrated solutions. To study the effect of high heme concentration on the lipid oxidation by MDCM homogenate, several different levels of myoglobin were added to the reaction mixture. As shown in Figure 2, addition of myoglobin increased the oxygen uptake rate when its concentration was less than 10 μM, but completely inhibited oxidation at concentrations above 60 μM. When the oxygen uptake rate was plotted against the molar ratio of linoleate to hemoprotein, the rate increased to a linoleate: heme ratio of 500:1, then decreased sharply after the ratio reached about 350:1 by adding more myoglobin. A complete inhibition occurred when the ratio reached 89:1. These results clearly indicate that the molar ratio of polyunsaturated fatty acids to hemoproteins must be determined to assess the ultimate importance of heme catalysis in MDCM.

Content of iron and polyunsaturated fatty acids

As shown in Table 5, MDCM contained 0.48 μmoles of total iron per g wet tissue. Approximately one-half of the total iron was in the form of nonheme iron and the other one-half in the form of heme iron. The molar concentration of total heme iron, 0.26 μmoles, is equivalent to 4.4 mg total pigments per g wet tissue, which agrees with the value of 4.7 mg/g reported by Froning et al. (1973) for the mechanically deboned broiler back meat. As previously reported by Froning and Johnson (1973), this study also revealed very high hemoglobin concentration as compared to myoglobin concentration.

To determine the molar ratio of polyunsaturated fatty acids to hemoprotein, fatty acid analyses were conducted and the molar concentration of polyunsaturated fatty acids was estimated. Total lipid content of MDCM studied was approximately 160 mg per g wet tissue (16%). Assuming that fatty acids account for about 90% of the weight of lipid, their weight content was estimated to be 144 mg. As shown in Table 6, approximately 24.5% of total fatty acids had two or more than two double bonds. This gives 35 mg (144 mg X 0.245) of potential substrate for lipid oxidation. Since linoleic acid is a

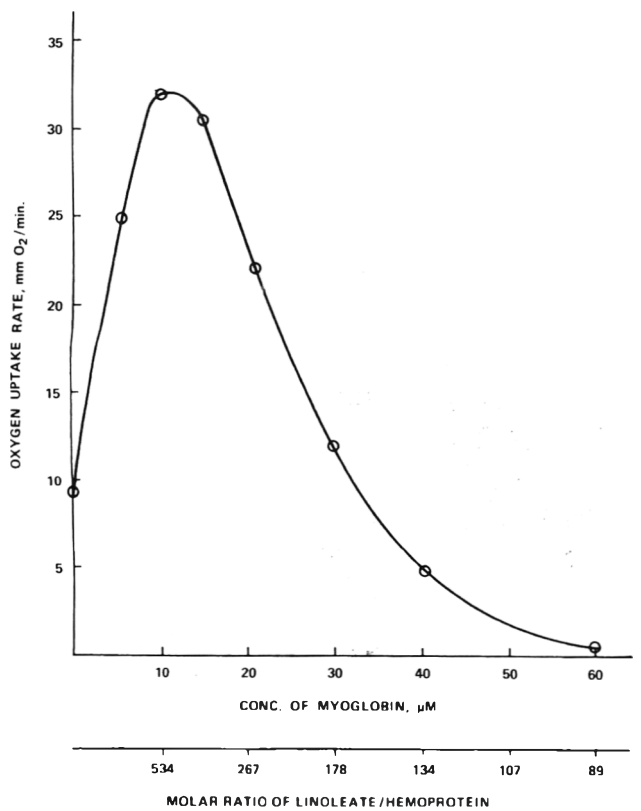


Fig. 2–Effect of added hemoprotein concentration on the oxygen uptake rate catalyzed by MDCM homogenate.

Table 4–Effect of ascorbic acid and chelating agent on the oxygen uptake rate catalyzed by H_2O_2-treated MDCM homogenate[a]

	Rate of oxygen uptake, mm O_2/min	
	pH	
Additives	6.0	7.0
Control (untreated homogenate)	20.4 ± 0.6	23.2 ± 1.1
H_2O_2-treated homogenate (I)	2.0 ± 0.1	1.5 ± 0.1
(I) + Ascorbic acid (1 mM)	16.9 ± 0.9	11.5 ± 0.6
(I) + EDTA (0.6 mM)	0.3 ± 0.02	0.2 ± 0.03
(I) + Ascorbic acid (1 mM) + EDTA (0.6 mM)	2.2 ± 0.2	3.2 ± 0.2

[a] Mean ± S.E., n = 2

Table 5–Iron content of MDCM[a]

	Iron (μmoles/g wet wt of MDCM)			
		Heme iron		
Total iron	Nonheme iron	Hemoglobin	myoglobin	total
0.48 ± 0.05 (100%)	0.22 ± 0.05 (46%)	0.20 ± 0.03 (42%)	0.06 ± 0.01 (12%)	0.26 ± 0.03 (54%)

[a] Mean ± S.E., n = 3

Table 6–Fatty acids of MDCM total lipids[a]

Fatty acid[b]	Normalized area percent
14:0	0.86 ± 0.02[d]
14:1	0.20 ± 0.01
16:0	20.36 ± 0.52
16:1	5.96 ± 0.06
18:0	6.94 ± 0.02
18:1	40.55 ± 0.15
18:2	20.30 ± 0.67
18:3	1.12 ± 0.01
18:4[c]	0.11
20:0	0.55
20:1[c]	0.11
20:2	0.14
20:3[c]	0.56 ± 0.01
20:4[c]	1.52 ± 0.01
20:5[c]	0.72 ± 0.01
Percent fatty acids having 2 or more double bonds	24.48 ± 0.65

[a] Each fatty acid expressed as a percent of total fatty acids
[b] Carbon chain length:number of double bonds
[c] Identified from plot of log of retention time vs carbon chain length
[d] Average of duplicate GLC runs

predominant polyunsaturated fatty acid, the molar concentration of total substrate was estimated by dividing 35 mg with the molecular weight of linoleic acid, 280, to yield 125 μ-moles.

From the above results, the molar ratio of polyunsaturated fatty acids to hemoproteins was calculated; 125 μmoles ÷ 0.26 μmoles = 480. According to Figure 2, the molar ratio of 480/1 would give a maximum oxygen uptake rate. It has been reported by other investigators that in many cases MDPM contains more than 16% lipid, depending on skin content (Satterlee et al., 1971). A higher lipid content would further increase the molar ratio of substrate to catalyst, thus providing conditions in which hemoproteins could function as strong pro-oxidants.

It was concluded from the foregoing data that hemoproteins (myoglobin and hemoglobin) were the predominant, if not exclusive, catalysts of lipid oxidation in MDCM. Furthermore, the relative concentration ratio of polyunsaturated fatty acids to hemoproteins was in the range where heme catalyzed lipid oxidation would occur at or close to maximum rate. Two conceivable ways to minimize the off-flavor development in MDPM would be: (1) the decrease in molar ratio of substrate to hemoprotein by several possible methods so that hemoprotein can act as an antioxidant rather than a pro-oxidant; and (2) the change in reaction environment by adding various inhibitors or antioxidants to inhibit the catalytic function of hemoproteins. Partial removal of lipids or hemoproteins by centrifugation or by low temperature rendering would change the molar ratio of substrate to hemoprotein and extend the shelf life of products. The addition of antioxidants such as Tenox 2 or Tenox 20 would be another effective way to prevent the off-flavor development due to lipid oxidation.

REFERENCES

Berner, D.L., Conte, J.A. and Jacobson, G.A. 1974. Rapid method for determining antioxidant activity and fat stability. J. Am. Oil Chem. Soc. 51: 292.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911.

Cameron, B.F. 1965. Determination of iron in heme compounds. 2. Hemoglobin and myoglobin. Anal. Biochem. 11: 164.

DeMan, J.M. 1964. Determination of the fatty acid composition of milk fat by dual column temperature programmed gas liquid chromatography. J. Dairy Sci. 47: 546.

Dimick, P.S., MacNeil, J.H. and Grunden, L.P. 1972. Poultry product quality. Carbonyl composition and organoleptic evaluation of mechanically deboned poultry meat. J. Food Sci. 37: 544.

Froning, G.W. and Johnson, F. 1973. Improving the quality of mechanically deboned fowl meat by centrifugation. J. Food Sci. 38: 279.

Froning, G.W., Satterlee, L.D. and Johnson, F. 1973. Effect of skin content prior to deboning on emulsifying and color characteristics of mechanically deboned chicken back meat. Poultry Sci. 52: 923.

Johnson, P.G., Cunningham, F.E. and Bowers, J.A. 1974. Quality of mechanically deboned turkey meat: Effect of storage time and temperature. Poultry Sci. 53: 732.

Lewis, S.E. and Wills, E.D. 1963. Inhibition of the autoxidation of unsaturated fatty acids by hematin proteins. Biochem. Biophys. Acta 70: 336.

Liu, H. 1970a. Catalysts of lipid peroxidation in meats. 1. Linoleate peroxidation catalyzed by MetMb or Fe (II)-EDTA. J. Food Sci. 35: 590.

Liu, H. 1970b. Catalysts of lipid peroxidation in meats. 2. Linoleate oxidation catalyzed by tissue homogenates. J. Food Sci. 35: 593.

Liu, H. and Watts, B.W. 1970. Catalysts of lipid oxidation in meats. 3. Catalysts of oxidative rancidity in meats. J. Food Sci. 35: 596.

Moerck, K.E. and Ball, H.R. Jr. 1974. Lipid autoxidation in mechanically deboned chicken meat. J. Food Sci. 39: 876.

Rickansrud, D.A. and Henrickson, R.L. 1967. Total pigments and myoglobin concentration in four bovine muscles. J. Food Sci. 32: 57.

Satterlee, L.D., Froning, G.W. and Janky, D.M. 1971. Influence of skin content on composition of mechanically deboned poultry meat. J. Food Sci. 36: 979.

Tappel, A.L. 1953a. Linoleate oxidation catalysts occurring in animal tissues. Food Res. 18: 104.

Tappel, A.L. 1953b. Oxidative fat rancidity in food products. 1. Linoleate oxidation catalyzed by hemin, hemoglobin, and cytochrome C. Food Res. 18: 560.

Tappel, A.L. 1962. Hematin compounds and lipoxidase as biocatalysts. In "Symposium on Foods: Lipids and Their Oxidation," p. 122. Avi Publishing Co., Westport, Conn.

Wills, E.D. 1965. Mechanisms of lipid peroxide formation in tissue: Role of metals and hematin proteins in the catalysis of oxidation of unsaturated fatty acids. Biochem. Biophys. Acta 98: 238.

Wills, E.D. 1966. Mechanisms of lipid peroxide formation in animal tissues. Biochem. J. 99: 667.

Ms received 3/8/75; revised 5/7/75; accepted 5/14/75.

MARK A. KANTOR[1] and NORMAN N. POTTER
Dept. of Food Science, Cornell University, Ithaca, NY 14853

PERSISTENCE OF ECHOVIRUS AND POLIOVIRUS IN FERMENTED SAUSAGES.
Effects of Sodium Nitrite and Processing Variables

INTRODUCTION

MEAT is one of the more likely foods to become contaminated with viruses. Sullivan et al. (1970) isolated poliovirus and echovirus from 3 of 12 market samples of ground beef, and Larkin et al. (1972) found enteroviruses in 22% of the market samples they tested. These relatively high proportions of positive samples, plus the common practices of undercooking meat and eating it in the rare condition, stress the importance of viruses in meat as a potential threat to public health. Fermented sausages pose a special problem in this regard, since both dry and semidry varieties do not receive extensive heating during preparation and processing.

Viruses resistant to food processing conditions adequate to control bacteria of public health concern have been studied by Strock and Potter (1972), Gough (1973) and others. Kaplan and Melnick (1954) and Wallis and Melnick (1962) demonstrated the protective effects of lipids and salts, respectively, on the thermal stability of viruses in food. Filppi and Banwart (1974) showed that the rate of thermal inactivation of poliovirus inoculated into ground beef was inversely proportional to the beef's fat content. Herrmann and Cliver (1973) reported high titer recovery of coxsackievirus from fermented thuringer sausage after processing when the meat was experimentally contaminated.

The controversial use of nitrates and nitrites in meat has received attention from several points of view, but no studies have appeared on their possible effects upon virus. Although these curing additives inhibit the outgrowth of *Clostridium botulinum* spores (Johnson and Loynes, 1971; Pivnick et al., 1970), and contribute desirable flavor and color to meat (Simon et al., 1973; Wasserman and Talley, 1972), formation of carcinogenic nitrosamines by reaction of nitrites with secondary amines has also been well documented (Sen et al., 1970; Wolff and Wasserman, 1972). The existence of nitrosamines in frankfurters was reported by Wasserman et al. (1972), who found 11–84 ppb of dimethylnitrosamine in 3 of 40 commercial samples of frankfurters, and the likelihood exists that nitrosamines also are present in fermented sausages. Removing nitrite from meat, or lowering its permitted levels, continues to be an issue of major concern.

One objective of the present investigation was to determine the fate of poliovirus and echovirus in different types of fermented sausages during varied processing conditions and storage. A second objective was to determine the specific effects of sodium nitrite on the persistence of poliovirus and echovirus in fermented sausages. If nitrite were to inhibit virus survival in meat, this could be another factor relevant to its continued use.

EXPERIMENTAL

Viruses and tissue culture

Poliovirus type 1 (Po-1), strain CHAT and echovirus type 6 (EC-6), strain D'Amori were obtained from the American Type Culture Collection. They were propagated and titrated on African green monkey kidney cells (*Cercopithecus aethiops*, cell line Vero) obtained from the Veterinary Virus Research Institute at Cornell University. The procedures for media preparation, cell maintenance, cell passing and virus propagation used in our laboratory have already been described (Strock and Potter, 1972), but several changes and additions were made, as subsequently described.

Media and reagents

Gamma-globulin-free calf serum (GIBCO 624) was omitted from media formulations, and Fungizone (GIBCO 529L) was added. The composition of media were as follows:

Medium A, for the growth of tissue culture cells: 76% Eagle's minimum essential medium (MEM), 10% lactalbumin hydrolysate, 10% fetal calf serum, 2% penicillin-streptomycin, 2% Fungizone.

Medium B, for the maintenance of tissue culture cells: 82% Eagle's MEM, 10% lactalbumin hydrolysate, 4% fetal calf serum, 2% penicillin-streptomycin, 2% Fungizone.

Medium C, for the propagation and titration of viruses: 80% Eagle's MEM, 10% lactalbumin hydrolysate, 6% fetal calf serum, 2% penicillin-streptomycin, 2% Fungizone.

Medium D, for the dilution of virus suspensions prior to titration: 86% Eagle's MEM, 10% lactalbumin hydrolysate, 2% penicillin-streptomycin, 2% Fungizone.

Cell growth, maintenance and passage

Cells were grown as monolayers at 37°C in glass Roux bottles and Blake bottles, containing 80 ml and 25 ml of Medium A, respectively. The monolayers could be maintained active with Medium A for about 1 wk after their formation. When it was desired to maintain the cells for a longer period, Medium A was poured off and replaced with 100 ml of Medium B in Roux bottles, and with 30 ml of Medium B in Blake bottles, which maintained cells for an additional 2–3 wk. Cells were passed as previously described.

Virus propagation

Viruses were propagated on cells in Blake bottles, following the previously described method. If the virus suspension were held in frozen storage before being added to the monolayer, cytopathic effect (CPE) generally occurred after about 36 hr for both poliovirus and echovirus. If viruses had not previously been frozen, but were transferred successively from one Blake bottle to another, then CPE occurred after about 18 hr for both viruses.

Virus titration

All virus titrations were conducted in Linbro Disposo-Trays (Linbro IS-FB-96-TC) using the appropriate tray covers (Linbro 55-Sterile). Serial threefold dilutions of the virus suspensions in Medium C were made in quadruplicate employing 0.050 ml capillary microdiluters. The trays were incubated in a 5% CO_2 atmosphere at 37°C for 3 days. CPE was readily observed by first fixing the cells with methyl alcohol for 5–10 min, and then staining with Giemsa stain for 30 min. Viruses were determined quantitatively using the Kaerber method (Jawitz et al., 1972) in which the titer is the reciprocal of the dilution which causes CPE in 50% of tissue cultures (TCD_{50} = "tissue culture infective dose fifty").

Formulation, preparation and processing of sausages

The formulations for b.c. (beef casing) salami and cervelat are shown in Table 1.

All meat used in preparing the sausages was purchased from a local supermarket. The meat was kept frozen until ready for use, and was then thawed at room temperature. The beef chuck steak was ground through a 3/16 in. plate, and the pork chops were ground through a 3/8

[1] Present address: Colgate-Palmolive Co., Research & Development Dept., 909 River Road, Piscataway, NJ 08854

Table 1—Formulation of sausages

Ingredient	B.c. salami (g)[a]	Cervelat (g)[b]
Beef chuck steak	1000	–
Ground beef	1400	2400
Pork chops	1600	600
Salt (commercial, uniodized table salt)	140	90
Dextrose (D-glucose, anhydrous)	30	30
Sucrose	30	–
Ground black pepper	–	11.24
White pepper	7.50	–
Garlic powder	0.62	–
Sodium erythorbate	2.18	1.64

[a] To this were added Lactacel DS starter culture and different levels of sodium nitrite
[b] To this were added Lactacel MC starter culture and different levels of sodium nitrite

in. plate, using the meat grinder attachment of a Hobart Laboratory Mixer (Model N-50). The meat and spices were thoroughly blended with the dough hook attachment of the Hobart mixer. The b.c. salami emulsion was then divided into 20 192-g portions, and the cervelat emulsion into 12 240-g portions. Each portion was placed into a separate plastic bag. Virus suspension of high titer, sodium nitrite solution (yielding final levels of 37.5 ppm, 75.0 ppm, and 150.0 ppm in the meat), and Lactacel DS (*Lactobacillus plantarum*) or Lactacel MC (*Pediococcus cerevisiae* and *Lactobacillus plantarum*) starter cultures (Merck and Co., Inc.) were then successively added to each bag, and the bags were hand kneaded for 5 min after each addition to ensure homogeneity. Previous experiments using replicate virus titrations demonstrated that this method was effective in achieving homogeneity. The emulsions were then stuffed into type 65-24 Brechteen collagen casings and links were tied, producing sausages weighing 150g and measuring 1 in. × 4 in. The b.c. salami was fermented for 3 days at 20°C, followed by drying at 4°C and 55% relative humidity for 3 wk. The cervelat underwent a 24-hr fermentation at 30°C and then heat treatments of 60°C for 5 min and 30 min in a water bath, followed by a 1 wk drying period as before. Samples of the sausages were taken at various intervals during processing and drying and analyzed for virus, moisture, pH, residual nitrite and bacterial plate counts.

Extraction of virus from sausage

To extract virus from sausage meat for titration, 10g of meat, taken from various locations within a sausage, was combined with 90 ml of phosphate buffered saline in a homogenizer flask. The jacket surrounding the flask was filled with crushed ice and the meat was homogenized in a VirTis Homogenizer (model 45) at speed 80 for 5 min. A quantity of 4.5 ml of the homogenate was transferred to a 1 cm × 1.0 cm screw cap centrifuge tube and 0.5 ml chloroform was added. The tube was then vibrated on a Vortex-Genie Mixer for 3 min at speed 5 to evenly disperse the fat and lean. After refrigerating the tube at 4°C for 15 min the tube was centrifuged in a clinical centrifuge with 13 cm conical head (Safeguard. Clay-Adams, Inc.) at 2800 rpm for 5 min. A quantity of 3 ml of the supernatant was pipetted into another similar centrifuge tube. This tube was also refrigerated for 15 min and then centrifuged at 3000 rpm for 5 min. Dilutions of the clarified virus suspension were prepared in Medium D and titrated in the titration trays as previously described.

Analytical tests

The AOAC procedures were followed for the determination of moisture and residual nitrite (AOAC, 1970). In the nitrite analysis, hot

Table 2—Persistence of EC-6 in b.c. salami of differert nitrite levels and processing conditions

Time	Added $NaNO_2$ (ppm)	% Moisture	pH	Plate counts/g APT	Plate counts/g LBS	Residual NO_2 (ppm)	EC-6 titer (TCD_{50}/g)
Initial time	0	57.9	5.80	6.1×10^6	3.5×10^6	–	6.6×10^7
	37.5		5.75	1.1×10^7	6.4×10^6	6.2	2.0×10^8
	75.0		5.80	5.9×10^6	5.4×10^6	23.6	1.1×10^8
	150.0		5.80	7.9×10^6	6.1×10^6	59.9	3.8×10^7
1 day at 20°C	0	59.6	5.60	1.3×10^8	6.6×10^7	–	6.0×10^7
	37.5		5.70	1.7×10^8	1.0×10^8	6.2	4.6×10^7
	75.0		5.70	1.9×10^7	1.4×10^7	22.1	4.6×10^7
	150.0		5.75	1.1×10^7	8.0×10^6	45.3	4.8×10^7
2 days at 20°C	0	59.4	5.60	3.8×10^8	1.4×10^8	–	6.5×10^7
	37.5		5.50	2.5×10^8	1.2×10^8	7.6	6.1×10^7
	75.0		5.50	1.7×10^8	1.5×10^8	12.0	1.1×10^8
	150.0		5.45	1.4×10^8	1.2×10^8	23.6	4.8×10^7
3 days at 20°C	0	58.7	5.20	2.7×10^8	1.1×10^8	–	4.8×10^7
	37.5		5.20	2.7×10^8	3.5×10^7	4.7	5.6×10^7
	75.0		5.20	8.0×10^7	9.0×10^7	6.2	2.9×10^7
	150.0		5.20	6.0×10^7	1.2×10^8	6.2	4.8×10^7
+7 days at 4°C	0	56.7	5.40	1.8×10^8	4.5×10^7	–	1.3×10^7
	37.5		5.35	8.5×10^7	1.7×10^7	3.3	2.3×10^7
	75.0		5.30	1.6×10^7	8.0×10^6	3.3	1.4×10^7
	150.0		5.25	1.4×10^7	5.0×10^6	3.3	3.8×10^7
+13 days at 4°C	0	52.1	5.25	3.6×10^8	1.0×10^8	–	4.6×10^7
	37.5		5.20	2.0×10^8	6.5×10^7	3.3	3.8×10^7
	75.0		5.20	7.7×10^7	5.3×10^7	6.2	3.5×10^7
	150.0		5.20	7.2×10^7	7.5×10^7	6.2	2.9×10^7
+21 days at 4°C	0	24.9	5.40	2.1×10^8	1.6×10^8	–	8.3×10^7
	37.5		5.35	2.0×10^8	1.1×10^8	1.8	1.7×10^8
	75.0		5.40	3.5×10^7	8.5×10^6	6.2	8.3×10^7
	150.0		5.35	4.3×10^7	1.6×10^7	12.0	6.6×10^7

alkaline water, produced by adding 5 ml of 0.1N NaOH to 1500 ml of nitrite-free water and heating to 80°C, was used to extract the nitrite. The nitrite-containing solutions were filtered through Whatman no. 1 and then through Whatman no. 42 paper. A 10-ml aliquot of each sample was then pipetted into a 50-ml volumetric flask containing 1 ml of sulfanilic acid and 1 ml of alpha-naphthylamine (may contain the carcinogenic beta-naphthylamine and caution is required) solutions. A standard curve was prepared as described in the AOAC method.

The pH of sausages was determined directly on the meat in three different locations and the average reading was taken.

Samples for bacterial plate counts were dispersed by adding 1.00g of meat to 99 ml of phosphate buffer (pH 7.2) in milk dilution bottles to which 15 glass beads were added. Bottles were uniformly shaken 100 times and appropriate dilutions were made. Total counts were enumerated on APT agar, and total Lactobacilli-Pediococci counts on LBS agar (Lactobacillus selective medium). All plates were incubated at 30°C for 3 days.

RESULTS & DISCUSSION

AS SEEN IN TABLES 2, 3, 4 and 5, both EC-6 and Po-1 persisted in high titers and were virtually unaffected by the wide range of processing conditions employed in simulating the commercial manufacture of b.c. salami and cervelat. The only exception was a decrease in titer of about 90% which occurred in the cervelat maintained at an internal temperature of 60°C for 30 min, a heat treatment more than sufficient to inactivate trichina.

Although it has been reported that enteroviruses are rapidly inactivated by desiccation (Plotkin et al., 1962), both EC-6 and Po-1 were persistent with no apparent loss of titer in the dried b.c. salami samples containing only 25% moisture. In a few cases, the virus titers actually seemed to increase at these lower moisture levels, but this was due to the higher per cent solids in the samples, resulting in concentration of the viruses.

The stability of both viruses in both fermented sausages was sustained although large numbers of bacteria were present throughout the processing and storage of the sausages. Neither during the fermentation period when actively metabolizing bacteria were producing lactic acid, enzymes and other products, nor following fermentation, did any of these potentially destructive agents affect virus titers.

The presence of sodium nitrite in all added levels, the intermediary nitrite compounds involved in color formation and other reactions, and the amounts of residual nitrite, all were without measurable effect on virus titers. It was suspected that inactivation of viruses might occur due to the affect of nitrous acid, a known mutagen and inactivator of certain viruses (Luria and Darnell, 1967), but this did not appear to be the case, confirming observations of Heidelbaugh and Graves (1968) with foot-and-mouth disease virus. The added nitrite also did not influence final bacterial counts, but the observations of Skjelkvale and Tjaberg (1974) were confirmed, in that samples of salami containing higher levels of nitrite tended to have lower bacterial counts during the first few days after preparation. Possible explanations for the changes in residual nitrite levels as observed in these experiments, have been discussed by Hill et al. (1973).

The results of this study, as well as those of Herrmann and Cliver (1973), demonstrate that certain enteroviruses are remarkably stable in fermented sausages through all stages of preparation and processing. Enterovirus levels in commercial sausage meat are very much lower than used in this study, but the minimal infective dose of enteroviruses for human infec-

Table 3—Persistence of Po-1 in b.c. salami of different nitrite levels and processing conditions

Time	Added $NaNO_2$ (ppm)	% Moisture	pH	Plate counts/g		Residual NO_2 (ppm)	Po-1 titer (TCD_{50}/g)
				APT	LBS		
Initial time	0	58.5	5.60	1.3×10^7	5.5×10^6	–	4.9×10^7
	37.5		5.70	8.0×10^6	8.0×10^6	3.3	2.5×10^8
	75.0		5.90	9.0×10^6	1.5×10^6	16.3	2.5×10^8
	150.0		5.90	6.5×10^6	1.5×10^6	51.1	2.5×10^8
1 day at 20°C	0	58.2	5.50	6.5×10^7	2.5×10^7	–	3.5×10^8
	37.5		5.70	2.4×10^7	5.5×10^6	14.9	2.4×10^9
	75.0		5.70	1.7×10^7	9.0×10^6	17.8	1.7×10^8
	150.0		5.70	6.0×10^6	4.5×10^6	41.0	8.3×10^7
2 days at 20°C	0	56.6	5.40	1.7×10^8	1.3×10^8	–	2.5×10^8
	37.5		5.40	1.1×10^8	6.8×10^7	3.3	6.0×10^8
	75.0		5.50	5.2×10^7	4.7×10^7	4.7	2.5×10^8
	150.0		5.50	1.0×10^6	$< 1.0 \times 10^6$	20.7	2.0×10^8
3 days at 20°C	0	57.4	5.00	2.9×10^7	1.5×10^7	–	2.5×10^8
	37.5		5.10	8.0×10^7	3.9×10^7	0.4	2.0×10^8
	75.0		5.10	8.6×10^7	4.1×10^7	3.3	2.0×10^8
	150.0		5.20	1.1×10^7	1.1×10^7	3.3	7.8×10^8
+10 days at 4°C	0	55.9	5.15	TMTC	5.3×10^6	–	6.0×10^8
	37.5		5.10	1.3×10^7	1.4×10^7	4.7	2.0×10^8
	75.0		5.20	2.5×10^7	3.1×10^7	6.2	2.9×10^8
	150.0		5.25	2.3×10^7	2.3×10^7	12.0	2.5×10^8
+17 days at 4°C	0	53.0	5.25	7.2×10^6	5.2×10^6	–	1.7×10^8
	37.5		5.15	9.8×10^6	8.4×10^6	7.6	1.7×10^8
	75.0		5.15	3.3×10^7	3.4×10^7	9.1	1.7×10^8
	150.0		5.35	3.0×10^7	3.2×10^7	10.5	1.7×10^8
+ 26 days at 4°C	0	25.1	5.10	1.4×10^7	3.1×10^6	–	3.4×10^8
	37.5		5.00	1.1×10^7	6.7×10^6	0.4	2.5×10^8
	75.0		5.10	1.8×10^7	1.3×10^7	6.2	7.3×10^8
	150.0		5.25	1.6×10^7	1.2×10^7	6.2	7.3×10^8

Table 4–Persistence of EC-6 in cervelat of different nitrite levels and processing conditions

Time	Added $NaNO_2$ (ppm)	% Moisture	pH	Plate counts/g		Residual NO_2 (ppm)	EC-6 titer (TCD_{50}/g)
				APT	LBS		
Initial time	0	51.6	5.70	3.1×10^7	1.2×10^7	–	6.6×10^7
	37.5		5.85	1.3×10^7	8.1×10^6	9.1	1.7×10^8
	75.0		5.85	9.6×10^6	4.3×10^6	35.2	2.9×10^8
	150.0		5.85	6.8×10^6	4.5×10^6	70.0	8.3×10^7
24 hr fermentation	0	–	4.95	4.8×10^8	4.1×10^8	–	2.0×10^8
	37.5		5.00	1.2×10^8	1.2×10^8	–	1.5×10^8
	75.0		5.05	1.5×10^8	1.2×10^8	3.3	6.6×10^7
	150.0		5.05	1.6×10^8	1.3×10^8	17.8	8.3×10^7
+5 min at 60°C	0	–	–	–	–	–	5.0×10^7
	37.5		–	–	–	–	1.1×10^8
	75.0		–	–	–	–	4.8×10^7
	150.0		–	–	–	–	2.8×10^7
+30 min at 60°C	0	–	–	–	–	–	5.1×10^6
	37.5		–	–	–	–	1.8×10^7
	75.0		–	–	–	–	1.6×10^7
	150.0		–	–	–	–	1.8×10^7
+1 wk at 4°C	0	47.9	5.10	2.8×10^7	2.6×10^7	–	4.8×10^7
	37.5		5.30	3.3×10^7	2.4×10^7	3.3	1.4×10^6
	75.0		5.35	9.5×10^7	9.7×10^7	3.3	9.1×10^6
	150.0		5.50	3.5×10^7	3.4×10^7	9.1	1.6×10^7

Table 5–Persistence of Po-1 in cervelat of different nitrite levels and processing conditions

Time	Added $NaNO_2$ (ppm)	% Moisture	pH	Plate counts/g		Residual NO_2 (ppm)	Po-1 titer (TCD_{50}/g)
				APT	LBS		
Initial time	0	58.5	5.75	1.5×10^7	5.5×10^6	–	2.5×10^8
	37.5		5.70	6.4×10^6	3.2×10^6	27.9	2.0×10^8
	75.0		5.75	1.0×10^7	4.8×10^6	61.3	2.0×10^8
	150.0		5.75	8.7×10^6	2.7×10^6	109.2	1.1×10^8
24 hr fermentation	0	–	4.85	3.8×10^8	4.2×10^8	–	4.4×10^8
	37.5		5.10	4.3×10^7	5.7×10^7	9.1	1.7×10^8
	75.0		5.15	4.7×10^7	5.6×10^7	9.1	1.7×10^8
	150.0		5.10	4.8×10^7	5.5×10^7	19.2	2.0×10^8
+5 min at 60°C	0	–	–	–	–	–	2.8×10^7
	37.5		–	–	–	–	5.6×10^7
	75.0		–	–	–	–	3.2×10^7
	150.0		–	–	–	–	4.8×10^7
+30 min at 60°C	0	–	–	–	–	–	6.0×10^6
	37.5		–	–	–	–	2.3×10^7
	75.0		–	–	–	–	4.6×10^7
	150.0		–	–	–	–	1.5×10^7
+1 wk at 4°C	0	52.1	4.90	1.5×10^7	9.6×10^7	–	2.8×10^7
	37.5		5.10	6.2×10^7	6.7×10^7	3.3	1.6×10^7
	75.0		5.15	4.5×10^7	5.1×10^7	6.2	3.2×10^7
	150.0		5.15	3.3×10^7	4.7×10^7	9.1	1.9×10^7

tion also may be extremely small (Koprowski et al., 1956; Plotkin et al., 1959), adding to the significance of their stability.

While nitrite levels to be permitted in cured meats in the future continue to receive study, with respect to fermented sausages, at least, an increased hazard from poliovirus and echovirus, due to reduction in nitrite levels does not seem likely.

REFERENCES

AOAC. 1970. "Official Methods of Analysis," 11th ed. Assoc. of Official Agricultural Chemists, Washington, D.C.

Filppi, J.A. and Banwart, G.J. 1974. Effect of the fat content of ground beef on the heat inactivation of human poliovirus. J. Food Sci. 39: 865.

Gough, R.E. 1973. Thermostability of Newcastle disease virus in liquid whole egg. The Veterinary Record. 93: 632.

Heidelbaugh, N.D. and Graves, J.H. 1968. Effects of some techniques

applicable in food processing on the infectivity of foot-and-mouth disease virus. Food Technol. 22: 120.
Herrmann, J.E. and Cliver, D.O. 1973. Enterovirus persistence in sausage and ground beef. J. Milk and Food Tech. 36: 426.
Hill, L.H., Webb, N.B., Moncol, N.D. and Adams, A.T. 1973. Changes in residual nitrite in sausage and luncheon meat products during storage. J. Milk and Food Tech. 36: 515.
Jawitz, E., Melnick, J.L. and Adelberg, E.A. 1972. "Review of Medical Microbiology." Lange Medical Publications, Los Altos, Calif.
Johnson, M.A. and Loynes, R. 1971. Inhibition of Clostridium botulinum by sodium nitrite as affected by bacteriological media and meat suspensions. Can. Inst. Food Tech. J. 4: 179.
Kaplan, A.S. and Melnick, J.L. 1954. Effect of milk and other dairy products on the thermal inactivation of coxsackievirus. Amer. J. of Public Health and the Nation's Health. 44: 1174.
Koprowski, H., Norton, T.W., Jerris, G.A. and Nelson, T.L. 1956. Clinical investigations of attenuated strains of poliomyelitis virus. Use as a method of immunization of children with living virus. J. Amer. Med. Assoc. 160: 954.
Larkin, E.P., Tierney, J.T., Reyes, A.L., Novelli, R.M.E., Sullivan, R. and Read, R.B. Jr. 1972. Microbiological content of ground beef: a survey of a large metropolitan area. Abstr. 72nd Annu. Meet. Amer. Soc. Microbiol. E112.
Luria, S.E. and Darnell, J.E. Jr. 1967. "General Virology," 2nd ed. John Wiley and Sons., Inc., N.Y.
Pivnick, H., Johnston, M.A., Thacker, C. and Loynes, R. 1970. Effect of nitrite on destruction and germination of Clostridium botulinum and putrefactive anaerobes 3679 and 3679h in meat and in buffer. Can. Inst. Food Tech. J. 3: 103.
Plotkin, S.A., Carp, R.I. and Graham, A.F. 1962. The polioviruses of man. Ann. N.Y. Acad. Sci. 101: 357.
Plotkin, S.A., Koprowski, H. and Stokes, J. 1959. Clinical trials in infants of orally administered attenuated poliomyelitis virus. Pediatrics. 23: 1041.
Sen, N.P., Smith, D.C., Schwinghamer, L. and Howsam, B. 1970. Formation of nitrosamines in nitrite-treated fish. Can. Inst. Food Tech. J. 3: 66.
Simon, S., Ellis, D.E., MacDonald, B.D., Miller, D.G., Waldman, R.C. and Westerberg, D.O. 1973. Influence of nitrite and nitrate curing ingredients on quality of packaged frankfurters. J. Food Sci. 38: 919.
Skjelkvale, R. and Tjaberg, T.B. 1974. Comparison of salami sausage produced with and without addition of sodium nitrite and sodium nitrate. J. Food Sci. 39: 520.
Strock, N.R. and Potter, N.N. 1972. Survival of poliovirus and echovirus during simulated commercial egg pasteurization treatments. J. Milk and Food Tech. 35: 247.
Sullivan, R., Fassolitis, A. and Read, R.B. Jr. 1970. Method for isolating viruses from ground beef. J. Food Sci. 35: 624.
Wallis, C. and Melnick, J.L. 1962. Magnesium chloride enhancement of cell susceptibility to poliovirus. Virology. 16: 122.
Wasserman, A.E., Fiddler, W., Doerr, R.C., Osman, S.F. and Dooley, C.J. 1972. Dimethylnitrosamine in frankfurters. Food Cosmet. Toxicol. 10: 681.
Wasserman, A.E. and Talley, F. 1972. The effect of $NaNO_2$ on the flavor of frankfurters. J. Food Sci. 37: 536.
Wolff, I.A. and Wasserman, A.E. 1972. Nitrates, nitrites and nitrosamines. Science. 177: 15.
Ms received 2/11/75, revised 3/26/75; accepted 3/30/75.

The authors extend their appreciation to the Merck Chemical Div. of Merck & Co., Rahway, N.J., for providing samples of their starter cultures, Lactacel DS and Lactacel MC. We also thank the Brechteen Co., Mt. Clemens, Mich., for providing the collagen casings used in this work.

B. D. EAKES and T. N. BLUMER
Dept. of Food Science, North Carolina State University, Raleigh, NC 27607
and R. J. MONROE
Dept. of Statistics, North Carolina State University, Raleigh, NC 27607

EFFECT OF NITRATE AND NITRITE ON COLOR AND FLAVOR OF COUNTRY-STYLE HAMS

INTRODUCTION

RECENT technological advancements in the curing of country-style ham have been accompanied by a sizeable increase in production and consumption of this cured meat product. In many southeastern states the production of country-style hams constitutes a significant part of the pork industry.

The curing procedure which involves the addition of nitrates and nitrites has become a controversial topic in the food industry. Nitrite performs three main functions in cured meats. It is responsible for the development of cured meat color through its reduction to nitric oxide and reaction with the meat pigment myoglobin (Fox, 1966). Secondly, nitrites have been shown to contribute to the development of cured flavor in some processed products (Cho and Bratzler, 1970; Wasserman and Talley, 1972; Simon et al., 1973). Thirdly, the germination, outgrowth and toxin production of *Clostridium botulinum* is inhibited by nitrite (Christiansen et al., 1973; Greenberg, 1973; Hustad et al., 1973).

Cured meat provides an environment conducive to many chemical and biochemical reactions. The reaction of nitrites and secondary or tertiary amines under acidic conditions may result in the formation of a class of compounds known as nitrosamines. The potential toxicity of these compounds and carcinogenic activity of dimethylnitrosamine in rats has been demonstrated (Barnes and Magee, 1954; Magee and Barnes, 1956). Further studies have confirmed that nitrosamine formation may occur in cured meats (Fazio et al., 1971; Sen, 1972; Fiddler et al., 1972) and at conditions similar to those in the human stomach (Lijinsky and Epstein, 1970).

This demonstration of a possible health hazard due to nitrates and nitrites has made it necessary to reevaluate the merits of nitrate and nitrite in cured meats and to determine the specific levels required to manufacture quality meat products, prevent botulinal toxic production and minimize potential nitrosamine formation. Differences in curing, heating and storage characteristics of various cured meat products also make it necessary to establish effects and residual levels of nitrates and nitrites for each type of product.

This study was undertaken to determine the effect of nitrate and/or nitrite on the color and flavor of country-style hams after four storage periods. Residual levels of nitrate and nitrite were determined at each sampling period.

EXPERIMENTAL

64 PAIRED HAMS were purchased fresh from a local meat packer and processed in the University Meats Laboratory on four different dates. A half-replicated 2^5 factorial design in 16 units as described by Cochran and Cox (1957) was used for this study. All hams were randomly assigned to treatment, method of cure and storage time. Least significant differences were used to evaluate differences between treatment means (Snedecor and Cochran, 1967).

Curing, aging and sampling

Curing mixtures of salt and sugar; salt, sugar and nitrate; salt, sugar and nitrite; salt, sugar, nitrate and nitrite represented the four treatments. Hams were either brine pumped or dry cured and held for 30, 60, 90 or 100 days. Dry-cured hams were rubbed with a mixture composed of 78.5% table grade granulated NaCl, 19.7% white cane sugar and 1.5% KNO_3 and/or 0.3% $NaNO_2$. Sodium erythorbate (0.5%) was added to the dry cure mix in reps 2, 3 and 4. This mixture was applied at the rate of 84 g/kg of meat. One-third of the curing mixture required for each ham was applied on the first, third and tenth days of cure. Pumped hams were injected with 75° brine at the rate of 10% of the fresh ham weight. Brine solutions were composed of 8.1% table grade granulated NaCl, 2.2% white cane sugar and 0.2% KNO_3 and/or 0.1% $NaNO_2$ dissolved in 1 kg boiled, cooled water. In reps 2, 3 and 4, 0.2% of sodium erythorbate was added to the above solution. The surface of the pumped hams was rubbed with 33g table grade NaCl/kg of meat immediately after pumping and then cured 4 days/kg at 3–5°C.

After curing, excess salt was removed from the surface and hams were placed in stockinettes and hung at 11 ± 2°C, relative humidity 60 ± 3%, for 30 days to effect salt equalization. They were then aged at 27 ± 3°C, relative humidity 55 ± 4% and air flow 10.5 m/min for 30 or 40 days.

At sampling, hams were cut ventral and parallel to the ischium (aitch bone) and a 2.5-cm slice was removed for proximate and residual nitrate and nitrite analysis. A 3.8-cm slice was removed for reflectance spectra studies, 2.5-cm slice for nitrosopigment determination and 0.63-cm slice for organoleptic evaluation. Hams sampled after cure represented 30 days from fresh state; after salt equalization, 60 days; and hams sampled after 30 and 40 days in aging represented 90 and 100 days, respectively. At each sampling period hams were subjectively evaluated for color of uncooked lean, marbling, firmness and aroma.

Determination of chemical characteristics

Percent fat and moisture were determined on duplicate homogenized samples (AOAC, 1965). Duplicate samples agreed within 0.50%.

Sodium chloride was determined according to the method of Glasstone (1946) as modified by Graham and Blumer (1972).

The pH of each sample was determined by blending 5g of muscle tissue and approximately 175 ml of distilled water and measuring the resulting slurry with a pH meter equipped with glass electrode.

Residual nitrate and nitrite were determined according to the method of Follett and Ratcliff (1963) with the modification suggested by Landmann (1966). Preliminary recovery studies conducted in this laboratory indicated that it is mandatory to add an additional 5 ml of pH 9.6 buffer immediately after heating to obtain maximum recovery of nitrate. This modification was employed throughout the analyses.

Quantitation of nitrosopigments was performed according to techniques described by Hornsey (1956). The value of the constant (K) used in the calculation of ppm acid haematin and ppm nitrosopigments was changed in accord with Arganosa and Henrickson (1969). All grinding and extraction of samples for analyses were carried out in the dark at 3–5°C to minimize pigment fading.

Reflectance spectroscopy also was used to determine the degree of pigment conversion. Measurements were made between wavelength 400 and 700 nm using a Bausch and Lomb Model 505 Recording Spectrophotometer with reflectance attachment. U.S.P. magnesium carbonate served as a standard. Samples at least 20 mm in thickness were cut perpendicular to the muscle fiber and placed in a sample holder made from black bakelite. Five freshly exposed surfaces of each ham were measured. Two measurements were made on the semimembranosus muscle, one each on the biceps femoris, semitendinosus and the knuckle area (vastus lateralis, rectus femoris, vastus intermedius and vastus

Table 1—Mean values for chemical characteristics of country-style hams by treatment, method of cure and processing period

Variable	Treatment				Method of cure		Processing time (in days)			
	Salt + sucrose	NO_3 only[a]	NO_2 only[a]	NO_3 + NO_2[a]	Brine	Dry cure	30	60	90	100
% Moisture	66.17	66.60	65.91	66.43	68.48[b]	64.07[c]	68.80[b]	67.93[c]	64.59[d]	63.42[e]
% Fat	4.73	5.69	4.91	4.31	4.51	5.29	4.64	4.98	5.38	4.56
% NaCl	4.93	4.86	4.86	,5.14	5.14[b]	4.75[c]	4.09[b]	4.71[c]	5.36[d]	5.73[d]
pH	6.19	6.02	6.30	6.09	6.09	6.28	6.09	6.32	6.09	6.12

[a] In addition to treatment ingredients listed, curing mixtures contained salt and sucrose.
[b,c,d,e] All means in the same row of treatment, method of cure, or processing time with the same superscript are not significantly different ($P < 0.05$)

medialis muscles). A mean value for the semimembranosus muscle was established and the mean of the four muscle areas of the ham was used in computations. The spectra of a red tile standard was run with each ham to monitor instrument variation and adjustments for tile value variation were made in the calculations. To remove the effect of light scattering by the muscle matrix, K/S values were calculated from 570 and 650 nm % reflectance readings (Judd and Wyszecki, 1963, Table D). The ratio of K/S values at 570/650 was used as an indication of the degree of nitrosation of pigments in samples of various treatments (Wodicka, 1956; Giddy, 1966).

Determination of organoleptic properties

Hams that had been in cure for 60, 90 or 100 days were evaluated in taste panel studies by two or three experienced taste panel members. Slices 0.63 cm in thickness were broiled in a conventional oven for approximately 12 min at a distance of 15 cm from the heat source. Evaluations for saltiness, elasticity, crumbliness, softness, juiciness, acidity, aged flavor, color and the presence of off-flavors were made on a 1-to-7 scale.

Effect of temperature on nitrate and nitrite depletion

A model system was used for a preliminary study of nitrate and nitrite depletion. Table grade granulated salt and sugar were added to 10-g samples of ground fresh pork in the same proportion as in ham processing. Nitrate and nitrite were added at levels of 500 and 200 ppm, respectively. Samples were stored at 4°C and 29°C. Residual nitrate and nitrite was measured on samples stored at 4°C at intervals of 2, 4, 6, 8 and 12 days. Samples stored at 29°C were measured at 2 and 4 days. Analyses were performed according to the procedure of Follett and Ratcliff (1963).

RESULTS & DISCUSSION

Chemical characteristics

A comparison of the effect of treatment, curing method and processing time on the chemical characteristics of country-style ham is presented in Table 1. Variations in curing mixture components did not significantly affect any of the chemical characteristics. Curing method was shown to have an effect upon percent moisture and salt but not on fat or pH. Brine pumped hams had a higher percent water and a slightly higher level of salt than dry-cured hams. The average percent water decreased progressively from the cured to the aged state with the greatest loss occurring during the first 4 wk of aging. Fat percentage is generally inversely related to the water content; however, this trend appeared to be variable possibly due to the influence of initial fat levels in the hams that were sampled at 100 days. Percentage of NaCl increased in hams over the processing period and is correlated with the loss in moisture. Proximate analyses are typical of commercially produced country-style ham that is aged 30–40 days at 27 ± 3°C and 55% RH.

Color

The nitrosation of the muscle pigment was determined at each sampling period by reflectance spectral studies and subjective evaluation of uncooked muscle and by pigment extraction and subjective evaluation of cooked muscle. Reflectance

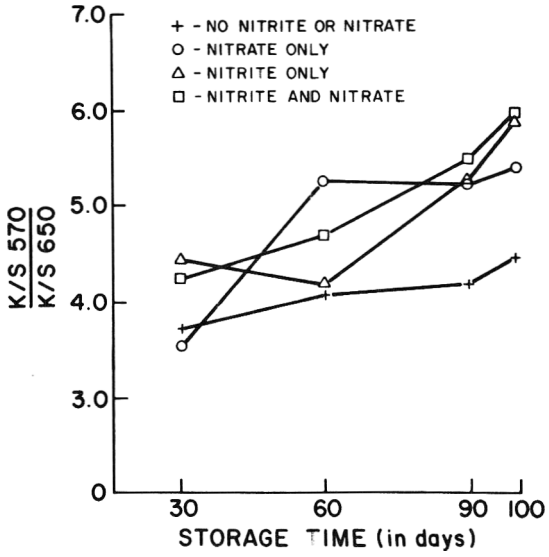


Fig. 1—Effect of curing treatment and processing time on the development of cured meat color in country-style hams as measured by reflectance spectra ratios.

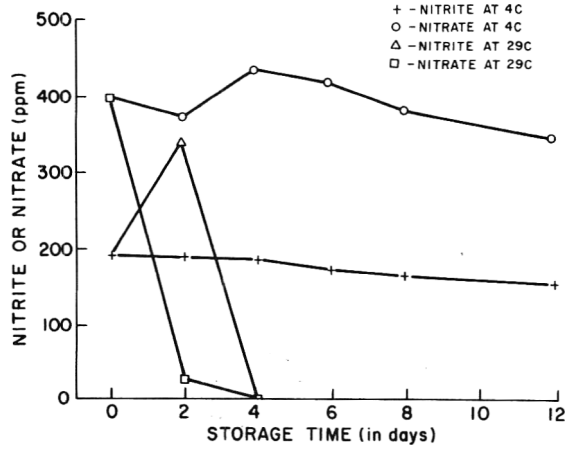


Fig. 2—Depletion of nitrate and nitrite in a ground pork model system during storage at 4°C and 29°C

ratios indicative of cured pigment formation are plotted by treatment over days in Figure 1 with increases in ratios representing greater color development. There was no difference in level of color development by curing method; therefore, this variable is not shown. Significantly higher levels of color development were obtained by the addition of nitrate and/or nitrite over the treatment in which only salt and sugar were added. However, similar levels of color were obtained with either nitrate, nitrite or a combination of these compounds. These results were confirmed by pigment extraction techniques (values not shown) and by panel evaluation of cooked muscle color (Table 2). Panel scores for cooked color indicated a slightly lower level of cured pigment in hams cured with salt and sucrose only as compared to those containing nitrate and a combination of nitrate and nitrite. Some color development during broiling may be expected as a result of protein denaturation and nonenzymatic browning reactions. These reactions could have affected panel judgments. Similar effects of nitrate and nitrite on uncooked ham color also have been reported recently by Kemp et al. (1974). An increase in color development with length of processing period also was apparent. This increase in color is anticipated due to the decrease in moisture over this period resulting in a corresponding increase in pigment concentration and the elevation of pH due to denaturation of proteins. Subjective evaluations of color of uncooked muscle indicate that color differences due to nitrate and/or nitrite were not detectable and all hams were rated acceptable.

Flavor

Organoleptic evaluations of hams processed 60, 90 and 100 days by an experienced panel indicated that there were no statistically significant flavor differences between treated and untreated samples. However, the panel gave slightly higher aged flavor ratings to samples that contained a combination of nitrate and nitrite (Table 2). In a recent study, Kemp et al. (1974) demonstrated that flavor and overall satisfaction scores for country-style ham were significantly increased by the presence of nitrate or nitrite. However, these researchers also found that highly acceptable hams could be produced with salt and sugar only. Previous studies have shown that the development of aged flavor in country-style hams is accompanied by an increase in concentration of salt, free amino acids, free fatty acids, peroxides, glycerol, water soluble organic matter and palmitoleic, linoleic and linolenic acid (Blumer, 1954; McCain et al., 1968; Craig et al., 1964; Kelly, 1965). Aged flavor of this product is probably attributable to increases of these flavor components and any effects of nitrate and nitrite per se on flavor may be partially masked by these components. Aged flavor development increased with number of days in aging and the typical aged flavor became apparent during the first 30 days under controlled temperature and aging conditions (Table 3). Some variability was noted among effects of treatment on tenderness factors. Samples with added nitrite were more elastic, cohesive and harder than those cured with salt and sugar only. These differences were variable and quite small and were probably not indicative of the desirability of addition or of deletion of nitrite from a technological point of view. The presence or absence of nitrate and/or nitrite did not affect the occurrence of off-flavors as evaluated by panel members.

Depletion of nitrate and nitrite

Quantitative dietary intake of nitrate and nitrite may be of importance when one considers the possibility of in vivo nitrosamine formation and residual nitrite also may contribute to nitrosopyrrolidine formation during broiling or frying of ham slices. Thus it appeared expedient to obtain a preview of the depletion of nitrate and nitrite at this stage of the study. A preliminary study of the effect of temperature on the depletion rate of nitrate and nitrite was performed using a ground

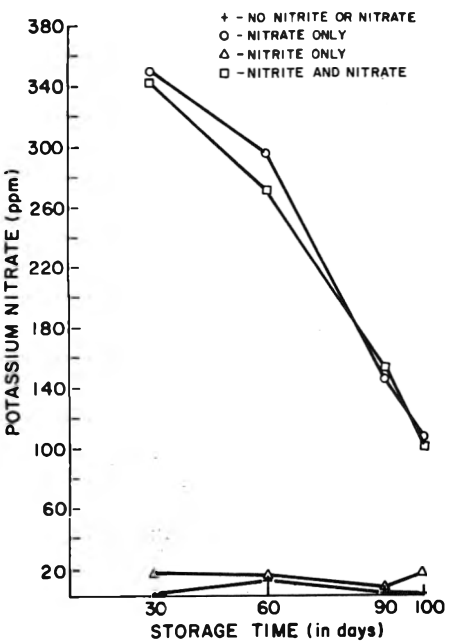


Fig. 3—Residual nitrate in country-style ham as affected by curing treatment and processing time.

Table 2—Effect of combinations of nitrate and nitrite in the curing treatment on organoleptic properties of country-style ham

	Curing treatment			
Factor	Salt + sucrose	NO_3 only[a]	NO_2 only[a]	$NO_3 + NO_2$[a]
Saltiness[b]	3.4	3.4	3.8	3.6
Elasticity[c]	3.0[d]	3.2[d,e]	4.1[e]	3.9[d,e]
Crumbliness[c]	5.0[d]	5.1[d]	4.0[e]	4.4[d,e]
Softness[c]	4.9[e]	4.7[d,e]	3.9[d]	3.8[d]
Juiciness[c]	5.1	5.0	4.9	4.7
Aged flavor[b]	2.6	2.7	2.6	3.0
Acidity[c]	4.0[d,e]	4.4[e]	3.7[d]	4.0[d,e]
Cooked color[b]	3.2[d]	3.4[d,e]	3.9[e]	3.6[d,e]

[a] In addition to treatment ingredients listed, curing mixtures contained salt and sucrose.
[b] Scale from 1 to 7 with 4 being ideal, intensity increases as value increases
[c] Scale from 1 to 7, intensity increases as value increases
[d,e] All means in a row with the same superscripts are not significantly different ($P < 0.05$).

Table 3—Effect of processing time on development of aged flavor in country-style ham

Processing time (in days)	Avg panel score[a]
60	2.0[b]
90	3.2[c]
100	3.3[c]

[a] Scale from 1 to 7 with 4 being ideal
[b,c] All means with the same superscript are not significantly different ($P < 0.05$).

pork model system. Results are presented in Figure 2. Nitrate and nitrite were depleted after 4 days storage at 29°C. Rapid depletion of nitrate after 2 days at 29°C and a corresponding increase in nitrite is indicative of microbial nitrate reduction. A gradual decrease in nitrate and nitrite concentration was observed in samples stored 12 days at 4°C.

Residual levels of nitrate and nitrite in hams were measured at 30, 60, 90 and 100 days of processing. Nitrite concentration was low at the end of 30 days at 4°C and the maximum amount quantitated was 18 ppm at 30 days. Residual nitrite concentrations in country-style ham that had been processed for 150 days were recently reported by Kemp et al. (1974). Levels ranged from 4.45 ppm in hams cured with salt and sugar only to 14.18 ppm in hams cured with 1 oz sodium nitrite/100 lb of meat. These residuals are higher than levels observed in hams that were processed for 100 days and analyzed in this study. However, the initial concentration added by Kemp et al. (1974) was twice as great and curing mixtures did not contain sodium ascorbate. Brown et al. (1974) have reported that that addition of ascorbate in brines used to produce packer-style hams results in a lower residual nitrite level and higher levels of ascorbate result in increased nitrite depletion.

Levels of nitrite were observed to decrease more rapidly in hams than in the ground pork model system. The use of sodium erythorbate in the curing mixture and greater activity of reducing systems in intact muscle are probably responsible for the increased rate of nitrite depletion in the intact ham muscle tissue (Kolari and Aunan, 1972; Mirvish et al., 1972; Olsman and Krol, 1972; Brown et al., 1974).

The concentration of nitrate, added either alone or with nitrite, decreased with time in cure with a more rapid depletion rate during aging at 27°C than during equalization at 11°C (Fig. 3). Acceleration of the depletion rate is a result of increased microbial reduction of the nitrate due to the increase in storage temperature. Extrapolation of model system depletion rates to 30 days revealed similar nitrate depletion in both the model system and the intact ham muscles.

Low levels (0–14 ppm) of nitrate were recovered from hams to which only nitrite was initially added. This occurrence has also been observed in other processed meats and may partially result from the conversion of nitrite to nitrate with the autocatalytic oxidation of the iron in meat pigments (Möhler), 1970; Herring, 1973). Studies of Möhler (1970) revealed that the formation of nitrate was correlated with the concentration of pigment, but it was independent of experimental conditions and amount of nitrite added. In contrast, Herring (1973) demonstrated that the accumulation of nitrate was proportional to amount of nitrite added in bacon. During storage the accumulation of nitrate may also be attributed to the disproportionation of nitrous acid to nitric oxide and nitrate and from the oxidation of nitric oxide by atmospheric oxygen in aqueous systems to give nitrate and nitrite (Herring, 1973; Ingram, 1973). Analytical methods may also contribute to this recovery and determination of nitrate below 10 ppm is not very precise.

REFERENCES

AOAC. 1965. "Official Methods of Analysis," 10th ed. Association of Official Analytical Chemists, Washington, D.C.

Arganosa, F.C. and Henrickson, R.L. 1969. Cure diffusion through pre- and post-chilled porcine muscles. Food Technol. 23: 1061.

Barnes, J.M. and Magee, P.N. 1954. Some toxic properties of dimethylnitrosamine. Brit. J. Ind. Med. 11: 167.

Blumer, T.N. 1954. Chemical compounds associated with aged flavor. Ph.D. thesis, Michigan State University, East Lansing.

Brown, C.L., Hedrick, H.B. and Bailey, M.E. 1974. Characteristics of cured ham as influenced by levels of sodium nitrite and sodium ascorbate. J. Food Sci. 39: 977.

Cho, I.C. and Bratzler, L.J. 1970. Effect of sodium nitrite on flavor of cured pork. J. Food Sci. 35: 668.

Christiansen, L.N., Johnston, R.W., Kautter, D.A., Howard, J.W. and Aunan, W.J. 1973. Effect of nitrite and nitrate on toxin production by Clostridum botulinum and on nitrosamine formation in perishable canned comminuted cured meat. Appl. Micro. 25: 357.

Cochran, W.G. and Cox, G.M. 1957. "Experimental Designs," 2nd ed. John Wiley and Sons, Inc., New York.

Craig, H.B., Blumer, T.N., Clawson, A.J. and Smart, W.W.G. 1964. Chemical, physical and organoleptic evaluation of aged country-style ham from hogs fed rations with and without peanut oil. J. Ani. Sci. 23: 956.

Fazio, T., White, R.H. and Howard, J.W. 1971. Analysis of nitrite and/or nitrate processed meats for N-nitrosodimethylamine. J. Assoc. Off. Anal. Chem. 54: 1157.

Fiddler, W., Diotrowski, E.G., Pensabene, J.W. and Wasserman, A.E. 1972. Some current observations on the occurrence and formation of N-nitrosamines. Proc. 18th Meeting Meat Res. Workers, p. 416, Guelph, Canada.

Follett, M.J. and Ratcliff, P.W. 1963. Determination of nitrite and nitrate in meat products. J. Sci. Food Agr. 3: 138.

Fox, J.B. Jr. 1966. The chemistry of meat pigments. J. Agr. Food Chem. 14: 207.

Giddy, C. 1966. The changes in meat pigments during sausage making processes. J. Sci. Food Agric. 17: 14.

Glasstone, S. 1946. "The Elements of Physical Chemistry." D. Van-Nostrand Co., New York.

Graham, P.P. and Blumer, T.N. 1972. Effect of prefreezing hams on quality attributes of dry cured product. J. Milk and Food Technol. 35: 98.

Greenberg, R.A. 1973. The effect of nitrite on botulinal toxin formation in bacon. Proc. Meat Ind. Res. Conf., p. 69, AMIF, Chicago, Ill.

Herring, H.K. 1973. Effect of nitrite and other factors on the physicochemical characteristics and nitrosamine formation in bacon. Proc. Meat Ind. Res. Conf., p. 47, AMIF, Chicago, Ill.

Hornsey, H.C. 1956. The colour of cooked cured pork. 1. Estimation of the nitric oxide-haem pigments. J. Sci. Food Agric. 7: 534.

Hustad, G.O., Cerveny, J.D., Trenk, H., Diebel, R.H., Kautter, D.A., Fazio, T., Johnston, R.W. and Kolari, O.E. 1973. Effect of sodium nitrite and sodium nitrate on botulinal toxic production and nitrosamine formation in wieners. Appl. Micro. 26: 22.

Ingram, M. 1973. The microbiological effects of nitrite. Proc. Int. Symp. Nitrite Meat Prod., p. 63, Zeist, The Netherlands.

Judd, D.B. and Wyszecki, G. 1963. "Color in Business, Science and Industry," 2nd ed. John Wiley and Sons, Inc., New York.

Kelly, S.M. 1965. The changes in relative amounts of long chain fatty acids during storage of ham fat and their possible relationship to flavor. M.S. thesis, North Carolina State University, Raleigh.

Kemp, J.D., Fox, J.D. and Moody, W.G. 1974. Cured ham properties as affected by nitrate and nitrite and fresh pork quality. J. Food Sci. 39: 972.

Kolari, O.E. and Aunan, W.J. 1972. The residual levels of nitrite in canned meat products. Proc. 18th Meat Res. Workers, Vol 2, p. 422, Guelph, Canada.

Landmann, W.A. 1966. Determination of nitrite and nitrate in meat products. J. Assoc. Off. Anal. Chem. 49: 875.

Lijinsky, W. and Epstein, S.S. 1970. Nitrosamines as environmental carcinogens. Nature 225: 21.

Magee, P.N. and Barnes, J.M. 1956. The production of malignant primary hepatic tumors in the rat by feeding dimethylnitrosamine. Brit. J. Cancer 10: 114.

McCain, G.R., Blumer, T.N., Craig, H.B. and Steel, R.G.D. 1968. Free amino acids in ham muscle during successive aging periods and their relation to flavor. J. Food Sci. 33: 142.

Mirvish, S.S., Wallcave, L., Eagen, M. and Shubik, P. 1972. Ascorbate-nitrite reaction: possible means of blocking the formation of N-nitroso compounds. Science 177: 65.

Möhler, L. 1970. Formation of curing pigment in beef muscle. 1. Oxidation of muscle pigment and nitrite. Z. Lebensmittel-Untersuch. U. Forsch. 142: 169.

Olsman, W.J. and Krol, B. 1972. Depletion of nitrite in heated meat products during storage. Proc. 18th Meat Res. Workers, Vol 2. p. 409, Guelph, Canada.

Sen, N.P. 1972. The evidence for the presence of dimethylnitrosamine in meat products. Fd. Cosmet. Toxicol. 10: 219.

Simon, S., Ellis, D.E., MacDonald, B.D., Miller, D.G., Waldman, R.C. and Westerberg, D.O. 1973. Influence of nitrite and nitrate curing ingredients on quality of packaged frankfurters. J. Food Sci. 38: 919.

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

Wasserman, A.E. and Talley, F. 1972. The effect of sodium nitrite on the flavor of frankfurters. J. Food Sci. 37: 536.

Wodicka, V.O. 1956. The nature and properties of the pigment of cured meats. Ph.D. thesis, Rutgers—The State University. New Brunswick, New Jersey.

Ms received 1/24/75; revised 3/31/75; accepted 4/8/75.

Paper No. 4583 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, N.C. Data are from M.S. thesis of the senior author.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named, nor criticism of similar ones not mentioned.

B. D. EAKES and T. N. BLUMER
Dept. of Food Science, North Carolina State University, Raleigh, NC 27607

EFFECT OF VARIOUS LEVELS OF POTASSIUM NITRATE AND SODIUM NITRITE ON COLOR AND FLAVOR OF CURED LOINS AND COUNTRY-STYLE HAMS

INTRODUCTION

CURRENT federal regulations for levels of sodium nitrite in cured meat products were established in 1925 as a direct result of studies conducted by Kerr et al. (1926). In this extended study it was confirmed that color and quality comparable to that of hams cured with nitrate could be attained when one-fourth to 1 ounce of nitrite/100 lb of meat was substituted for nitrate. The residual nitrite level was arbitrarily set at 200 ppm since this represented the maximum content found in any part of the nitrite cured hams.

Nitrite gained acceptance as an additive due to the prior knowledge that meats cured with nitrate contained nitrite and that it did not constitute any apparent health hazard (Kerr, 1926). The toxicity of nitrosamines has been demonstrated by Barnes and Magee (1954) and it was later shown that dimethylnitrosamine possessed carcinogenic activity in rats (Magee and Barnes, 1956). The possible health hazard due to the reaction of nitrite and nitrosatible amines in foods to form nitrosamines has made it necessary to reevaluate the functions of nitrate and nitrite in cured meats, to justify levels of addition, and to search for possible substitutes. These functions and levels must be established for a wide variety of processed meats due to variations in additives, processing conditions and subsequent time and temperature of storage of different types of products. Due to the importance of the issue, an expert panel was established by the United States Department of Agriculture to consider information regarding functions and levels of these two compounds and to make recommendations for their reduction or elimination. Thus, the importance of this study appears obvious.

In a related study (Eakes, 1974) it was determined that hams cured with nitrate and nitrite either alone or in combination in the curing mixture had more acceptable color than hams cured with salt and sucrose only. In this study, a pork loin (longissimus dorsi muscle) model system and country-style hams were used to determine the minimum level of nitrate and nitrite necessary to attain acceptable color and flavor compared with those traditionally employed.

EXPERIMENTAL

Curing and aging of loins

36 porcine longissimus dorsi muscles separated from loins, individually wrapped and stored at −20°C were removed and allowed to thaw at 3°C. Loins were divided into two replicates with 20 and 16 loins in Rep 1 and 2, respectively. Rep 1 consisted of five treatments, with four loins in each. Treatment 1, the control, was composed of a curing mixture of 78.5% table grade granulated salt, 19.7% white cane sugar, 1.5% KNO_3 and 0.3% $NaNO_2$. The mixture was applied at the rate of 68g/kg of meat. In treatment 2, only dry granulated table grade salt was applied to loins at the rate of 62g/kg of meat. Treatments 3, 4 and 5 were dry cured with salt, and aqueous solutions (25 ml) of nitrate and/or nitrite were applied to the loin surfaces at levels of 10, 40, 70 and 100 ppm. Treatment 3 contained nitrate only, treatment 4 nitrite only, and treatment 5 nitrate and nitrite. In treatment 5 the nitrite concentration was equal to 16.67% of total ppm added and nitrate was equal to 83.33%. These represent the same proportions used in the control treatment. Rep 2 consisted of only four treatments since treatment 2 (salt only) of Rep 1 was eliminated from the study because the results showed this treatment to be inferior to the others. Other treatments remained the same as in Rep 1 except that nitrate and nitrite concentrations used were 40, 70, 100 and 130 ppm. Immediately after application of cure mix, loins were placed at 3°C for 7 days to allow for absorption of cure. They were then removed, weighed, stockinetted and hung at 11 ± 2°C, RH 60 ± 3% for 5 days equalization and at 27 ± 3°C, RH 55 ± 4% for 4 days aging.

Curing and aging of hams

16 paired hams were purchased from a local meat packer and cured in the University Meats Laboratory with salt, sugar and four levels of nitrate and/or nitrite. Cure mix for treatment 1 (Control) of the hams was the same as that stated above for treatment 1 of the loins. Treatment 2, 3 and 4 cure mix was composed of 78.5% salt and 19.7% sugar. These mixtures were applied at the rate of 8.4% of ham weight. One-third of the curing mixture required for each ham was applied on the first, third and tenth days of cure. By observing the results of the loin study, 70, 100, 130 and 160 ppm of nitrate and/or nitrite were placed in aqueous solutions (25 ml) and injected into the hams of treatments 2, 3 and 4 prior to applying salt and sugar in a dry form. Treatment 2 contained nitrate, treatment 3 contained nitrite and a combination of nitrate and nitrite was used in treatment 4. When a combination of nitrate and nitrite was added, nitrate represented 83.33% of total ppm with the remaining being nitrite. Hams were placed in cure, equalization and aging for a period of 30 days each for a total processing time of 90 days. Conditions maintained during each period were the same as those indicated for the loins.

Chemical analyses and subjective evaluations

Analyses for percent moisture, fat and salt were performed according to procedures described by Butz et al. (1974).

The pH of each sample was determined on a meat-water slurry composed of 5g of muscle tissue and 175 ml distilled water that had been blended for 5 min in a Waring Blendor.

Residual nitrate and nitrite were determined according to the method of Follett and Ratcliff (1963) with the modification suggested by Landmann (1966). Previous studies conducted in this laboratory indicated that it is mandatory to add an additional 5 ml of pH 9.6 buffer immediately after heating to obtain maximum nitrate recovery. The latter modification was performed throughout the analyses.

Development of nitrosopigments was monitored according to extraction techniques described by Hornsey (1956) and by reflectance spectrophotometry. A ratio of K/S values for percent reflectance readings at 570 and 650 nm was used to determine the degree of pigment nitrosation (Judd and Wyszecki, 1963, Table D; Wodicka, 1956; Giddy, 1966).

Subjective evaluations of color of lean were made at time of sampling. Color was scored from extremely light to extremely dark, 1 to 5, respectively with 3 being ideal.

Organoleptic evaluations

All hams were evaluated by six experienced taste panel members. Slices 0.63 cm in thickness were broiled in a conventional oven for approximately 12 min at a distance of 15 cm from the heat source. Evaluations for saltiness, elasticity, crumbliness, softness, juiciness, acidity, aged flavor, color, cured flavor and off flavors were made on a 1 to 7 scale.

Table 1—Average weight loss, percent water and salt and pH of porcine L. dorsi muscle after 16 days in processing and of hams after 90 days

Variable	Loins					Hams			
	Control[a]	Salt	NO_3 only[b]	NO_2 only[b]	NO_3+ NO_2[b]	Control[a]	NO_3 only[c]	NO_2 only[c]	NO_3+ NO_2[c]
% Wt loss	26.33	23.44	23.27	21.94	24.12	18.93	20.74	18.60	18.21
% Moisture	52.00	54.92	56.14	51.22	53.20	60.90	62.58	60.18	60.69
% Salt	7.89	7.36	8.62	9.38	8.82	5.08	5.31	5.85	5.30
pH	5.6	5.7	5.8	5.8	5.4	5.9	5.9	5.8	5.8

[a] Control treatment contained salt, sucrose, nitrate (1,256 ppm) and nitrite (251 ppm).
[b] In addition to treatment ingredients listed, curing mixtures contained salt.
[c] In addition to treatment ingredients listed, curing mixtures contained salt and sucrose.

RESULTS & DISCUSSION

Chemical characteristics

Mean values for chemical characteristics and weight losses of cured and aged porcine L. dorsi muscle and country-style hams are presented in Table 1. Lower percent moisture in the aged loin samples as compared to hams is attributed to the greater exposure of muscle surface areas of the former. Percentage weight loss and percentage salt are seen to increase as moisture decreases. Chemical characteristics of all country-style hams regardless of curing treatment conform to normally expected ranges for commercially produced hams cured under similar conditions.

Color

The study using pork loins was conducted to determine the effect of low levels of nitrate and/or nitrite on color and to estimate a range of desirable levels for the ham study. The salt only treatment in Rep 1 did not produce acceptable color and was deleted from subsequent experiments. It is known that hams may be cured with salt only and an acceptable color can be attained after a long aging period; however, the red color in hams cured with salt only is not stable during cooking as it is with those cured with nitrate or nitrite. Table 2 shows reflectance ratios (K/S 570/K/S 650) for loins treated with various levels of nitrate and nitrite. An increase in this ratio is indicative of greater color development. Reflectance spectral analyses of controls revealed that a ratio of 2.84 or greater for loin samples and 2.90 or greater for ham samples indicates acceptable color. Although all values are not shown, reflectance measurements were confirmed by extracted pigment concentrations and subject color scores. These measurements closely agreed with reflectance ratios and it appeared redundant to include these values. Comparisons for controls and various levels indicate that 70 ppm of nitrate and/or nitrite produce acceptable color in most samples analyzed. Increased levels produced higher concentrations of nitrosopigment; however, these levels of pigment were not considered necessary for color acceptability. The average percent moisture for loins treated with 70 ppm nitrite and/or nitrate was 53.15%. Previous studies in this laboratory have confirmed that the level of color is affected by percent moisture and this factor may influence optimum levels of nitrate and nitrite.

Reflectance ratios from hams treated with various levels are also presented in Table 2. As in loins, an increase in concentration of nitrate or nitrite resulted in higher levels of color development and comparisons between reflectance ratios and other color evaluations for controls and treatment levels (Fig. 1 and Table 3) revealed that hams injected with 70 ppm nitrate and/or nitrite were acceptable from a color standpoint. In studies on the use of various levels of nitrite in packer-style hams, Brown et al. (1974) found that 182 ppm nitrite resulted in darker, more typical cured meat color than did 91 ppm of nitrite. Each value in Table 2 and Figure 1 represents one ham and some variability results from this nonreplicated experimental design. The average percent moisture for hams containing 70 ppm nitrate and/or nitrite was 59.00% which is typical of commercially produced country-style ham.

Overall comparisons of color evaluations confirmed that

Table 2—Effect of level of nitrate and/or nitrite added on development of cured meat color in pork loins and country-style hams as measured by reflectance spectra ratios[a]

NO_3 and/or NO_2 added (ppm)	Loins				Hams			
	Control[b]	NO_3[c]	NO_2[c]	NO_3+[c] NO_2	Control[b]	NO_3[d]	NO_2[d]	NO_3+[d] NO_2
	2.84	—	—	—	2.90	—	—	—
10		2.82	2.38	1.80		—	—	—
40		2.30	2.50	2.70		—	—	—
70		2.91	3.00	3.02		3.92	3.75	2.93
100		2.25	3.73	3.18		3.31	3.25	2.79
130		2.70	3.58	3.59		3.76	3.17	3.36
160		—	—	—		3.51	3.33	3.36

[a] Reflectance spectra ratios equal to or greater than control ratios indicate acceptable color development.
[b] Control treatment contained salt, sucrose, nitrate (1,256 ppm) and nitrite (251 ppm).
[c] In addition to treatment ingredients listed, curing mixtures contained salt.
[d] In addition to treatment ingredients listed, curing mixtures contained salt and sucrose.

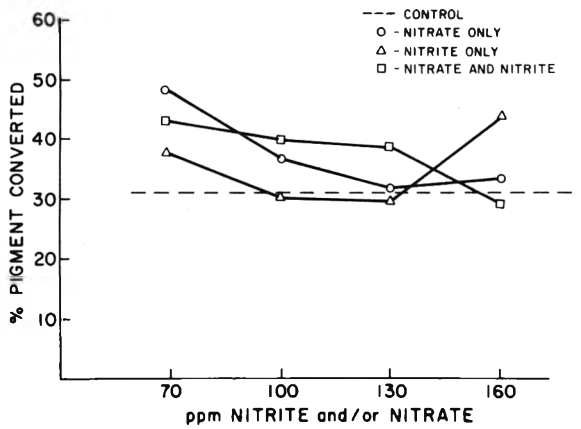


Fig. 1—Effect of nitrate and/or nitrite added on development of cured meat color in country-style hams as measured by pigment extraction techniques.

levels of nitrate and nitrite lower than those currently employed may be added for development of acceptable color but the data do not strongly support the use of one specific treatment. The decision as to the use of either nitrate, nitrite or a combination is largely based on preference of the individual processor and is shown to be of little consequence from a technological point of view. However, this study does not include microbiology of the ham in regard to protection from harmful microorganisms due to the presence of nitrite. This type of ham, because of high NaCl concentration and comparatively low water activity would normally not be considered conducive to the growth of most anaerobes.

Flavor

All hams were evaluated by experienced taste panelists and results of these organoleptic evaluations are tabulated in Table 3. Flavor differences as affected by treatment were small due to the presence of other compounds associated with aged flavor development that may partially mask flavor differences due to nitrate and nitrite per se. At a level of 70 ppm, hams treated with nitrite only and with nitrate and nitrite were slightly more acceptable from an aged flavor standpoint; however, cured flavor scores for the same samples indicate greater acceptability of hams containing nitrate only and nitrate and nitrite. Flavor evaluations over all concentrations revealed greatest acceptability of hams treated with nitrate only or a combination of nitrate and nitrite. Previous studies have shown that nitrite is the active agent in cured flavor development in all meat frankfurters and nitrate has been shown to have no effect (Simon et al., 1973); however, nitrite had no effect in all beef frankfurters. Brown et al. (1974) have also reported an increase in the flavor of packer-style hams by the addition of 91 ppm nitrite in the curing brine; however, there were no flavor differences between hams treated with 91 and 182 ppm. Country-style ham has a very distinctive aged flavor that is probably influenced by increases in salt, free amino acids and free fatty acids as well as nitrate that is gradually reduced to nitrite.

Residual nitrate and nitrite

Residual nitrate and nitrite in loins was determined after 16 days in curing and aging and in hams after 90 days (Tables 4 and 5). Both the loins and hams contained minimal amounts of nitrite at sampling. The depletion rate of nitrate was variable and did not appear to be affected by initial level added or pH. Reduction of nitrate is a result of microbial action and variations in residual levels is probably dependent upon the population of nitrate reducing organisms within the product and upon storage time and temperature. Nitrate was observed in samples to which nitrite was initially added. This result has been noted in other processed meats to which nitrate was added (Möhler, 1970; Herring, 1973). The accumulation of nitrate has been attributed to oxidation and to the disproportionation of nitrous acid in the cured meat to yield nitric

Table 3—Effect of curing treatment and level of nitrate and/or nitrite on sensory attributes of country-style hams

Treatment	Levels (ppm)	Saltiness[c]	Elasticity[d]	Crumbliness[d]	Softness[d]	Juiciness[d]	Aged flavor[c]	Acidity[d]	Nuttiness[e]	Cooked color[c]	Cured flavor[c]
Control[a]		3.9	3.0	4.8	4.9	4.7	3.7	4.8	4.3	3.5	3.9
Nitrate only[b]	70	4.0	3.8	3.7	3.8	4.8	3.5	4.0	4.0	2.8	3.8
	100	3.2	3.8	3.8	4.2	4.5	3.3	3.8	4.3	3.2	3.5
	130	3.8	4.2	4.2	3.8	4.5	3.7	4.8	4.5	3.3	4.0
	160	3.5	3.5	3.0	3.0	3.8	4.0	4.3	4.8	3.8	4.2
	Mean	3.6	3.8	3.7	3.7	4.4	3.6	4.2	4.4	3.3	3.9
Nitrite only[b]	70	4.0	2.5	5.2	5.3	4.8	3.7	4.0	4.3	4.0	3.5
	100	3.7	2.7	5.2	5.5	4.8	3.0	4.5	4.2	3.5	3.3
	130	3.8	3.2	3.8	4.7	4.8	3.7	4.3	4.7	3.8	3.8
	160	4.2	4.7	4.2	4.0	4.3	3.3	4.2	4.3	3.7	3.5
	Mean	3.9	3.3	4.6	4.9	4.7	3.4	4.2	4.4	3.8	3.5
Nitrate and nitrite[b]	70	4.0	4.0	4.2	4.2	4.5	3.8	4.3	4.3	3.8	3.8
	100	4.2	3.3	4.7	4.5	4.3	3.8	4.2	4.5	3.5	3.5
	130	3.5	4.8	3.3	3.3	4.5	3.7	3.7	4.2	3.0	3.8
	160	3.5	2.2	5.5	5.2	3.8	3.3	4.0	4.3	3.7	3.8
	Mean	3.8	3.6	4.4	4.4	4.3	3.6	4.0	4.3	3.5	3.7

[a] Control treatment contained salt, sucrose, nitrate (1,256 ppm) and nitrite (251 ppm).
[b] In addition to treatment listed, curing mixtures contained salt and sucrose.
[c] Scale from 1 to 7 with 4 being ideal; intensity increases as value increases
[d] Scale from 1 to 7; intensity increases as value increases
[e] Scale from 4 to 7 with 4 being neutral; intensity increases as value increases

Table 4—Initial levels and residual nitrate and nitrite in loins stored 16 days[a]

KNO_3 added				$NaNO_2$ added				KNO_3 + $NaNO_2$ added			
Initial		Residual		Initial		Residual		Initial		Residual	
NO_3	NO_2	NO_3	NO_2	NO_3	NO_2	NO_3	NO_2	NO_3	NO_2	NO_3	NO_2
10	–	0	1	–	10	0	2	8	2	4	1
40	–	28	0	–	40	23	4	33	7	17	3
70	–	5	0	–	70	18	2	58	12	33	3
100	–	53	0	–	100	38	0	83	17	55	2
130	–	85	0	–	130	13	5	108	22	106	7

[a] All values are given in parts per million

Table 5—Initial levels and residual nitrate and nitrite in country-style hams stored 90 days[a]

KNO_3 added				$NaNO_2$ added				KNO_3 + $NaNO_2$ added			
Initial		Residual		Initial		Residual		Initial		Residual	
NO_3	NO_2	NO_3	NO_2	NO_3	NO_2	NO_3	NO_2	NO_3	NO_2	NO_3	NO_2
70	–	38	0	–	70	17	0	58	12	54	0
100	–	33	0	–	100	24	5	83	17	61	0
130	–	127	0	–	130	10	0	108	22	120	0
160	–	149	10	–	160	32	0	133	27	142	0

[a] All values are given in parts per million

oxide and nitrate. Nitric oxide may also be oxidized in aqueous systems to yield nitrate and nitrite (Möhler, 1970; Herring, 1973; Ingram, 1973).

It is evident from this study that levels of nitrate and nitrite can be significantly reduced without adverse effects on flavor and color of country-style hams. However, the effect of low levels on the growth of pathogenic microorganisms should also be assessed in order to continue to insure a high quality product from the standpoint of food safety. In addition, low levels of nitrate and/or nitrite in the curing mixture should be evaluated under commercial processing conditions to evaluate properly the quality of the resulting product.

REFERENCES

Barnes, J.M. and Magee, P.N. 1954. Some toxic properties of dimethylnitrosamine. Brit. J. Ind. Med. 11: 167.

Brown, C.L., Hedrick, H.B. and Bailey, M.E. 1974. Characteristics of cured ham as influenced by levels of sodium nitrite and sodium ascorbate. J. Food Sci. 39: 977.

Butz, R.G., Blumer, T.N., Christian, J.A., Swaisgood, H.E. and Lucas, H.L. 1974. Factors responsible for white film formation on cut surfaces of dry-cured hams. J. Food Sci. 39: 516.

Eakes, B.D. 1974. Effect of various levels of sodium nitrate and sodium nitrite on the color and flavor of country-style hams. M.S. thesis, North Carolina State University, Raleigh, N.C.

Follett, M.J. and Ratcliff, P.W. 1963. Determination of nitrite and nitrate in meat products. J. Sci. Food Agric. 3: 138.

Giddy, C. 1966. The change in meat pigments during sausage making processes. J. Sci. Food Agric. 7: 14.

Herring, H.K. 1973. Effect of nitrite and other factors on the physiochemical characteristics and nitrosamine formation in bacon. Proc. Meat Ind. Res. Conf., p. 47, AMIF, Chicago, Ill.

Hornsey, H.C. 1956. The red colour of cooked cured pork. 1. Estimation of the nitric oxide-haem pigments. J. Sci. Food Agric. 7: 534.

Ingram, M. 1973. The microbiological effects of nitrite. Proc. Int. Symp. Nitrite Meat Prod., p. 63, Zeist, The Netherlands.

Judd, D.B. and Wyszecki, G. 1963. "Color in Business, Science and Industry," 2nd ed. John Wiley and Sons, Inc., New York.

Kerr, R.H., Marsh, C.T.N., Shroeder, W.F. and Boyer, A.E. 1926. The use of sodium nitrite in the curing of meat. J. Agr. Res. 33: 541.

Landmann, W.A. 1966. Determination of nitrite and nitrate in meat products. J. Assoc. Off. Anal. Chem. 49: 875.

Magee, P.N. and Barnes, J.M. 1956. The production of malignant primary hepatic tumors in the rat by feeding dimethylnitrosamine. Brit. J. Cancer 10: 114.

Möhler, K. 1970. Formation of curing pigment in beef muscle. 1. Oxidation of muscle pigment and nitrite. Z. Lebensmittel-Untersuch. U. Forsch. 142: 169.

Simon, S., Ellis, D.E., MacDonald, B.D., Miller, D.G., Waldman, R.C. and Westerberg, D.O. 1973. Influence of nitrite and nitrate curing ingredients on quality of packaged frankfurters. J. Food Sci. 38: 919.

Wodicka, V.O. 1956. The nature and properties of the pigment of cured meats. Ph.D. thesis, Rutgers—The State University, New Brunswick, N.J.

Ms received 1/24/75; revised 3/31/75; accepted 4/8/75.

Paper No. 4582 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, N.C. Data are from M.S. thesis of the senior author.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named, nor criticism of similar ones not mentioned.

J. I. GRAY[1] and L. R. DUGAN JR.
Dept. of Food Science & Human Nutrition, Michigan State University, East Lansing, MI 48824

INHIBITION OF N-NITROSAMINE FORMATION IN MODEL FOOD SYSTEMS

INTRODUCTION

CARCINOGENIC N-nitrosamines are classically produced by the electrophilic reaction between nitrite or nitrous acid and secondary and tertiary amines under acidic conditions. There is now no doubt that N-nitrosamines can be formed in food systems if the concentrations of nitrite and nitrosatable amines are high enough and the conditions are appropriate (Fan and Tannenbaum, 1973).

Nitrites are used in many countries as deliberate food additives. These serve to stabilize the color of cured meats, contribute flavor and protect against the danger of botulism (Cho and Bratzler, 1970; Duncan and Foster, 1968; Wasserman and Talley, 1972). In addition, nitrates which are widely distributed in vegetables such as spinach, beets, celery and lettuce can undergo bacterial reduction to produce significant concentrations of nitrite (Sinios and Wodsak, 1965; Phillips, 1968).

Amines or amine precursors such as proteins, amino acids, phospholipids or quaternary ammonium compounds are present in most foods and may be available for reaction with nitrite. Secondary amines have been reported in fish (Gruger, 1972; Wick et al., 1967), vegetables (Phillips, 1968; Preusser, 1966) and fruit juices (Stewart et al., 1964). In certain instances, a single meal may have as much as 100 mg of secondary amine (Sebranek and Cassens, 1973). Lijinsky and Epstein (1970) speculated that the pyrolysis of protein and cooking of foods might produce nitrosatable secondary amines such as pyrrolidine and piperidine. This was confirmed by Gray and Dugan (1975) who reported the formation of N-nitrosopyrrolidine when collagen was heated at elevated temperatures in frying oil in the presence of nitrite. The potential precursor role of phospholipids in N-nitrosamine formation was demonstrated by Mohler and Hallermayer (1973). They produced parts per million (ppm) quantities of N-nitrosamines by boiling lecithin and nitrite in buffer solutions in the pH range 3.5–7. Fiddler et al. (1972) showed that quaternary ammonium compounds such as neurine, choline, acetylcholine, carnitine and betaine all formed trace amounts of N-nitrosamines.

Since the presence of nitrite is a requisite for N-nitrosamine formation, any compound that could compete successfully with the secondary amine for the available nitrite would reduce the possibility of N-nitrosamine formation. Mirvish et al. (1972) and Fan and Tannenbaum (1973) working with model systems have demonstrated that ascorbic acid can block the N-nitrosation reaction by reacting with nitrite. Sodium ascorbate or its isomer, erythorbate is used in cure mixtures to accelerate cure color formation. Fiddler et al. (1973) showed that frankfurters prepared with either 550 or 5500 ppm ascorbate and 1500 ppm nitrite and processed for 2 hr had no N-nitrosamine present, in comparison with the approximate 10 ppb present in the samples made with nitrite alone. While the mechanism for the inhibition of the N-nitrosation reaction is not completely understood, it appears that these reductants compete for the nitrite, thereby making it less available for reaction with secondary amines.

Urea and ammonium sulfamate (Mirvish et al., 1972) and tannic acid (Bogovski et al., 1971) have been shown to inhibit the formation of N-nitrosamines. The latter workers also noted an apparent inhibiting effect of milk on the N-nitrosation reactions in their model systems. Similar results were obtained by Fan and Tannenbaum (1973) who showed that the rate of formation of N-nitrosomorpholine in milk was retarded compared with the reaction in buffer solution at pH 6.0 and 25°C. Knowles (1974) also made the observation that nitrite interaction with a wide variety of smoke phenols may occur in bacon during production and frying.

In this paper, the effect of certain compounds, some of which are endogenous to cured meat systems and some which may be added for preservative or other purposes, on the N-nitrosation reaction was investigated in both aqueous and low moisture carboxymethylcellulose systems.

EXPERIMENTAL

Preparation of model systems

Low moisture carboxymethylcellulose (CMC) systems. A system containing secondary amine (or amino acid) $NaNO_2$, various concentrations of the compound under investigation, 2.5g CMC 7HF (Hercules, Inc.) and 25C ml buffer was prepared as described earlier (Gray and Dugan, 1974). Two buffer systems (biphthalate for pH 3.5 and citrate for pH 5.5, both 0.1M) were used. The mixture was freeze dried for 24 hr in a Virtis RePP model No. 42 sublimator at a pressure of 5μ and a platen temperature of 24°C. Residual moisture content was approximately 3%. The concentrations of the reactants used in this study are indicated later.

The dried system was macerated to a fine powder and doubly wrapped in aluminum foil before heating in an oven at the required temperature. The N-nitrosamine was extracted from the matrix by blending with 250 ml distilled water and 10g K_2CO_3 for 1 min. The aqueous solution was extracted with dichloromethane (one 200 ml aliquot followed by two 150 ml aliquots).

Aqueous systems. A system containing secondary amine, $NaNO_2$, various concentrations of the compounds under investigation and 60 ml buffer solution was heated in a 125 ml stoppered iodine flask in a water bath at 69°C for 3 hr. On cooling, the contents were extracted with three 100 ml aliquots of dichloromethane.

A system containing secondary amine, $NaNO_2$, test compound, 15g Mazola corn oil and 60 ml biphthalate buffer was heated at 69°C for 3 hr. The reaction mixture was shaken constantly during the reaction period. The system was cooled in a refrigerator for 30 min to separate the oil and water phases. The separated phases were added to 350 ml 3M NaOH and vacuum distilled. The distillate, collected in a receiving flask at −8°C, was extracted with three 100 ml aliquots of dichloromethane.

A system incorporating soluble starch (Difco) to inhibit separation of oil from water was prepared using 5g corn oil, 15g soluble starch and 75 ml biphthalate buffer, plus the required reactants. The system was shaken for 4 hr at 50°C and 8 hr at room temperature. The reaction mixture was distilled under vacuum after the addition of 3N NaOH.

Quantitation of N-nitrosamine

The dichloromethane extracts, after drying over anhydrous

[1] Present address: Dept. of Food Science, University of Guelph, Guelph, Ontario, Canada

Na_2SO_4, were concentrated to approximately 5 ml in a Kuderna-Danish concentrator. Hexane (1 ml) was added and the extract was further concentrated under a stream of nitrogen to a volume of 1 ml. A Beckman GC-5 gas chromatograph equipped with dual hydrogen flame detectors and a stainless steel column (6 ft × 1/8 in. o.d.) of Carbowax 20M on 80-100 mesh Chromosorb W was used for analysis. The chromatograph was operated isothermally at 90°C for dimethylnitrosamine (DMN) and 140°C for N-nitrosopyrrolidine (N-Pyr) analyses, with gas flows of 26.0, 20.0 and 300 ml/min for nitrogen, hydrogen and compressed air, respectively. Mass spectrometry and comparison of GLC retention data with authentic N-nitrosamines were used for positive identification of the above compounds.

RESULTS & DISCUSSION

SINCE N-NITROSAMINES are formed in cured meat systems by the reaction between secondary amines and nitrite, it is reasonable to assume that any compound capable of reacting with nitrite may inhibit the N-nitrosation of the amine by competing for the available nitrite. The compounds selected to investigate this inhibitory action are either endogenous to a cured meat system, contain functional groups corresponding to endogenous reductants in meat systems or may be a preservative to prolong shelf life of the product.

The formation of N-nitrosamines was investigated in both low moisture CMC and aqueous systems. A pH of 3.5 was used in some cases because it facilitates the formation of N-nitrosamine from the secondary amine and a pH of 5.5 which is found in many meat systems. A temperature of 69°C was used since it is commonly used in the pasteurization of hams. The inhibitory effects of ascorbic acid and sodium bisulfite on the N-nitrosation of dimethylamine (DMA) in both systems are shown in Table 1. The concentration of DMA and $NaNO_2$ were 1 mM and 5 mM, respectively and essentially complete inhibition of the reaction was achieved when the ratio of ascorbic acid (or bisulfite) to nitrite was larger than 2:1. This is in agreement with the data of Fan and Tannenbaum (1973) who studied the effect of ascorbic acid on the formation of N-nitrosomorpholine in a model system. The percentage conversion of the amine to the N-nitrosamine varied with the pH and with the system. In the aqueous system, 40.26 and 6.2% conversions were obtained at pH 3.5 and 5.5 respectively; in the low moisture CMC system, conversions of 44.6 and 17.7% were obtained at pH 3.5 and 5.5.

Mirvish et al. (1972) studied the role of ascorbic acid as a possible means of preventing the formation of N-nitrosamine and concluded that the blocking of the N-nitrosation reaction by ascorbate was probably due to competition for the available nitrite, or more correctly, nitrous anhydride. In this study, at the pH values used, the proportion of the ascorbate anion is very significant and reaction with nitrite is very rapid. The ascorbate anion is 240 times more rapidly nitrosated than ascorbic acid, due presumably to the greater nucleophilic activity of the anion (Mirvish et al., 1972).

Sodium bisulfite was equally effective in inhibiting the formation of DMN, over 99% inhibition being achieved in both systems when the bisulfite:nitrite concentration ratio was greater than 2:1. Sulfur dioxide is used in the food industry to inhibit both enzymatic and nonenzymatic browning, to inhibit and control microorganisms and as an antioxidant and reducing agent (Roberts and McWeeny, 1972). Up to 450 ppm SO_2 is permitted in sausages or sausage meat in the United Kingdom (Roberts and McWeeny, 1972).

Smaller concentrations of tannic acid were used as a result of its high molecular weight. Table 2 indicates that greater inhibition was achieved at the lower pH, where almost complete inhibition was achieved in both systems. Tannic acid has been implicated as a possible means of blocking the N-nitrosation of secondary amines (Bogovski et al., 1971). They demonstrated that the highest concentrations of DMN formed in apple juice were only 5% of the amount formed in water under the same conditions and in most cases the amount was less than 1%. Tannic acid is a natural constituent of apple juice, beer and tea and can be hydrolyzed to give gallic acid (Cram and Hammond, 1959). As a result of this reaction, propyl gallate was one of the phenolic-type compounds investigated in this study.

Antioxidant compounds were also effective in blocking DMN formation, hydroquinone and α-tocopherol being especially effective (Table 3). These were investigated in an

Table 1—Effect of ascorbic acid and sodium bisulfite on N-nitrosamine formation in model systems

System	Conc of compound mM	% Inhibition (DMA → DMN) Ascorbic acid pH 3.5	Ascorbic acid pH 5.5	$NaHSO_3$ pH 3.5	$NaHSO_3$ pH 5.5
Low moisture CMC	0				
	2.5	60.8	71.9	68.2	75.8
	5.0	91.3	92.0	94.4	90.5
	10.0	99.0	95.0	99.1	95.8
	20.0	99.4	99.5	99.5	99.2
Aqueous	0				
	2.5	67.4	71.6	66.2	74.3
	5.0	96.7	97.9	95.1	97.3
	10.0	98.5	99.1	98.4	99.1
	20.0	99.5	99.8	99.4	99.5

Table 2—Effect of tannic acid on N-nitrosamine formation in model systems containing $NaNO_2$ (5 mM) and secondary amine (1 mM)

System	Conc of Tannic acid mM	% Inhibition (DMA → DMNA) pH 3.5	pH 5.5
Low moisture CMC	0		
	0.01	6.0	3.0
	0.10	58.5	63.1
	0.50	84.3	82.2
	1.00	97.5	87.3
	2.00	99.5	90.6
Aqueous	0		
	0.01	1.8	0.0
	0.10	59.8	67.8
	0.50	97.0	82.9
	1.00	99.2	85.4

Table 3—Effect of phenolic compounds on N-nitrosamine formation in aqueous model systems (pH 3.5)

Compound	Concentration	% Blockage
Vanillin	5 mM	46.70
	10 mM	60.53
Thymol	5 mM	54.68
	10 mM	56.64
Hydroquinone	5 mM	98.82
	10 mM	99.10
α-Tocopherol	5 mM	92.80

aqueous system containing 1 mM DMA and 5 mM $NaNO_2$ and heated at 69°C for 2 hr. Hydroquinone is representative of more complex quinoid-type reductants that occur in animal tissues such as the K-vitamins, co-enzyme Q and ubiquinone (Fox and Ackerman, 1968). These workers investigating the mechanism of the formation of nitric oxide myoglobin and the role of various reductants, determined that hydroquinone (R) reacted with nitrous acid to give R-NO which then released nitric oxide for reaction with metmyoglobin. The cleavage which releases nitric oxide is a one electron transfer, presumably resulting in the formation of a semiquinone. α-Tocopherol which possesses a chroman ring as well as the phenolic functional group probably functions in the same manner, causing reduction of the nitrite to nitric oxide.

Since it appeared that a reducing function was effective in blocking N-nitrosamine formation, a series of nitrogen and sulfur compounds was investigated. This system was composed of DMA (1 mM), $NaNO_2$ (5 mM), compound under investigation (5 mM) and 50 ml buffer solution and was heated at 69°C for 3 hr. A greater inhibition of the N-nitrosation reaction was achieved at pH 3.5 (Table 4). The very small amount of blocking by urea was expected since Mirvish et al. (1972) reported very low inhibition by urea of the N-nitrosation of morpholine and piperazine in the pH range 2–4. Increasing the pH to 5.5 also decreased the degree of inhibition. Ammonium sulfamate was very effective at the lower pH, 99% blocking being achieved. A very marked reduction in inhibition was obtained at pH 5.5 which again is supported by the data of Mirvish et al. (1972). Urea and ammonium sulfamate have been shown to react rapidly with nitrite and the sulfamate has been used to quench the N-nitrosation reaction prior to analysis (Issenberg and Tannenbaum, 1971).

Table 4–Effect of nitrogen and sulfur compounds on formation of N-nitrosamines in an aqueous system

Compound	% Inhibition (DMA → DMN) pH 3.5	pH 5.5
Urea	24.0	13.4
Ammonium sulfamate	99.0	63.1
Methionine	90.2	55.0
Glutathione	98.7	88.0
Cysteine	99.0	88.1
2-Mercaptoethanol	75.8	–

Table 5–Effect of NADH on N-nitrosamine formation in an aqueous system

Secondary amine	Conc of NADH (μM)	% Blocking
Dimethylamine	10	33.0
	25	59.6
	50	93.0
Pyrrolidine	10	28.0
	25	62.3
	50	93.1
Piperidine	10	33.1
	25	63.2
	50	91.0

Table 6–Effect of inhibitory reagents on N-nitrosamine formation in a low moisture CMC system

System	N-Nitrosopyrrolidine mg	% Conversion Proline → N-Pyr	% Blocking
Control	4.35	1.74	–
Ascorbic acid	0.02	0.008	99.5
Propyl gallate	0.02	0.008	99.5
L-cysteine	0.076	0.030	98.3

The inhibiting effect of the amino acids cysteine, glutathione and methionine also varied with the pH of the system (Table 4). The readily oxidized cysteine and glutathione gave essentially total inhibition at pH 3.5 but only 88% at pH 5.5. Mirna and Hofmann (1969) investigated the reaction of nitrite with sulfhydryl compounds in an acid medium and showed that the formation of the nitrosothiol derivative was pH dependent. With glutathione, no reaction took place at pH 7.4, even after heating for 15 min at 100°C; at pH 5.0, a small glutathione-SH consumption was observed but at pH 2.3 at room temperature, practically all the glutathione-SH was consumed. Fox and Ackerman (1968) estimated that the titratable reductant concentration in meat ranged as high as 100 mM. Free sulfhydryl groups have been found in the range 21–25 mM (Hamm and Hofmann, 1966; Hofmann, 1971) which is about the concentration of cysteine in meat (AMIF, 1971) and is more than adequate to account for total nitrite depletion.

Methionine which does not possess a free sulfhydryl group did not give as good an inhibitory effect as did cysteine or glutathione. Approximate inhibitions of 90 and 55% at pH 3.5 and 5.5 respectively were obtained. Since nitrite is a strong oxidant it is possible that the nitrite oxidized the amino acid to its sulfoxide or sulfone being itself reduced to nitric oxide.

2-Mercaptoethanol only gave a 75.8% inhibition at pH 3.5 which was probably due to its extreme volatility. Theoretically a complete inhibition, like that obtained for cysteine, would be expected at the lower pH.

The role of β-nicotinamide adenine dinucleotide, reduced form, (NADH) as a blocking agent was also investigated in an aqueous system containing secondary amine (50 μM), $NaNO_2$ (50 μM) in the biphthalate buffer (pH 3.5). The system was heated for 12 hr at 50°C. Table 5 shows that NADH at 50μM was an effective blocking agent. NAD is present in tissues ranging from 0.5–1.0M but it is not known what proportion is in the reduced form under the specified conditions (Fox and Nicholas, 1974).

Since the concentrations of the reactants used in the majority of experiments were much greater than generally encountered in most food systems, the inhibitory effect of ascorbic acid, bisulfite and ammonium sulfamate was investigated using much smaller concentrations. In a system containing 25 μM DMA, 25 μM $NaNO_2$ and 25 μM of the compound under investigation in a biphthalate buffer system (pH 3.5), inhibition of 98.9–99.5% was achieved.

The reaction between proline and $NaNO_2$ at high temperatures in a low moisture CMC system has been shown to produce N-Pyr (Ender and Ceh, 1971; Gray and Dugan, 1975). A CMC system containing proline (2.5 mM), $NaNO_2$ (12.5 mM) and 12.5 mM of the compound under test (ascorbic acid, cysteine, and propyl gallate) was slurried in biphthalate buffer, pH 3.5, freeze dried and then heated at 185°C for 45 min. The temperature used was optimum for the formation of N-Pyr (Gray and Dugan, 1975). Table 6 shows that the three compounds investigated were very effective at blocking N-Pyr formation in this system which involves a heat stress.

An interesting effect was noted in the formation of N-Pyr in a system containing corn oil and buffer solution. The sys-

tem contained 15g corn oil, 65 ml biphthalate buffer (pH 3.5), pyrrolidine (1 mM), $NaNO_2$ (5 mM) and 5 mM ascorbic acid or monotertbutylhydroquinone (TBHQ) and propyl gallate or α-tocopherol. When the latter compound was used, no blocking of the N-nitrosation reaction occurred, apparently due to tocopherol being completely isolated in the oil phase and unable to affect the nitrite-pyrrolidine reaction. On the other hand, ascorbic acid, TBHQ and propyl gallate gave 95–99% inhibition. The solubilities of TBHQ and propyl gallate in corn oil are low, TBHQ being only 10% soluble at 25°C while propyl gallate is insoluble at the same temperature. These two antioxidants also have slight solubilities in water which was improved when 15 ml ethanol were added to the system (Eastman Chemical Products Inc. Technical Bulletin).

No N-Pyr was detected in the oil phase even when no reductant was used. When 5 mg N-Pyr was added to the oil-buffer system and shaken at 69°C for 3 hr, no N-nitrosamine was detected in the oil layer. Although N-Pyr is fat soluble, a 100% partition in the aqueous phase was observed in this study.

The emulsifying properties of soluble starch was also employed to give a homogeneous system with oil and water. This system was agitated on a mechanical stirrer for 4 hr at 50°C and at room temperature for 8 hr before analysis. Approximately 99% inhibition was achieved with α-tocopherol which supports the previous conclusions about the oil-water systems. Similar inhibition was achieved with ascorbic acid, propyl gallate and butylated hydroxytoluene.

CONCLUSIONS

THESE STUDIES have shown that any compound which can react with a nitrite can be utilized to at least partially inhibit the N-nitrosation reaction between secondary amine and sodium nitrite. Reducing agents such as cysteine and glutathione, endogenous to meat systems and ascorbic acid which is added to frankfurters (547 ppm) could be utilized to reduce N-nitrosamine formation. These reducing compounds would also serve to preserve the red color of minced meat or sausages. Rikert et al. (1957) have discussed the use of cysteine and ascorbic acid in reducing metmyoglobin, despite their two electron transfer mechanism. Bauernfeind and Pinkert (1970) also praised the use of ascorbic acid in meat as it lowers the pH of the system, thereby benefitting the rate of curing.

The tocopherol, propyl gallate and TBHQ studies indicate that the phenolic functional groups are also capable of blocking the N-nitrosation reaction. When a single antioxidant is added to a food, it must not exceed 0.01% (100 ppm), based on the fat content of the food. Since frankfurters contain about 30% fat, it is possible that the incorporation of antioxidant in the frankfurter emulsion could play an important role in preventing excess nitrite from reacting with available secondary amines. Simon et al. (1973) have shown that BHA and BHT do not adversely affect the flavor when added to all-meat frankfurters.

REFERENCES

AMIF. 1971. Meat Sci. Rev. 5, American Meat Institute Foundation.

Bauernfeind, J.C. and Pinkert, D.M. 1970. Food Processing with added ascorbic acid. Adv. Food Res. 18: 220.

Bogovski, P., Castegnaro, M., Pignatelli, B. and Walker, E.A. 1971. The inhibiting effect of tannins on the formation of nitrosamines. Presented at the International Agency for Research on Cancer and the Deutsches Krebsforschungszentrum Joint Meeting on the Analysis and Formation of Nitrosamines, Heidelberg, Oct., 1971.

Cho, I.C. and Bratzler, L.J. 1970. Effect of sodium nitrite on flavor of cured pork. J. Food Sci. 35: 668.

Cram, D.J. and Hammond, G.S. 1959. "Organic Chemistry," 2nd ed., p. 651. McGraw and Hill.

Duncan, C.L. and Foster, E.M. 1968. Role of curing agents in the preservation of shelf-stable canned meat products. Appl. Microbiol. 16: 401.

Ender, F. and Ceh, L. 1971. Conditions and chemical reaction mechanisms by which nitrosamines may be formed in biological products with reference to their possible occurrence in food products. Z. Lebensm.-Unters. u. Forsch. 145: 133.

Fan, T.Y. and Tannenbaum, S.R. 1973. Natural inhibitors of nitrosation reactions: The concept of available nitrite. J. Food Sci. 38: 1067.

Fiddler, W., Pensabene, J.W., Doerr, R.C. and Wasserman, A.E. 1972. Formation of N-nitrosodimethylamine from naturally occurring quaternary ammonium compounds and tertiary amines. Nature 236: 307.

Fiddler, W., Pensabene, J.W., Peotrowski, I.G., Doerr, R.C. and Wasserman, A.E. 1973. Use of sodium ascorbate or erythorbate to inhibit formation of N-nitrosodimethylamine in frankfurters. J. Food Sci. 38: 1084.

Fox, J.B. and Ackerman, S.A. 1968. Formation of nitric oxide myoglobin: Mechanisms of the reaction with various reductants. J. Food Sci. 33: 364.

Fox, J.B. and Nicholas, R. 1974. Nitrite in meat. Effect of various compounds on loss of nitrite. J. Agr. Food Chem. 22: 302.

Gray, J.I. and Dugan, L.R. 1974. Formation of N-nitrosamines in low moisture systems. J. Food Sci. 39: 474.

Gray, J.I. and Dugan, L.R. 1975. Formation of N-nitrosopyrrolidine from proline and collagen. J. Food Sci. 40: 484.

Gruger, E.H. 1972. Chromatographic analyses of volatile amines in marine fish. J. Agr. Food Chem. 20: 781.

Hamm, R. and Hofmann, K. 1966. Schwefelhaltige Verbindungen des Freisches. 3. Mitterlung Bestimmung von Sulfydrylund Disulfid-Gruppen in Myofibrillen und Muskelgewebe mit Hilfe der amperometrischen Titration. Z. Lebensm. Untersuch. und Forsch. 130: 133.

Hofmann. K. 1971. Bestimmung von SH-Gruppen im Muskelgewebe durch indirekte amperometrische Titration mit Sibernitrat unter Anwendung von Kaliumjodid. A. Lebensm. Unters. u. Forsch. 147: 68.

Issenberg, P. and Tannenbaum, S.R. 1971. Approaches to the determination of volatile and nonvolatile N-nitroso compounds in food and beverages. Presented at the International Agency for Research in Cancer and the Deutsches Krebsforschungszentrum Joint Meeting on the Analysis and Formation of Nitrosamines, Heidelberg, Oct., 1971.

Knowles, M.E. 1974. Nitrosation of phenols in smoked bacon. Nature 249: 672.

Lijinsky, W. and Epstein, S.S. 1970. Nitrosamines as environmental carcinogens. Nature 225: 21.

Mirna, A. and Hofmann, K. 1969. Uber den Verbleib von Nitrit in Fleischwaren. 1. Umsetzung von Nitrit mit Sulfydryl-Verbindungen. Fleischwirtschaft 49: 1361.

Mirvish, S.S., Wallcave, L., Eagen, M. and Shubik, P. 1972. Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitroso compounds. Science 177: 65.

Mohler, K. and Hallermayer, E. 1973. Formation of nitrosamines from lecithin and nitrite. Z. Lebensm. Unters. u. Forsch. 151: 52.

Phillips, W.E.J. 1968. Naturally occurring nitrate and nitrite in foods in relation to infant methaemoglobinaemia. Food Cosmet. Toxicol. 9: 219.

Preusser, E. 1966. Aliphatic amines in the seeds of fruits and in germinating plants of Vicia faba and Zea mays. Biol. Zbl. 85: 19.

Rikert, J.A., Bressler, L., Ball, C.D. and Steir, E.G. 1957. Factors affecting quality of prepackaged meat. 2. Color studies B. Effects of storage time, storage temperature, antioxidants, bacteria, light, freezing and fat upon color of product. Food Technol. 11: 567.

Roberts, A.C. and McWeeny, D.J. 1972. The use of sulphur dioxide in the food industry: a review. J. Food Technol. 7: 221.

Sebranek, J.G. and Cassens, R.G. 1973. Nitrosamines: a review. J. Milk Food Technol. 36: 76.

Simon, S., Willis, D.E., MacDonald, B.D., Miller, D.G., Waldman, R.C. and Wasterberg, D.O. 1973. Influence of nitrite and nitrate curing ingredients on quality of packaged frankfurters. J. Food Sci. 38: 919.

Sinios, A. and Wodsak, W. 1965. Die Spinatvergiftung des Sauglings. Dt. med Wschr. 90: 1856.

Stewart, I., Newhall, W.F. and Edwards, G.J. 1964. The isolation and identification of l-synephrine in the leaves and fruits of citrus. J. Biol. Chem. 239: 930.

Wasserman, A.E. and Talley, F. 1972. The effect of sodium nitrite on the flavor of frankfurters. J. Food Sci. 37: 536.

Wick, E.L., Underriner, E. and Paneras, E. 1967. Volatile constituents of fish protein concentrate. J. Food Sci. 32: 365.

Ms received 2/23/75; revised 5/24/75; accepted 5/28/75.

Michigan Agricultural Experiment Station Journal Article #7143.

Investigation supported in part by research grant FD-00364 from the Food and Drug Administration of the U.S. Department of Health, Education and Welfare.

APPLIED SCIENCE and ENGINEERING

E.A. MULLEY,[1] C.R. STUMBO and W. M. HUNTING
Dept. of Food Science & Nutrition, University of Massachusetts, Amherst, MA 01002

KINETICS OF THIAMINE DEGRADATION BY HEAT.
A new method for studying reaction rates in model systems and food products at high temperatures

INTRODUCTION

ONE OF THE FIRST recorded properties of thiamine, the antineuritic vitamin, was its relative ease of destruction by heat. Williams (1938) reported that heating of thiamine in neutral solutions resulted in cleavage of the molecule at the methylene bridge to yield pyrimidine and thiazole fragments. This was confirmed by Watanabe (1939a) who, after heating aqueous thiamine solutions at 248°F, identified the breakdown products to be 2-methyl-5-hydroxymethyl-6-amino pyrimidine hydrochloride and 4-methyl-B-hydroxyethyl thiazole.

The most reliable and satisfactory method of approaching vitamin B_1 destruction is through simple reaction kinetics (Farrer, 1955). In order to accurately study the effects of a heat treatment, it is desirable to obtain nearly instantaneous and uniform heating to the required temperature, a definite holding time at the required temperature, and nearly instantaneous cooling to room temperature. However, in all the kinetic studies to date, small-sized containers were used in which the food mass reached the desired temperature only after a lag period.

A search of the literature indicates that there is an almost complete lack of data on the effect of heat on thiamine at temperatures above 250°F. Not only are the available data limited to temperatures of 250°F and below, but they are incomplete and in some cases, contradictory. Such a situation indicated the need for a comprehensive study of the thermal destruction characteristics of thiamine both in buffered solutions and in food systems over a temperature range which is being used in the food industry today.

The objective of this study was to determine the rates of destruction of thiamine hydrochloride in phosphate buffer (pH 6.0) and selected low-acid foods over the temperature range 250–280°F using a thermoresistometer. The low-acid food systems chosen were pea puree, beef puree and peas-in-brine puree.

EXPERIMENTAL

Preparation of samples

Buffer system. A stock thiamine solution was prepared by dissolving 200 mg U.S.P. crystalline thiamine chloride hydrochloride (which had been dried for at least 24 hr over phosphorus pentoxide in a desiccator) in 10 ml of 25% (v/v) ethanol. The buffered solution was made by diluting 50 μl of the stock thiamine to 25 ml with 1/10M phosphate buffer (pH 6.0).

Pea puree system. A 15% pea puree was prepared by liquidizing 30g frozen green peas with 50 ml distilled water in an omnimixer and then making the volume up to 200 ml with the same solvent. A thiamine-fortified system has obtained by dissolving 80 mg thiamine hydrochloride in 50 ml of the diluted puree.

Beef puree system. An oven roast type of beef, purchased locally, was trimmed of excess fat and connective tissue and frozen and 15% beef puree prepared. A thiamine-fortified system was then obtained in a way similar to the pea puree system.

Peas-in-brine puree system. 50g frozen green peas were homogenized with 50 ml of 2% brine. A fortified system was prepared by diluting 1 ml of a stock thiamine solution (37.5 mg thiamine hydrochloride in 25 ml of 25% ethanol) to 25 ml with the peas-in-brine puree.

Thermal processing system. In 1948, Stumbo designed a thermoresistometer for studying the resistance of bacterial spores to temperatures in the higher range, from 220°F upward. Using this equipment, virtually instantaneous heating and cooling is obtainable, so that losses of thiamine during come-up times would have a negligible effect on the accuracy of the results. Also, heating times may be readily reproduced to within 0.0005 min and heating temperatures inside the chambers may be controlled to within ± 0.3F° (Stumbo, 1948).

Preliminary runs using the thermoresistometer in the conventional way (i.e., for the purpose of studying the heat resistance of spore suspensions), gave rise to variable results. Feliciotti (1955) encountered similar problems when he attempted to use the Pflug thermoresistometer to study the kinetics of thiamine degradation in buffer solutions and low-acid foods.

Since conventional methods were not successful, two modifications were employed in using the thermoresistometer for our purposes:

(1) Paper discs of 0.25 in. (0.625 cm) diameter were placed in the aluminum cups before the samples were delivered on to them. The discs were used to prevent any possible spattering of the sample which might have resulted from the sudden drop in pressure when the central chamber was exhausted at the end of the heating period; and

(2) At the conclusion of heating the plate on which the samples were placed was backed up to the entrance port and only the cup containing the paper disc and sample was picked up with a pair of forceps and dropped into a test tube containing the desired diluent. The boats, used for carrying the cups, were picked off the plate and discarded.

Three to six replicates, consisting of 20 μl each of the test solution, were simultaneously subjected, in the thermoresistometer, to each of the selected time-temperature treatments. Control samples, which were not heated, were handled in a similar way to the test ones.

[1] Present address: Nutrition Dept., General Mills, Inc., 9200 Wayzata Blvd., Minneapolis, MN 55440

Thiamine analysis

The "thiochrome method" for thiamine assay described by the Association of Vitamin Chemists (1966) was used for all thiamine analyses. Thiochrome procedures depend upon the oxidation of thiamine to thiochrome which fluoresces in UV light. Under standard conditions and in the absence of other fluorescing substances, the fluorescence is proportional to the thiochrome present and hence to the thiamine originally in solution.

Treatment of data

The order of the degradation reaction of thiamine by heat in the various systems under study was ascertained graphically by plotting different functions of the concentration against time of heating at constant temperature. A first order reaction was characterized by a straight line when the logarithm of the concentration was selected as the function in question. This plot yielded a rate of destruction curve. The reciprocal slope of this curve, designated by the letter 'D' represents the time required to destroy 90% of the material originally present. From the 'D' values obtained at different temperatures, it was possible to derive a thermal destruction (TD) curve. A curve of this type, obtained by plotting the logarithm of the 'D' values against the corresponding temperatures (where 'z' represents the slope and is the temperature difference effecting a tenfold change in 'D'), defines the thermal destruction characteristics at a given temperature as well as the sensitivity of the material to changes in temperature.

RESULTS & DISCUSSION

THE RATE OF DESTRUCTION curves for thiamine hydrochloride at 250, 260, 270 and 280°F in phosphate buffer (pH 6.0) and in pea, beef and peas-in-brine purees (as a semilog plot of thiamine retained vs. heating time at constant temperature) may be seen in Figures 1 through 4. The solid lines represent the portions of the curves fitted by linear regression. The extremely good fit of the line to the experimental points is strong evidence that the thermal destruction of thiamine in the systems under study is first order in nature.

Table 1 shows the times for 90% destruction (D values) of thiamine hydrochloride in phosphate buffer (pH 6.0) and in pea puree, beef puree and peas-in-brine puree at 250, 260, 270 and 280°F. An examination of the results reveals that the thermal lability of thiamine increases with rising temperature and that thiamine is more stable to heat in the food systems studied than it is in the phosphate buffer system.

Figure 5 gives the thermal destruction time curves for thiamine hydrochloride in the buffer and food systems as plots of the logarithm of the 'D' values against the corresponding temperatures of exposure. These curves are also the result of computerized regression analyses. The 'z' values (temperature difference in F° effecting a tenfold change in 'D') so generated range from 45 F° for phosphate buffer to 49F° for peas-in-brine puree. The temperature sensitivities of pea and beef purees are characterized by a 'z' of 48F°.

Various groups of workers who have studied the effect of temperature on the destruction of thiamine have discussed their results in terms of the Arrhenius equation, namely:

$$\log_{10} k = (-E_a/2.303R)1/T + C$$

in which k is the reaction rate constant, T the absolute temperature, R the gas constant in calories, E_a which is often defined as the energy of activation, a quantity characteristic of the

Table 1—Times for 90% destruction (D values) of thiamine hydrochloride in phosphate buffer (pH 6.0), pea puree, beef puree and peas-in-brine puree at temperatures between 250° F and 280° F

	Temperature			
	250° F D,min	260° F D,min	270° F D,min	280° D,min
Phosphate buffer	156.8	93.0	54.0	34.3
Pea puree	246.9	197.9	104.4	61.3
Beef puree	254.2	160.4	91.8	62.7
Peas-in-brine puree	226.7	145.4	76.1	59.1

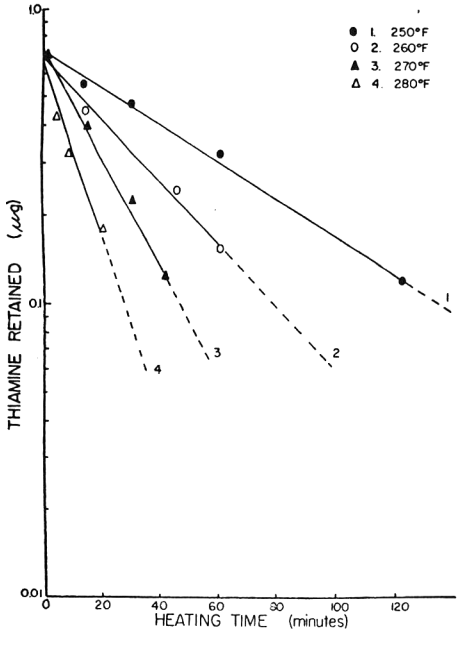


Fig. 1—Rate of destruction curves for thiamine hydrochloride in phosphate buffer (pH 6.0).

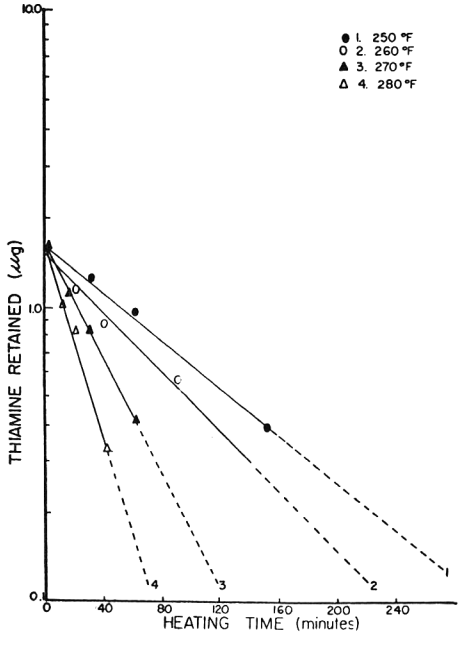


Fig. 2—Rate of destruction curves for thiamine hydrochloride in pea puree.

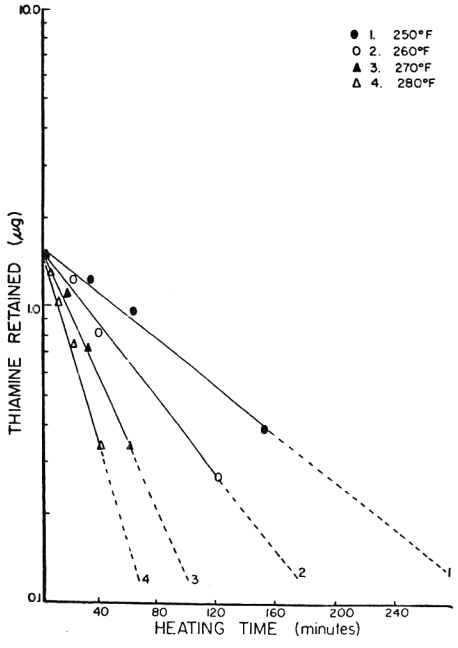


Fig. 3—Rate of destruction curves for thiamine hydrochloride in beef puree.

reaction that some believe to have the dimensions of energy, and C a constant of integration. Conformity with this equation is shown graphically by the linearity of the curve when log k is plotted against the reciprocal of the absolute temperature. From the slopes of the curves (which have been generated as described before) it was possible to calculate E_a for the thermal breakdown of thiamine in the four systems. Values of E_a so obtained ranged from 27.0 kcal/mole for peas-in-brine puree to 29.4 kcal/mole for phosphate buffer. The E_a values for beef puree and pea puree were intermediate, being 27.4 and 27.5 kcal/mole, respectively.

The fact that the thermal destruction of thiamine hydrochloride in buffered solutions follows a first order reaction rate is in accordance with the observations of several earlier authors (Watanabe, 1939a; Beadle et al., 1943; Farrer, 1941, 1945a). In the case of food products, however, deviations from first order reactions have been observed (Bendix et al., 1951).

The failure of some workers to obtain straight line relationships for the thermal degradation of thiamine over the entire heating interval because of initial irregularities might have been due to the fact that the equipment which they used to obtain their kinetic data could not be operated under ideal conditions. The inclusion of lag correction factors for come-up and cooling times may have introduced errors into the mathematics of the method. The extent of the control which the operator has over the thermoresistometer to ensure minimal lag periods and accurate holding times at the desired temperature are reflected in the consistency of the results which were obtained.

Some workers have noted that oxygen affects the first order reaction rate of thiamine destruction. They obtained straight line destruction rate curves only after oxygen was excluded from the reaction vessel (Farrer and Morrison, 1949; Sabri et al., 1968). Our observations, on the other hand, lend support to the conclusions of those authors who state that the destruction of thiamine is thermal—not oxidative (Williams and Spies, 1938; Farrer, 1955). Further study may be necessary to resolve this apparent anomaly regarding the influence of redox potential on thiamine degradation.

The present results were obtained only after a slight modification was included in the way in which the thermoresistometer was operated. It was postulated that, in the original method, that is, for obtaining the heat resistance of bacterial spores, copper ions, from the bronze conveyor plate, were contaminating the thiamine solutions and giving rise to inconsistent and erroneous results.

Booth (1943) was the first to recognize the influence of copper ions in accelerating the rate of destruction of thiamine at 212°F. Tanaka (1966a, b) successfully crystallized a copper-thiamine complex having an empirical composition of C12H16-1702N4SCu and in a further study (Tanaka, 1969) reported that the thiamine-copper complex showed thiamine decomposing power nearly corresponding to an equimolar concentration of copper ions. He suggested that, in a thiamine solution contaminated with copper, alternate formation and degradation of thiamine-copper complexes may occur and that this may be the cause of the gradual decomposition of thiamine by copper. In studying the influence of copper on the rate of destruction of aneurin in buffer solutions at 100°C, Farrer (1947) concludes: "In the absence of any indication of an oxidation of aneurin, there appears to be no simple explanation for the mechanism of the accelerating effect of copper."

The observation that thiamine in natural foods is more heat-resistant than thiamine in aqueous and buffered solutions indicates the existence of factors, other than heat, that can modify the reaction. McIntire and Frost (1944) showed that α and β amino acids and some of their derivatives have a marked stabilizing effect upon thiamine at pH 6.0. This effect became noticeable above pH 4.5–5.0 depending upon the concentration of thiamine and other factors and its magnitude increased with a decrease of acidity within the range studied, namely pH 4.5–7.0. Proteins are known to protect thiamine even though the protective mechanism involved has not been completely elucidated. Adsorption upon starch in foods may

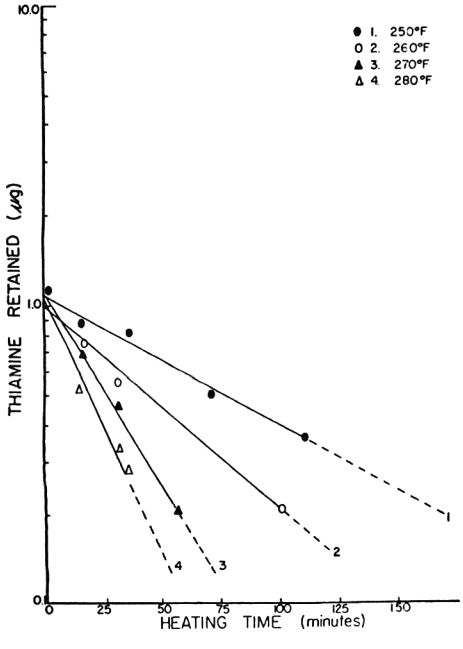


Fig. 4—Rate for destruction curves for thiamine hydrochloride in peas-in-brine puree.

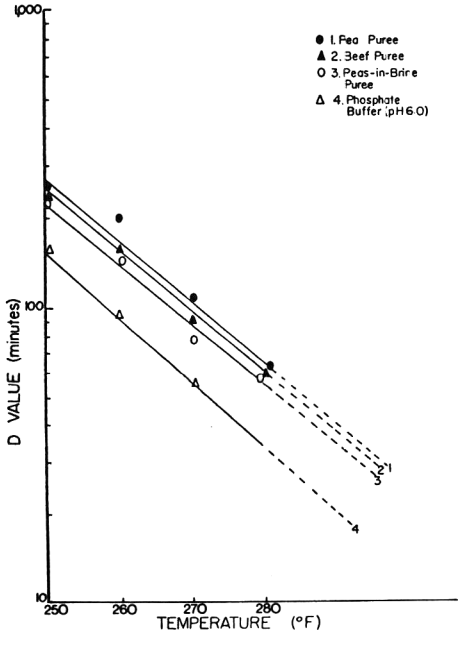


Fig. 5—Thermal destruction time curves for thiamine hydrochloride in buffer and purees.

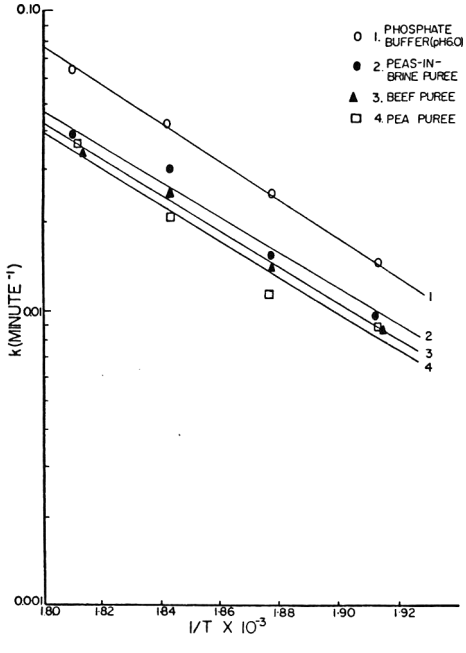


Fig. 6—Arrhenius plot for thermal degradation of thiamine hydrochloride.

also play an important role in causing the retention of thiamine during heating.

Variation in the proximate composition of foods does not seem to be of prime importance in accounting for differences in thiamine destruction rates (Feliciotti, 1955). The similarity in 'D' values for thiamine destruction in peas and beef appear to bear this out. The presence of sodium chloride in solution also does not appear to affect the rate of thiamine breakdown to any great extent.

Some investigators have attempted to explain the increased stability of thiamine in biological material in terms of the co-carboxylase form of the vitamin (Greenwood et al., 1943). Subsequent workers, however, have shown that co-carboxylase is a good deal less stable than thiamine (Booth, 1943; Lincoln et al., 1944; Farrer, 1945b). The effect of pH and form of the vitamin on the rate of thiamine destruction will be dealt with in a subsequent paper.

It is clear from the evidence available that the thermal destruction of vitamin B_1 in buffered and food systems can be followed by simple kinetic methods. First order reactions are relatively easy to follow and the result may be expressed as a rate constant, k; as a half-life time, $t_{1/2} = \log_e 2/k$; or as D, the decimal reduction time, $D = 2.303/k$. Whichever system is preferred, the result may then be compared with other results obtained under the same conditions. In addition, the kinetic approach is a neat and quantitative expression of the percentage retention (or loss) after a given time but, most of all, it is the first step in correlating losses with temperature as well as with time.

REFERENCES

Association of Vitamin Chemists. 1966. "Methods of Vitamin Assay," 3rd ed., p. 20. Interscience Publishers, New York.

Beadle, B.W., Greenwood, D.A. and Kraybill, H.R. 1943. Stability of thiamine to heat: Effect of pH and buffer salts in aqueous solutions. J. Biol. Chem. 149: 339.

Bendix, G.H., Heberlein, D.G., Ptak, L.R. and Clifcorn, L.E. 1951. Factors influencing the stability of thiamine during heat sterilization. Food Res. 16: 494.

Booth, R.G. 1943. The thermal decomposition of thiamine and co-carboxylase at varying hydrogen-ion concentrations. Biochem. J. 37: 518.

Farrer, K.T.H. 1941. The influence of pH on the destruction of aneurin at 100°. Australian Chem. Inst. J. and Proc. 8: 113.

Farrer, K.T.H. 1945a. The thermal destruction of vitamin B_1. 1. The influence of buffer salts on the rate of destruction of aneurin at 100°. Biochem. J. 39: 128.

Farrer, K.T.H. 1945b. The thermal destruction of vitamin B_1. 2. The influence of buffer salts on the rate of destruction of co-carboxylase at 100°. Biochem. J. 39: 261.

Farrer, K.T.H. 1947. The thermal destruction of vitamin B_1. 3. The influence of copper on the rate of destruction of aneurin in buffer solutions at 100°. Biochem. J. 41: 162.

Farrer, K.T.H. 1955. The thermal destruction of vitamin B_1 in foods. Advs. Food Res. 6: 257.

Farrer, K.T.H. and Morrison, P.G. 1949. The thermal destruction of vitamin B_1: The effect of temperature and oxygen on the rate of destruction of aneurin. Austr. J. Exp. Biol. Med. Sci. 27: 517.

Feliciotti, E. 1955. The thermal destruction of thiamine in buffer solutions and low-acid foods in the temperature range 228° to 300°F. Ph.D. thesis, University of Massachusetts, Amherst.

Greenwood, D.A., Beadle, B.W. and Kraybill, H.R. 1943. Stability of thiamine to heat. 2. Effect of meat-curing ingredients in aqueous solutions and in meats. J. Biol. Chem. 138: 49.

Lincoln, H., Hare, E.L. and Harrel, C.G. 1944. The loss of thiamine in cooking breakfast cereals. Cereal Chem. 21: 274.

McIntire, F.C. and Frost, D.V. 1944. Thiamine stability: Effect of amino acids and related compounds and of thiamine concentration. J. Am. Chem. Soc. 66: 1317.

Sabri, M.E., George, P.A. and Mohan Rao, V.K. 1968. In vitro interaction between thiamine and ascorbic acid. Indian J. Technol. 6: 272.

Stumbo, C.R. 1948. A technique for studying resistance of bacterial spores to temperatures in the higher range. Food Technol. 2: 228.

Tanaka, A. 1966a. Complex compounds of thiamine and heavy metals. 2. An improved method of crystallization of thiamine copper compound and some properties of the crystal. Bitamin 33: 19.

Tanaka, A. 1966b. Complex compound of thiamine and heavy metals. 3. Chemical composition of a crystalline thiamine-copper compound. Bitamin 33: 497.

Tanaka, A. 1969. Complex compounds of thiamine and heavy metals. 5. Model study of the behavior of thiamine in metal poisoning. 1. Mechanism of decomposition of thiamine and its significance in copper poisoning. Bitamin 39: 330.

Watanabe, A. 1939a. Kinetics of the thermal decomposition of thiamine hydrochloride in aqueous solutions. J. Pharm. Soc. Japan 59: 218.

Watanabe, A. 1939b. Hydrolytic decomposition of vitamin B_1. Pharm. Soc. Japan 59: 500.

Williams, R.R. 1938. The chemistry of thiamine. J. Am. Med. Assoc. 110: 727.

Williams, R.R. and Spies, T.D. 1938. "Vitamin B_1." The MacMillan Company, New York.

Ms received 1/15/75; revised 3/21/75; accepted 3/22/75.

E.A. MULLEY,[1] C. R. STUMBO and W. M. HUNTING
Dept. of Food Science & Nutrition, University of Massachusetts, Amherst, MA 01002

KINETICS OF THIAMINE DEGRADATION BY HEAT. Effect of pH and form of the vitamin on its rate of destruction

INTRODUCTION

THIAMINE occurs in natural foods and other biological material either in the free form or in a combined form as a protein complex, as a phosphorus-protein complex or as the pyrophosphoric acid ester, co-carboxylase (thiamine pyrophosphate). The relative amounts of any of these forms may vary in different types of products but remain relatively constant in any one product.

Thiamine in natural products is notably more resistant to heat than pure thiamine in aqueous solutions, thus indicating the existence of factors which can modify the reaction. Some investigators have attempted to explain the increased stability of thiamine in biological material in terms of the presence of the co-carboxylase form of the vitamin (Greenwood et al., 1943). However, Booth (1943), Lincoln et al. (1944), Farrer (1945b) and others later, agree that co-carboxylase is a good deal less stable than thiamine.

Feliciotti and Esselen (1957) after studying the thermal destruction rates of thiamine in pureed meats and vegetables suggested that its destruction in foods may be dependent on the inter-relationship of pH and the relative proportions of the 'free' and 'combined' form of the vitamin. Farrer (1955) is also of the opinion that, in meats, the main variable affecting thiamine losses is likely to be the proportion of co-carboxylase present. A study of the inter-relationship between pH and the form of the vitamin on its rate of destruction should provide insight into the justification for using thiamine hydrochloride as an index of the efficacy of heat processing.

EXPERIMENTAL

Preparation of samples

Stock solutions of thiamine hydrochloride and co-carboxylase were prepared by dissolving 37.5 mg of the chemical in 25 ml of 25% ethanol. In each case, the synthetic material had been dried for at least 24 hr over phosphorus pentoxide in a desiccator. Working solutions of thiamine hydrochloride and co-carboxylase were made up as follows:

100% thiamine hydrochloride (0% co-carboxylase):
1 ml stock thiamine hydrochloride diluted to 25 ml with buffer of desired pH

65% thiamine hydrochloride (35% co-carboxylase):
0.65 ml stock thiamine hydrochloride + 0.35 ml stock co-carboxylase diluted to 25 ml with buffer of desired pH

30% thiamine hydrochloride (70% co-carboxylase):
0.3 ml stock thiamine hydrochloride + 0.7 ml stock co-carboxylase diluted to 25 ml with buffer of desired pH

0% thiamine hydrochloride (100% co-carboxylase):
1 ml stock co-carboxylase diluted to 25 ml with buffer of desired pH

Phosphate buffer solutions (1/10M) of pH 4.5, 5.0, 5.5, 6.0 and 6.5 were used. The effect at hydrogen-ion concentrations of pH 7.0 and above was not investigated because thiamine is relatively unstable in neutral and alkaline solutions and foods possessing such pH values are not encountered frequently.

Thermal processing system

Stumbo's thermoresistometer (Stumbo, 1948) was used to conduct the kinetic studies on all the samples at each pH. Modifications adopted previously, as described earlier, were employed for this set of experiments also. Triplicates, consisting of 20 μl each of the test solution, were simultaneously subjected, in the thermoresistometer, to every time treatment at 265°F. Control samples, which were not heated, were handled in a similar way to the test ones.

Thiamine analysis

A micro-method for assaying thiamine in this series of experiments was developed. After heat processing, the test sample was collected in 2 ml 0.1N hydrochloric acid. The solution was transferred by rinsing with 2.7 ml HCl into a 50 ml stoppered centrifuge tube. Since synthetic chemicals were being employed, the acid extraction step was omitted. Enzyme hydrolysis was carried out with 0.3 ml freshly prepared enzyme suspension for at least 3 hr at 113–122°F. Conversion to thiochrome and measurement of fluroescence were done in the conventional way (Association of Vitamin Chemists, 1966). Blanks were run in every case.

Treatment of data

The kinetic rate data were obtained graphically by plotting the logarithm of the concentration against time at 265°F. This was done at each pH for thiamine hydrochloride and co-carboxylase as well as for mixtures of the two. The 'D' values obtained were plotted against pH in order to interpret the effect of the hydrogen ion concentration and the form of the vitamin on its rate of destruction.

RESULTS & DISCUSSION

THE RATE OF destruction curves at 265°F for thiamine hydrochloride, co-carboxylase and mixtures of the two in phosphate buffer at pH 4.5, 5.0, 6.0 and 6.5 may be seen in Figures 1 through 5. These curves are lines of best fit located by linear regression analysis.

At every pH, first order rates of reaction were observed for both thiamine hydrochloride and co-carboxylase as well as for mixtures of the two. The D values obtained from these curves are presented in Table 1. An examination of the results indicates that the rate of destruction is directly related to the amount of co-carboxylase present with the differences in degradation rates being magnified with a rise in pH. Lowering the pH from 5.0 to 4.5 does not appear to affect the times for 90% destruction for each system individually.

The sensitivity of thiamine to the action of alkali has been long recognized (Williams and Spies, 1938). The chemical properties of thiamine in relation to pH have been reviewed by Dwivedi and Arnold (1972). Williams and Ruehle (1935) first described the unusual behavior of thiamine chloride hydrochloride and other thiamine salts when titrated with base. The first step in this titration, with pK_1 at about 4.8, represents the titration of the protonated amino pyrimidine group (Structure I > II, Fig. 6). In the second titration step, with a midpoint at pH 9.2, two equivalents of base are taken up in a slow

[1] Present address: Nutrition Dept., General Mills, Inc., 9200 Wayzata Blvd., Minneapolis, MN 55440

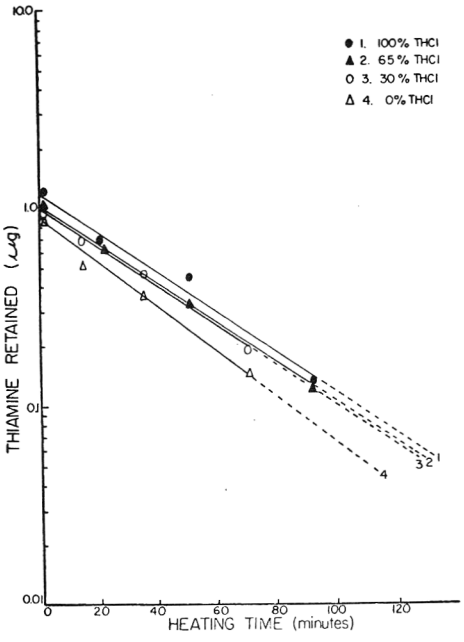


Fig. 1—Rate of destruction curves for thiamine hydrochloride and co-carboxylase in phosphate buffer (pH 4.5) at 265° F.

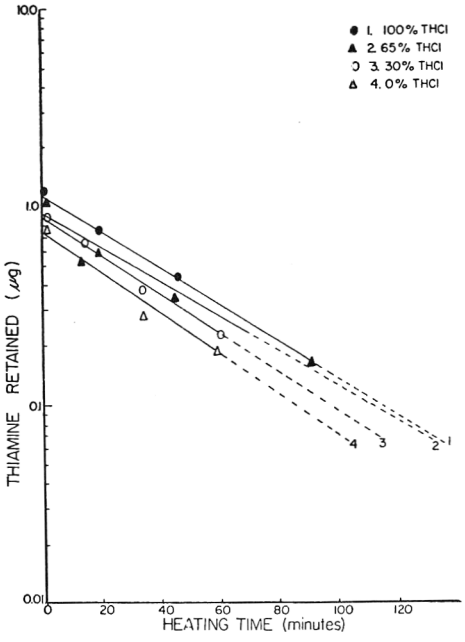


Fig. 2—Rate of destruction curves for thiamine hydrochloride and co-carboxylase in phosphate buffer (pH 5.0) at 265° F.

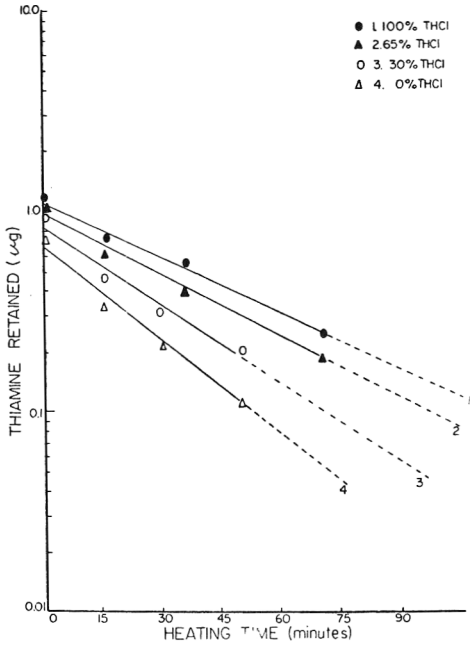


Fig. 3—Rate of destruction curves for thiamine hydrochloride and co-carboxylase in phosphate buffer (pH 5.5) at 265° F.

reaction with the formation of pseudo-base (Structure III). This intermediate undergoes a ring opening with further dissociation of a proton to give the ionized thiol form (Structure IV).

Molitor and Sampson (1936) stated that pure thiamine hydrochloride in aqueous solution at pH 3.5 may be heated to 248°F without undergoing decomposition. The fact that less thiamine breakdown occurred at pH 3.5 than at pH 5.0 or 6.0 suggests that the protonated form of thiamine (Structure I), which predominates at pH 3.5, is less prone to thermal destruction than is thiamine II. It has been observed that in the case of most amine salts, e.g. phenylhydrazine hydrochloride. the presence of the acid groups on the primary amine imparts increased stability to the molecule. (The pyrimidine moiety of the thiamine molecule has an amino group on carbon 6. This group endows the molecule with basic properties which are of great value in the preparation of the acid salt, thiamine chloride hydrochloride.) The mode of thiamine destruction ap-

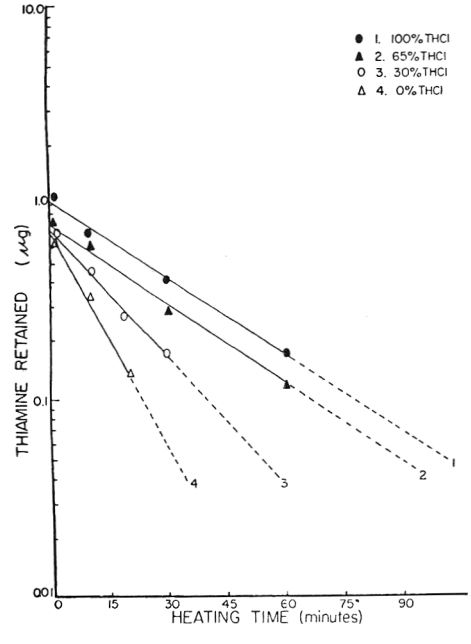


Fig. 4—Rate of destruction curves for thiamine hydrochloride and co-carboxylase in phosphate buffer (pH 6.0) at 265° F.

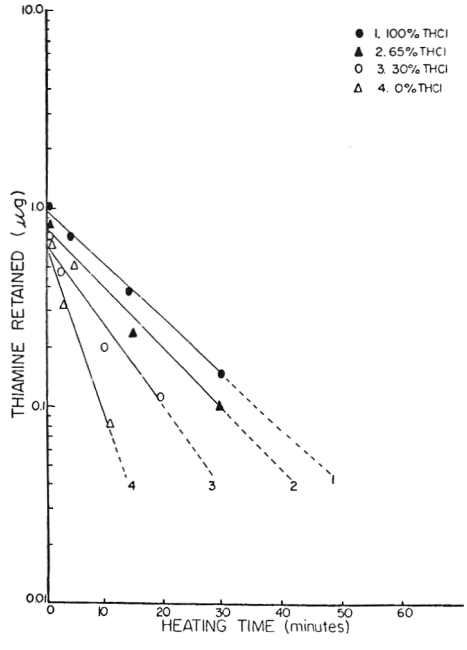


Fig. 5—Rate of destruction curves for thiamine hydrochloride and co-carboxylase in phosphate buffer (pH 6.5) at 265° F.

pears to be the same for both forms. The mechanism involves breaking of the C-N bond of the methylene "bridge" between thiazole and pyrimidine moieties of thiamine. A pyrimidine derivative, probably 2-methyl-4-amino-5-hydroxymethyl pyrimidine and 4-methyl-5-(B-hydroxyethyl) thiazole result from the breakdown (Dwivedi and Arnold, 1972).

The effects of pH on log D may be viewed diagrammatically in Figure 7. From this figure one may conclude that concentrations of co-carboxylase up to 35% in a thiamine hydrochloride-co-carboxylase mixture do not appear to influence the rate at which thiamine degrades at 265°F over the pH range of most food products (viz. pH 4.5–6.5). In drawing a curve through the points, it was observed that the data lends itself to interpretation by two straight lines. The changes in slope of the curves tend to occur at greater H-ion concentrations for co-carboxylase than for thiamine hydrochloride.

An examination of Figure 7 reveals that when the pH of the phosphate buffer solution exceeded 6.0, the stability of the thiamine molecule dropped suddenly as evidenced by the sharp decrease in the D value. Feliciotti (1955) hypothesizes that the pH of the phosphate buffer influences the thiamine molecule by neutralization of the hydrochloride. At a pH level below 6.2, the dynamic equilibrium of the solution results in the availability of partial protective action to the thiamine molecule. As the pH of the buffer was gradually increased, there was an equally gradual drop in the stability of thiamine. When the pH of the buffer solution went over 6.2 and the vitamin was completely without protective action, the stability of the molecule dropped suddenly, as indicated by the large increase in the k value. The basis of his reasoning relates to the potentiometric curve obtained when the vitamin was titrated against an equimolar solution of sodium hydroxide. Williams and Ruehle (1935) reported that the addition of an equimolar concentration of alkali resulted in the complete neutralization of the hydrochloride, thereby liberating the monochloride. This neutralization was found to be over at approximately pH 6.2, as indicated by the sudden rapid change in the pH of the solution.

In studying the influence of buffer salts on the rate of destruction of aneurin at 212°F Farrer (1945a) also observed that when log k was plotted against pH for each buffer solution, the curve for the Sorensen phosphate buffer consisted of two separate and distinct straight lines. However, that author explained the matter in terms of a change in the ionic constitution of the buffer medium.

In the case of co-carboxylase it may be seen that the change in slope in the curve log D vs. pH occurs at a lower pH than it does for thiamine hydrochloride. While there is no experimental evidence on which to rely, it might be hypothesized that the potentiometric titration curve of co-carboxylase with sodium hydroxide is shifted in such a way that the stabilizing

Table 1—D values for thiamine hydrochloride and co-carboxylase in phosphate buffer between pH 4.5 and pH 6.5 at 265° F

pH		0%	30%	65%	100%
	T-HCl	100%	70%	35%	0%
	CoCar	D,min	D,min	D,min	D,min
4.5		88.6	100.1	100.8	99.5
5.0		97.6	102.2	114.3	107.3
5.5		64.4	76.6	97.0	107.7
6.0		27.7	46.8	73.6	76.0
6.5		11.6	24.6	32.8	36.0

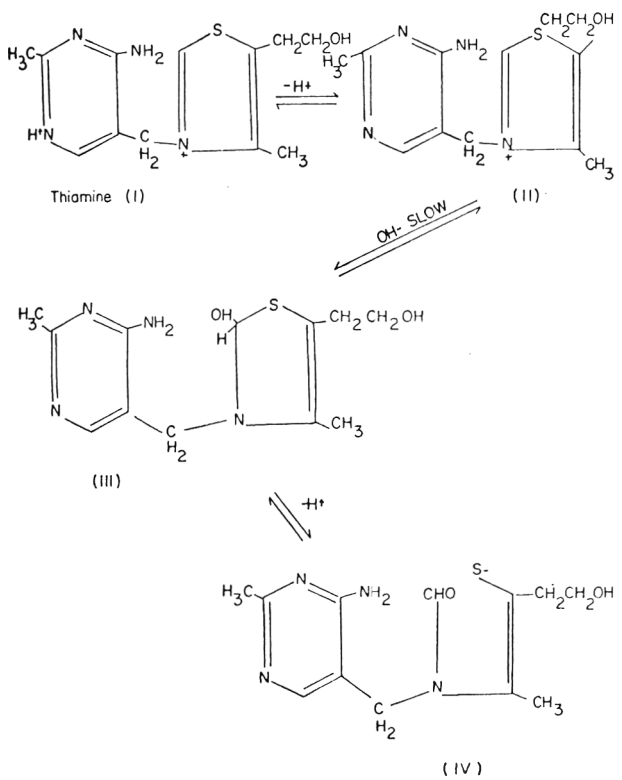


Fig. 6—Acid-base equilibria of thiamine.

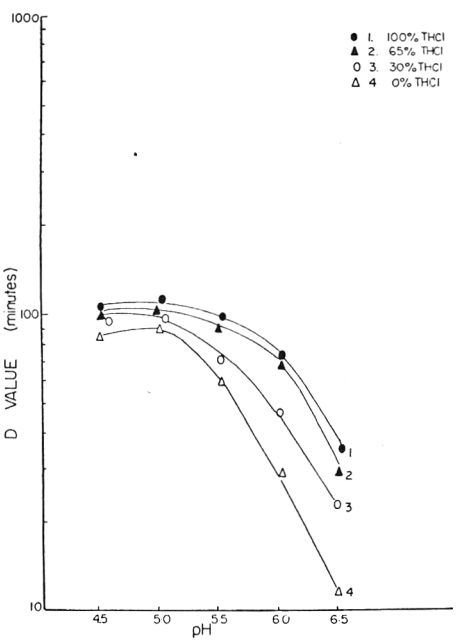


Fig. 7—Effect of pH on D values of thiamine hydrochloride and co-carboxylase in phosphate buffer.

influence on the molecule is removed at a lower pH than in the case of thiamine hydrochloride. However, it would appear that co-carboxylase concentrations of less than 35% in a thiamine hydrochloride-co-carboxylase mixture are without influence as far as altering the effect of pH on the rate of the thermal destruction of thiamine in phosphate buffer.

From this work it is apparent that, under identical heating conditions, co-carboxylase is destroyed more rapidly than thiamine hydrochloride. Similar findings were reported by Farrer (1945, 1949). The parallel behavior of thiamine hydrochloride and co-carboxylase suggests that the same influences operate and, by inference, that the faster destruction of co-carboxylase is linked with the pyrophosphoric acid group which constitutes the only difference between the two molecules and which would appear to strain the co-carboxylase molecule in some way.

When both forms of thiamine are present together, the increased lability of co-carboxylase becomes apparent only when its concentration in the mixture exceeds 35%. If the present results could be extended to food systems, it would appear that the presence of up to one-third of the thiamine as co-carboxylase will not affect the kinetics of the thermal destruction of this vitamin. (Review of available information reveals that most foods fall into this category.) However, all the forms of thiamine are not known since there are no available analytical methods for differentiating them. So, with the present knowledge, it appears that co-carboxylase, in an amount normally present in foods, will not influence the use of thiamine as an index of the sterilization efficacy of canning.

REFERENCES

Association of Vitamin Chemists. 1966. "Methods of Vitamin Assay," 3rd ed, p. 120. Interscience Publishers, New York.

Booth, R.G. 1943. The thermal decomposition of thiamine and co-carboxylase at varying hydrogen-ion concentrations. Biochem. J. 37: 518.

Dwivedi, B.K. and Arnold, R.G. 1972. Chemistry of thiamine degradation: Mechanisms of thiamine degradation in a model system. J. Food Sci. 37: 886.

Farrer, K.T.H. 1945a. The thermal destruction of vitamin B_1. 1. The influence of buffer salts on the rate of destruction of aneurin at 100°C. Biochem. J. 39: 128.

Farrer, K.T.H. 1945b. The thermal destruction of vitamin B_1. 2. The influence of buffer salts on the rate of destruction of co-carboxylase at 100°C. Biochem. J. 39: 261.

Farrer, K.T.H. 1949. The thermal destruction of vitamin B_1. Further studies on the influence of the concentration of buffer salts on the rates of destruction of aneurin and co-carboxylase. Austr. J. Exp. Biol. Med. Sci. 27: 591.

Farrer, K.T.H. 1955. The thermal destruction of vitamin B_1 in foods. Advs. Food Res. 6: 257.

Feliciotti, E. 1955. The thermal destruction of thiamine in buffer solutions and low-acid foods in the temperature range 228° to 300°F. Ph.D. thesis, University of Massachusetts, Amherst.

Feliciotti, E. and Esselen, W.B. 1957. Thermal destruction rates of thiamine in pureed meats and vegetables. Food Technol. 11: 77.

Greenwood, D.A., Beadle, B.W. and Kraybill, H.R. 1943. Stability of thiamine to heat. 2. Effect of meat-curing ingredients in aqueous solutions and in meats. J. Biol. Chem. 138: 49.

Lincoln, H., Hare, E.L. and Harrel, C.G. 1944. The loss of thiamine in cooking breakfast cereals. Cereal Chem. 21: 274.

Molitor, H. and Sampson, W.L. 1936. Merck's Jber. 50: 51.

Stumbo, C.R. 1948. A technique for studying resistance of bacterial spores to temperatures in the higher range. Food Technol. 2: 228.

Williams, R.R. and Ruehle, A.E. 1935. Studies of crystalline vitamin B_1. 2. Presence of quaternary nitrogen. J. Am. Chem. Soc. 57: 1856.

Williams, R.R. and Spies, T.D. 1938. "Vitamin B_1." The MacMillan Company, New York.

Ms received 5/31/74; revised 3/19/75; accepted 3/22/75.

E.A. MULLEY,[1] C. R. STUMBO and W. M. HUNTING
Dept. of Food Science & Nutrition, University of Massachusetts, Amherst, MA 01002

THIAMINE: A CHEMICAL INDEX OF THE STERILIZATION EFFICACY OF THERMAL PROCESSING

INTRODUCTION

THE EFFECT of the heat sterilization process for canned foods on the quality and nutrient retention of the food has been a major concern of food processors ever since Nicholas Appert first discovered the art of canning for food preservation in 1809, and one of the more recent advances in the food industry has been the development of means to predict the retention of these factors in thermal processing.

The microbial safety of food is the most important criterion for determining the heat process and, to date, the efficiency of the thermal process has been tested by microbiological methods. However, the occurrence of contamination during sterility testing of foods has been recognized and documented (Denny, 1970; 1972). The inherent limitations of microbiological methods in determining the efficacy of thermal processing and the time, tedium and expense associated with these methods have prompted the present investigation.

Theoretical approach

While several mathematical procedures have been developed to aid in the prediction of nutrient retention in thermal processing (Ball and Olson, 1957; Hayakawa, 1969; Teixeira et al., 1969), the method of Jen et al. (1971) has been adopted in this study since it is reasonably simple and versatile in application and, although it may be readily computer programmed, all calculations involved in its use are easily carried out manually.

In conduction-heating canned foods, the volume of concern in sterilization lies within an iso-j region (a region in which j, a lag factor, has the same numerical value) that encloses somewhat less than one-tenth of the total can volume (Stumbo, 1973). This is not true when considering heat-vulnerable quality factors—very significant amounts of these may remain in the outermost regions of the container (Greenwood et al., 1944). Therefore in determining the effects of heat on these factors, no portion of the container contents may be ignored, that is, accurate integration of data encompassing all regions of the container must be carried out.

According to Jen et al. (1971), for conduction-heating products in cylindrical containers, when the logarithm of (1-v) was plotted against $(F_\lambda - F_c)$ all points representing corresponding values of F_λ and v, from the center to the wall of the container could be represented by a straight line. The equation ascribed to this line was:

$$\ln (1-v) = m (F_\lambda - F_c)$$

in which: 1 = volume of container (taken as unity); v = volume enclosed by any iso-j region; F_λ = F value of heat received by any iso-j region ('F' being the equivalent in minutes at 250°F of all heat considered, with respect to its capacity to destroy spores or vegetative cells of a particular organism); and F_c = F value of heat received by the geometrical center of the container.

[1] Present address: Nutrition Dept., General Mills, Inc., 9200 Wayzata Blvd., Minneapolis, MN 55440

Starting with the basic integral which simply specifies that the fraction of any given heat vulnerable factor remaining in the entire container at the end of a process must equal the sum of the fractions remaining at all points in the container,

$$10^{-F_s/D_r} \cdot 1 = \int_0^1 10^{-F_\lambda/D_r} \cdot dv$$

where F_s is the integrated lethal value of heat received by all points in a container during process and D_r the time required at 250°F to destroy 90% of the spores or vegetative cells of a given organism, and taking v = 0.19 (when $j_\lambda/j_c = 0.5$ and $g_\lambda/g_c = 0.5$) as a point of reference on the straight line, the integral was solved by substituting equivalent values from the equation of the straight line. (j_λ is defined as the j value of the cooling curve for an iso-j region enclosing 0.19 of container volume, j_c is the j of the cooling curve for the geometrical center of the container; g_c is the difference, in Fahrenheit degrees between retort temperature and the maximum temperature reached by the food at the geometrical center of the container and g_λ is the difference in Fahrenheit degrees between retort temperature and the maximum temperature reached by the food at any point in the container other than the geometrical center.) The following use equation was obtained:

$$F_s = F_c + D_r \log \frac{D_r + 10.93 (F_\lambda - F_c)}{D_r}$$

This equation is valid only when values of j_λ and g_λ are taken as equal to one-half the values of j_c and g_c, respectively. The reason for this is only one of convenience in problem solving. For further discussion concerning the derivation and application of this equation, see Stumbo (1973).

After verification of the kinetics of thiamine destruction by heat in the products under study, the validity of the hypothesis was tested by actual retorting of the canned material to simulate processes used in commercial practice. Since a still-retort was used, heat effects over the entire container have been integrated as explained above.

The expected retentions of thiamine in the processed products were calculated by the procedure of Jen et al. (1971) while the actual amount of thiamine retained was analyzed by the thiochrome method (Association of Vitamin Chemists, 1966). The correlation between the expected and actual thiamine retentions formed the basis for testing the applicability of this method as an index of the sterilizing value of a thermal process.

EXPERIMENTAL

Preparation of samples

Pea puree. The puree was prepared from frozen peas purchased from a local food market which were quickly thawed by placing them in boiling water for 1–2 min. After draining off the water, they were placed in a Waring Blendor in convenient batches and blended until smooth. Thiamine hydrochloride, dissolved in a few milliliters of distilled water, was added to the pea puree at the rate of 0.16 mg per gram puree. It may be noted that the addition of thiamine served only as an

index of sterilizing efficacy and had no relation, in that sense, to nutritional considerations.) The puree was blended thoroughly to ensure uniform distribution of the thiamine (analyses conducted on random samples confirmed the homogeneity of the mixture). 450g of the fortified puree were placed in each 307 × 409 can for processing.

Beef puree. Oven roasts, purchased locally, were trimmed of excess fat and connective tissue and frozen until ready for use. A convenient portion was thawed when needed, cut into cubes, comminuted and then pureed in a Waring Blendor. Thiamine hydrochloride was added, after dissolution in a small amount of distilled water, at the rate of 0.16 mg thiamine hydrochloride per gram of puree. 450g of the puree were weighed out into each 307 × 409 cans after ensuring that the thiamine hydrochloride had been thoroughly mixed with the beef.

Peas-in-brine. Peas-in-brine was chosen as the convection heating product since pea puree was selected to represent conduction-heating products.

To prepare 'peas-in-brine,' batches of 300g each of frozen green peas were placed in boiling water for a minute or two after which the water was drained off. The batch was then transferred to a 307 × 409 can and 150 ml of thiamine hydrochloride-enriched 2% brine were poured over the peas. Thiamine hydrochloride was added in an amount such that a can of 'peas-in-brine' contained 0.06 mg thiamine hydrochloride per gram of product.

Thermal processing set-up

All retort processes were carried out at about 250°F since that is considered to be the reference temperature for establishing a thermal process for commercial sterility.

In order to evaluate the sterilizing value of a thermal process or to calculate the predicted thiamine retention, heat penetration data have to be obtained to determine the thermal diffusivity of the product under investigation. For heat penetration studies the larger 603 × 700 can was used, so that the heat conducted through the lead-in wires dissipated to the surrounding food material before it could affect the temperature at the thermocouple junction.

Commercially-available Eklund thermocouples were mounted through the wall of the container so that bulging of the can ends during retorting would not displace the thermocouple junction from the geometrical center of the can. Another thermocouple was placed on the outer wall of the can to record the processing temperature in the retort. The lead wires from the thermocouple were connected to a pre-calibrated, recording potentiometer in order to obtain a continuous strip-chart recording of the temperature history under the desired operating conditions. The heat penetration data obtained on the 603 × 700 can were mathematically converted for use in the process simulation studies on the 307 × 409 container as described by Olson and Jackson (1942).

For the actual canning studies in which the hypothesis was being tested, products were packed in 307 × 409 cans and still retorted to certain pre-set conditions.

For each product, three processes of differing sterilizing efficiences were set up by choosing various heating times at 250°F so as to span the entire f_h/U_{250} table for z = 48 (which was found to be the 'z' value for thiamine in the products studied). These processes were meant to represent most of the ones in current use for low-acid foods.

For any given thermal process, two 307 × 409 cans of product were used, with one of them being equipped with a thermocouple arrangement, so that a permanent record of the processing conditions could be maintained. Processed containers of product were kept at 2°C overnight, prior to analysis.

Thiamine analysis

In order to experimentally determine the thiamine retention associated with any one thermal process, each can of product was blended separately prior to sampling. Greenwood et al. (1944) demonstrated conclusively that a vitamin gradient exists from the outside to the center of a processed can of meat, since the heat treatment received by the product in the center is markedly different in degree from that received near the wall.

5g of the well-blended product were weighed into small glass beakers using an analytical balance. Duplicate analyses were performed on each can of processed product as well as on the control (unheated) sample. The weighed sample was then transferred to a 100 ml volumetric flask using 75 ml 0.1N HCl. Acid extraction and enzyme digestion were performed in every case. The incubated suspensions, after being diluted in volume with distilled water, were filtered. 100 µl of filtrate were pipetted into a 50 ml centrifuge tube along with 5 ml distilled water. (Duplicate analyses were conducted in each case.) Conversion to and measurement of thiochrome and calculation of the thiamine present were carried out in the usual way. Blanks were run simultaneously in all cases.

Treatment of data

Using data obtained from the strip-chart recording, heat penetration curves for each product were plotted according to the method proposed by Stumbo (1973). Heat penetration parameters generated from the heating curves were employed in calculating sterility and thiamine retention for each process time at 250°F for particular products. These manual calculations have been backed up using the computer.

Actual thiamine retentions were calculated directly from the mean assay values for thiamine. Percentage retentions were obtained by comparing the concentrations of thiamine in a processed sample with those of its respective control. These values were then checked with predicted retentions to ascertain the validity of the thesis.

RESULTS & DISCUSSION

HEAT PENETRATION CURVES for pea and beef purees in 603 × 700 cans at 252°F are presented in Figures 1 and 2. In the case of peas-in-brine, heat penetration data obtained on the 307 × 409 can were used to predict thiamine retentions. Heat penetration data generated with the 603 × 700 can gave rise to erroneous f_h values for the 307 × 409 container. This is not an unusual observation for convection-heating foods since the size of the container exerts a large influence on the f_h value by altering the extent to which product movement may occur within the can. Conduction errors with the shorter thermocouples would be minimal because of the rapid rate of heat transfer within the can of a convection-heating product. Figure 3 shows the heat penetration curve for peas-in-brine in the 307 × 409 can also "still-retorted" at 252°F. The f_h and thermal diffusivities generated by these curves and calculated according to the method described by Stumbo (1973) are given in Table 1.

An examination of the heat penetration data for pea puree and peas-in-brine reveals that the problem of obtaining heat transfer from the walls of a container to the center of the product in conventional heat processing is greater in the case of pea puree than it is with peas-in-brine because of the absence of a freely circulating, efficient, liquid heat-transfer medium. As a result, the total heat treatment required to sterilize canned pureed products is considerably more severe than that to which liquid-packed canned foods are subjected.

The percent thiamine retention for each thermal process along with the predicted thiamine retentions which were calculated on the basis of kinetic and heat penetration data are given in Tables 2, 3 and 4. In the case of pea puree and peas-in-brine, analyzed values for thiamine were not more than about five percent below the predicted retention. Also included in the same tables are the sterilizing values of the processes with regard to two types of bacterial spores (characterized by different D and z values) of public health and commercial importance. The reason for including this information is in order to get a feeling for the range of severities of the heat processes and to visualize where commercial procedures would fit into the whole scheme.

In the case of beef puree, even though the semi-log heating curve is a straight line (as it is with pea puree and peas-in-brine), the f_h value and thermal diffusivity are not in the range expected of conduction-heating foods. It appears that the greater thermal diffusivity and more rapid heat transfer were due to a channeling effect within the solid slab of pureed meat, caused by the formation of a broth or gravy within the can. Liquid separation was more pronounced with extended processing times.

Rice (1971) states that when meat or meat products are hermetically sealed in a container and processed by heat to destroy microorganisms and to denature the several enzyme systems naturally present, no change occurs in the proximate composition or energy value of the total content of the container. However, during the processing, some water and fat,

along with small amounts of soluble substances may separate from the solid meat to form a broth of gravy.

Changes in the structure of the meat fibers with temperature have been examined using phase contrast and electron microscopy. Giles (1969) showed that meat fiber shrinkage and sarcomere shortening were closely related and that there was very little change in either at cooking temperatures of 140°F, but large changes at temperatures of 158°F or higher.

It is known that temperature changes the solubility of meat proteins (Bouton and Harris, 1972). Hamm and Deatherage (1960) found that coagulation of the myofibrillar proteins began between 86 and 104°F and was nearly completed by 131°F. The sarcoplasmic proteins were almost completely denatured by 143.6°F (Bendall, 1964). Hamm and Deatherage (1960) and Hamm and Iwata (1962) have shown that these heat-induced changes in protein solubility relate to changes in the water-holding capacity of the meat.

In commercially processed meat products (e.g., luncheon meat), the presence of polyphosphates and other salts enhance the water-holding capacity of the meat so that there is no obvious separation of broth which would detract from the appearance of the product. The absence of added salts in the present study resulted in the loss of water-holding capacity of the meat proteins on exposure to elevated temperatures for extended lengths of time. This in turn gave rise to a product which did not heat purely by conduction. In such cases, it is inaccurate to apply mathematical methods used in this study to predict thiamine retention or bacterial inactivation. An examination of Table 3 demonstrates the result. The poor correlation between predicted and actual thiamine retentions for the most severe process is to be expected for the reasons already enumerated. What is surprising is the closeness between predicted and actual retentions in the two other cases which might lead one to assume that thiamine retention could be used as an index in predicting the sterilizing efficiency of a commercial process for strained beef, which would be of the order of the mildest process used in the present investigation. While such a thought is tempting, it is to be hoped that the dangers of such a course have been exposed.

Table 1—Results from heat penetration tests[a] on pea puree, beef puree and peas-in-brine

Product	f_h (min)	Thermal diffusivity (in.²/min)	j_{ch}	j_{cc}
Pea puree				
603 X 700 can	178.0	0.0151	1.6	2.0
307 X 409 can	56.8			
Beef puree				
603 X 700 can	117.0	0.0226	2.1	2.2
307 X 409 can	37.9			
Peas-in-brine				
307 X 409 can	6.0		1.0	1.0

[a] Heat penetration curves based on the original data are shown in Figures 1, 2 and 3.

The results with pea puree and peas-in-brine, as seen in Tables 2 and 4, confirm the thesis that thiamine retention can be used as a chemical index of the sterilization efficacy of the canning process for both conduction- and convection-heating food products. In all cases, the actual retention of thiamine was never more that 5% below the predicted value. The actual thiamine retention would agree with mathematical or computer predictions only if the value of each physical parameter, each rate constant and every processing condition were precisely equal to the values supplied as input data to the computer model or mathematical calculation. In biological systems it is unlikely that such precision can often be obtained.

One of the input parameters that could influence the predicted thiamine retention is the retort temperature. It is recognized that an error of ± 0.5° in retort temperature could remain undetected throughout the process. Likewise, the process time could be controlled only to within ± 0.5 min. Other parameters, like the thermal diffusivity and the degradation

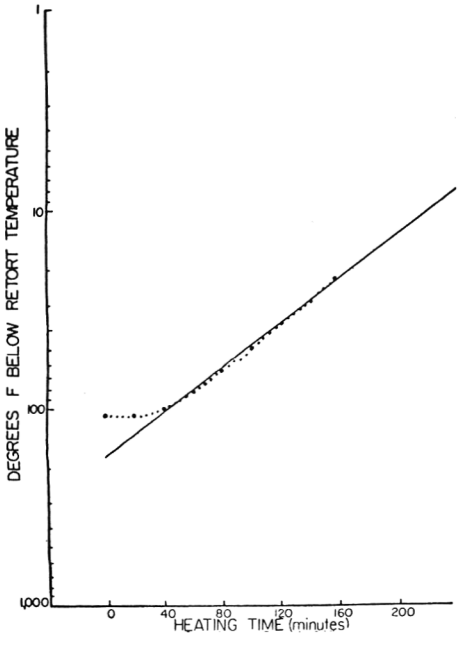


Fig. 1—Heat penetration curve for pea puree in 603 × 700 can "still-retorted" at 252° F.

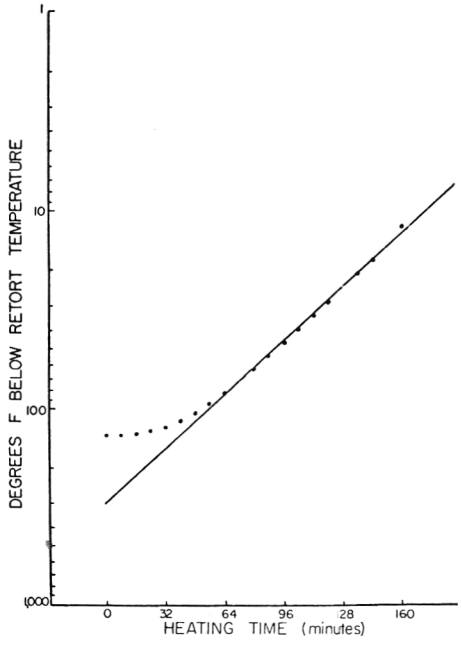


Fig. 2—Heat penetration curve for beef puree in 603 × 700 can "still-retorted" at 252° F.

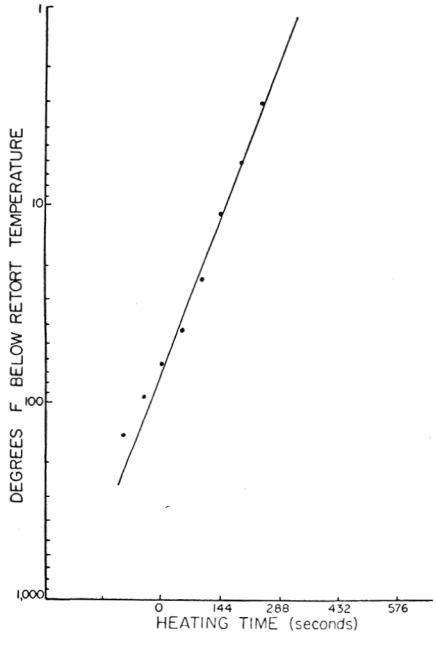


Fig. 3—Heat penetration curve for peas-in-brine in 307 × 409 can "still-retorted" at 252° F.

Table 2—Thiamine retention in pea puree processed in 307 X 409 cans at 252° F

F_s				
Sterility		Thiamine	Thiamine retention	
D = 0.2 z = 16	D = 3.2 z = 14	D = 234.0 z = 48	Predicted (%)	Actual (%)
15.4	17.6	66.4	52.0	48.3
1.6	3.6	31.6	73.3	71.1
		8.4	92.1	90.0

Table 3—Thiamine retention in beef puree processed in 307 X 409 cans at 252° F

F_s				
Sterility		Thiamine	Thiamine retention	
D = 0.2 z = 16	D = 3.2 z = 14	D = 243.5 z = 48	Predicted (%)	Actual (%)
34.7	37.8	71.4	50.9	40.6
14.9	17.4	50.8	61.9	57.7
2.7	4.6	27.7	77.0	76.6

Table 4—Thiamine retention in peas-in-brine processed in 307 X 409 cans at 252° F

F_s				
Sterility		Thiamine	Thiamine retention	
D = 0.2 z = 16	D = 3.2 z = 14	D = 190.8 z = 48	Predicted (%)	Actual (%)
27.2	28.3	25.6	73.4	69.7
16.7	17.2	16.4	82.1	79.8
3.5	3.3	5.7	93.4	88.0

rate of thiamine depend on experimental determinations and are susceptible to variations from observed values. Teixeira (1971) constructed a table to show the effect of each of these perturbations on the predicted thiamine retention. The results show a maximum possible variation of ± 6% in the thiamine retention predicted by the computer model for the remote possibility that all the errors are compounded in the same direction. He states: "The precision of the experimental analysis is of the same order of magnitude as the reliability of the computer model, and agreement between the two within ± 6% would establish the validity of the computer model." The maximum variation of +5% between the predicted and actual thiamine retentions is well within the permitted range and so confirms the validity of the thesis.

In conclusion, it may be reiterated that an approach, similar to the one described in this thesis, would be equally applicable to any factor that degrades by first order kinetics. The choice of index would depend on the amount of information available regarding its kinetics, the type of product being studied and the sterilization method in question. With the genesis of fresh knowledge, it should be possible to calculate heat inactivation curves for all thermolabile compounds and microorganisms in foods and to optimize a process for nutrient retention, enzyme inactivation and destruction of microorganisms.

In the present investigation, thiamine hydrochloride was blended uniformly with the contents of the entire container. The exposure of the chemical index to the constituents of the food material introduces several variables which might (or might not) have an effect on the kinetics of the degradative reaction. A set-up in which the indicator, contained in a pouch, could be placed at the slowest-heating point of a product before processing, so that steam (or electromagnetic radiation), but not the food constituents, could react with the index, represents a more efficient solution to the problem since, in that case, it would not be necessary to verify the degradation kinetics of the index in every product to be tested.

While the whole area of chemical indicators promises to be a fertile ground for research and patent hunters, its practical application may be just beginning. The authors believe that the use of a chemical index in sterilization processing has the potential of effecting a revolutionary change in the food and pharmaceutical industries.

REFERENCES

Association of Vitamin Chemists. 1966. "Methods of Vitamin Assay," 3rd ed, p. 120. Interscience Publishers, New York.

Ball, C.O. and Olson, F.C.W. 1957. "Sterilization in Food Technology." McGraw-Hill, Inc., New York, N.Y.

Bendall, J.R. 1964. "Meat Proteins: Proteins and Their Reactions," Ed. Schultz, H.W. and Anglemier, A.F., p. 225. Avi Publishing Co., Westport, Conn.

Bouton, P.E. and Harris, P.V. 1972. The effects of cooking temperature and time on some mechanical properties of meat. J. Food Sci. 37: 140.

Denny, C.B. 1970. Collaborative study of procedure for determining commercial sterility of low-acid canned foods. JAOAC 53: 713.

Denny, C.B. 1972. Collaborative study of a method for the determination of commercial sterility of low-acid canned foods. JAOAC 55: 613.

Giles, B.G. 1969. Changes in meat produced by cooking, p. 289. Fifteenth European Meeting of Meat Research Workers, Helsinki, Aug. 17—24.

Greenwood, D.A., Kraybill, H.R., Feaster, J.F. and Jackson, J.M. 1944. Vitamin retention in processed meat: Effect of thermal processing. Ind. Eng. Chem. 36: 922.

Hamm, R. and Deatherage, F.E. 1960. Changes in hydration solubility and changes of muscle proteins during heating of meat. Food Res. 25: 587.

Hamm, R. and Iwata, H. 1962. Chemische und physikalische veranderungen beim erhitzen von fleisch. 1. Wirkung des erhitzens in gegenwart von natriumchlorid auf wasserbingungsvermogen und pH-wert des rindermuskels. Z. Lebensm. Untersuch. Forsch. 116: 120.

Hayakawa, K. 1969. New parameters for calculating mass average sterilizing value to estimate nutrients in thermally conductive food. Can. Inst. Food Technol. J. 2: 165.

Jen, Y., Manson, J.E., Stumbo, C.R. and Zahradnik, J.W. 1971. A procedure for estimating sterilization of and quality factor degradation in thermally processed foods. J. Food Sci. 36: 692.

Olson, F.C.W. and Jackson, J.M. 1942. Heating curves: Theory and practical application. Ind. Eng. Chem. 34: 337.

Rice, E.E. 1971. Nutritional content and value of meat and meat products. In "Science of Meat Production," Ed. Price, J.F., 2nd ed, p. 287. Freeman, San Francisco, Calif.

Stumbo, C.R. 1973. "Thermobacteriology in Food Processing," 2nd ed. Academic Press, Inc., New York.

Teixeira, A.A. 1971. Thermal process optimization through computer simulation of variable boundary control and container geometry. Ph.D. thesis, University of Massachusetts, Amherst.

Teixeira, A.A., Dixon, J.R., Zahradnik, J.W. and Zinsmeister, G.E. 1969. Computer optimization of nutrient retention in the thermal processing of conduction-heated foods. Food Technol. 23: 845.

Ms received 5/31/74; revised 3/19/75; accepted 3/22/75.

CATHARINA Y. W. ANG, CHARLOTTE M. CHANG, A. E. FREY and G. E. LIVINGSTON
Food Science Associates, Inc., Dobbs Ferry, NY 10522

EFFECTS OF HEATING METHODS ON VITAMIN RETENTION IN SIX FRESH OR FROZEN PREPARED FOOD PRODUCTS

INTRODUCTION

ALTHOUGH the use of bulk packed prepared frozen foods is growing rapidly, there is a paucity of data on the comparative effects of various reconstitution methods on the retention of nutrients in such foods.

For individual serving portions, Causey and Fenton (1951a, b) compared the effects of reheating several frozen cooked vegetables and meat dishes using a Maxson oven, boiling water, a microwave oven, a household oven and a double boiler. They found that the average retention of ascorbic acid in vegetables was 70–82% and there were no significant differences among the reheating methods used. Little loss of thiamine was observed in any of the meat products using any of the reheating methods. However, different methods showed significant differences in thiamine retentions for some dishes. Other studies by Eheart (1969) indicated that frozen broccoli reheated in a "boil-in-bag" retained much more ascorbic acid than the freshly cooked or the frozen-pan reheated broccoli.

Kahn and Livingston (1970) studied the thiamine retentions in four frozen prepared food products reconstituted by three high speed heating methods. The average retentions of four products, i.e., beef stew, chicken a la king, shrimp newburg and peas in cream sauce, were 93.5% for microwave oven, 90% for infrared oven and 86% for the hot water immersion heating method. They also reported that hot-holding of freshly prepared products in hot water at 180°F (82.2°C) resulted in the retention of 78, 74 and 67% of thiamine after 1, 2 and 3 hr respectively. These studies were based on individual serving portion preparations and no statistical data were given. Earlier studies on the effect of hot-holding have been reviewed by Harris and Von Loesecke (1960) who stated that 94% of ascorbic acid and 66% of thiamine were lost after 3 hr steam table standing for a variety of vegetables.

Studying large scale preparation and reheating, the staff of the Catering Research Unit at the University of Leeds (1970) evaluated the nutrient content of a variety of hospital food items. They found that frozen prepared foods reheated in convection ovens in the "cook/freeze" system retained significantly higher amounts of ascorbic acid than foods in the conventional cooking system, but there were no significant differences in thiamine and riboflavin retentions. Slightly higher available lysine content was also reported for the "cook/freeze" system. Bowers and Fryer (1972) compared the retention of thiamine and riboflavin in cooked, cooked-reheated and cooked-frozen-reheated turkey muscles using gas and microwave ovens. Their results on a moisture-free, fat-free basis showed no significant differences in thiamine retention attributable to the two types of oven used, but a higher riboflavin content was found in muscle heated by gas as compared to muscle heated by microwave. Different treatments using the same oven had no significantly different effect on the retentions of thiamine and riboflavin.

The effects of microwave cooking on nutrient retention as reviewed by Livingston et al. (1973) and Ang and Livingston (1974) indicated that for some products, higher retentions of thiamine, riboflavin and ascorbic acid were obtained with the microwave cooking method, but for some others, the conventional methods were better than the microwave. Studies on buffer solutions suggested that the degree of thiamine destruction by microwave is due solely to the temperature effect (Goldblith et al., 1968), and no significant differences in the retention of thiamine and riboflavin could be demonstrated when electric and microwave were compared (Van Zante and Johnson, 1970).

The present study was initiated for the purpose of evaluating a broad spectrum of bulk packed frozen prepared food products, with respect to protein quality and vitamin retention, including thiamine, riboflavin, ascorbic acid and beta carotene, when these foods were reconstituted by different high speed heating methods and to compare the effects of these methods with the conventional hot-holding of freshly prepared products. This study was undertaken as a continued phase of Project ACTION (Auditing Convenience Techniques' Impact on Nutrition) (Co and Livingston, 1969; Kahn and Livingston, 1970) and has been conducted in collaboration with the USDA Protein Nutrition Laboratory, Human Nutrition Research Div., Beltsville, Md. The food preparation, reconstitution and vitamin analyses were carried out by the authors at Columbia University, and the protein nutritional value studies were carried out and reported by the collaborators (Bodwell and Womack, 1974).

Six food products, namely, mashed potatoes, pot roast with gravy, peas with onions, beans and frankfurters, diced carrots and frozen fried fish portions were studied. The heating procedures, equipment used and methods of product temperature measurements were those described in a separate paper (Ang et al., 1975). The raw materials used, preparation techniques and the analytical results are reported in this paper.

EXPERIMENTAL

Materials

Materials used in preparing or assembling products were procured in the form most commonly used by institutional operators, e.g., fresh beef and frankfurters, canned beans, frozen peas, onions and carrots, instant potato flakes and frozen precooked breaded fish. Products were prepared in the laboratory using published institutional recipes wherever applicable. 4–5 lb of product were packed in aluminum pans or polyester pouches as described by Ang et al. (1975) except for fried fish portions where no prepacking was required.

Mashed potatoes. Ingredients used for this product were dehydrated potato flakes (supplied by the Food Division, Borden, Inc., New York, N.Y.) instant nonfat dry milk (Carnation Company, Los Angeles, Calif.) and margarine (Mazola brand made by Best Foods, CPC International, Inc., Englewood Cliffs, N.J.). Sufficient quantities of each dry ingredient were thoroughly blended separately to provide a homogeneous dry mix which was then packed in plastic bags under nitrogen and stored in a refrigerator until the time of use.

Pot roast with gravy. U.S. Good grade, boneless top round fresh beef in 8–11 lb tied rolls was purchased from a local meat supplier. All the meat was received in one shipment and stored under refrigeration at 2–4°C until used (up to 48 hr). Shortening was used in roasting (Nutex brand, manufactured by Procter and Gamble Co., Cincinnati, Ohio). A concentrated au jus gravy was prepared by utilizing hydrolyzed plant proteins and other ingredients specified in a formula published by the

Nestle Company, Inc., White Plains, New York (Food Ingredients Division, 1967).

Vegetables. Commercially blanched and individual quick frozen dark skin Perfection peas, Crystal pearl onions and diced Emperator carrots were used for this study (supplied by the Green Giant Co., Le Sueur, Minn.).

Beans and frankfurters. Canned pea beans in tomato sauce and frozen frankfurters (24 in. long, 5/8 in. diam) made for use in the canned product were used (both were supplied by the Campbell Soup Company, Camden, N.J.).

Frozen fish portions. Fish samples including (1) frozen raw, (2) battered and breaded and (3) battered, breaded and fried were secured by the authors in the fish processing plant of Frionor Norwegian Frozen Fish, Ltd., New Bedford, Mass. (The batter mix, No. 4193 and the breading mix No. 8898 were products of the Modern Maid Food Products, Inc., Jamaica, N.Y.) Approximately 2,500 pieces of frozen raw cod fish portions weighing 58 ± 6g (size about 3 × 16 × 1 cm) were selected for further processing which consisted of battering, breading, deep frying for 45 sec at 375–400°F (191–205°C) and blast freezing at −40°C for 12 min. Two hundred portions of fish were collected at each of the above mentioned processing stages. Weights were recorded before and after each stage. All samples were stored in a freezer at −10°C until used.

General procedures for conventional heating methods

Prepared foods packed in half size disposable aluminum pans and covered (4–5 lb/pan depending on product) were held in a food warmer (Cres-cor model H-339-128-1, Crescent Metal Products, Inc., Cleveland, Ohio) for 30 min, 1-1/2 hr or 3 hr. The average warmer temperature was approximately 200–210°F (93.3–98.8°C) and food temperature was about 180°F (82.2°C). Six or more replicate pans of each product were prepared for each treatment.

Individual preparation and heating methods for each product are described below.

Mashed potatoes. Dehydrated potato flakes were reconstituted with milk, margarine and water according to the manufacturer's direction using a 5-gal steam-jacketed kettle (Market Forge Company, Everett, Mass.) and stirred for 5 min with a wire whip.

The product temperatures immediately after preparation ranged approximately between 145–148°F (63–64.5°C) and rose to 150°F (65.5°C), 164°F (73.5°C) and 175°F (79.4°C) after holding for 1/2, 1-1/2 and 3 hr respectively.

Pot roast with gravy. Fresh raw beef was cooked according to the Armed Forces Recipe (Armed Forces Recipe Service, 1969) with slight modification. Two rolls of meat were placed in a disposable aluminum roasting pan (12 × 20 × 4-1/2 in.) containing 1/2 lb shortening and heated in a convection oven (gas heated air flow AFS-100-FF, Wolf Range Co., Campton, Calif.) preset to 425°F (218.3°C). Four pans (total weight of meat was about 80 lb) were cooked per batch. The internal temperature of each roll of meat was monitored by a thermocouple connected to a recorder. The meat was turned once after 30 min of heating, salt and pepper being added at this time. The pans were then covered with aluminum foil or another disposable aluminum pan and heating was continued until the internal temperature reached 160°F (71.1°C). The heating time ranged from 3-1/2–5-1/2 hr depending on the size of the roll. The cooked beef was placed in a refrigerator (2–4°C) overnight before slicing. The drippings (6-1/2 gal) were collected and mixed with the diluted gravy (8 fl oz gravy concentrate in 10 gal hot water) to make the final gravy.

Each roll of roast beef was trimmed to remove excess visible fat and sliced in 1/8 in. thickness with an electric meat slicer. In order to randomize variability from round to round, slices from each roll were distributed into 10 aluminum pans, which were later used for different treatments. 2-1/2 lb of meat were collected into each pan. All pans were stored in a refrigerator until all the meat was cooked and the gravy was made; 2 lb of gravy were then added into each pan and all pans were chilled held in a refrigerator before reheating in a convection oven to 180°F.

Vegetables. 3 lb 10 oz of peas and 6 oz of onions (10% of onions per 4 lb pack) were mixed and packed into each pan or pouch. Carrots were packed directly into 4 lb packs and stored in the freezer.

Beans and frankfurters. Frozen frankfurters were first cut into segments approximately 1/2 in. long. 1 lb 4 oz of the cut segments were mixed with 3 lb 12 oz of canned beans to provide 5 lb per pan or pouch with a ratio of 3:1 beans to frankfurters.

The pans were first stored under refrigeration after packing, then heated in the convection oven to 180°F.

Frozen fried fish portions. Packaging into foil pans or pouches was not required for this product. For conventional heating, 12 portions of frozen fried fish (approximately 2 lb) were placed uncovered on large disposable aluminum pans (10 × 18 in.) and heated in the convection oven. Toward the end of heating, temperatures at different locations of various pieces were measured. A total of 14 pans were heated for each treatment in order to provide sufficient finished product for the protein quality studies. Net weights were determined before and after heating and holding.

Convenience food service handling methods

Prepared food products were packed either in disposable aluminum pans or pouches and stored in a freezer at −10°C until used. To simulate convenience food service handling these products were reheated in a convection oven, high pressure steamer, infrared oven (model HE-5000, Litton Industries, Minneapolis, MN 55411), and microwave oven (2450 MHz, 2.1 kw, Model 1104, N.V. Philips Gloeilampenfabrieken) and then held in a closed still food warmer at 180°F for 30 min to simulate the normal delay between reheating and consumption which would be encountered in a food service operation.

The particular precautions used in each case to insure uniform heating and internal end product temperatures have been described by Ang et al. (1975).

Methods of analysis

Sufficient amount of each raw ingredient (5 lb or more) was homogenized and analyzed. Frozen raw materials were first thawed at room temperature for 4 hr before blending. Immediately after heating, the food was blended in a 1-gal blender and samples were taken for vitamin analyses. The remaining samples (both raw and cooked) were placed on aluminum or stainless steel trays to 1/2 in. depth, covered with aluminum foil, and stored in a freezer. They were later freeze dried in a Stokes vacuum dryer for 2–3 days. Moderate heat not exceeding 100°F (38°C) was applied on the second day to accelerate drying. Dried food samples were then comminuted using a food grinder and vacuum packed into plastic pouches for shipping to the collaborators for protein quality studies. Samples for moisture analysis were weighed into moisture dishes and dried under a vacuum of 20 in. Hg at 70°C until a constant weight was obtained, whereas samples for other nutrients were weighed into tightly covered beakers and stored in the freezer. Approximately 100g of each of the carrot samples were packed into plastic bags, sealed, frozen and shipped under dry ice to the collaborators for carotene assay. Ascorbic acid was generally determined on the day following sampling, whereas thiamine and riboflavin were determined 2–3 days later.

Analytical methods used for reduced ascorbic acid, thiamine and riboflavin were the photometric, thiochrome and fluorometric methods, respectively, of the "Methods of Vitamin Assay" (Vitamin Chemists Association, 1966). Preliminary studies showed that the enzymes polidase –s of the Schwarz/Mann Co. (New York) and clarase-30 of the Miles Laboratories, Inc. (Elkhart, Ind.) were satisfactory for the thiamine assay. The procedure of Bligh and Dyer (1959) was used for fat determination and carotene content was determined by the method of Sweeney and Marsh (1970). Duncan's new multiple-range test (Steel and Torrie, 1960) was used in statistical analyses.

RESULTS & DISCUSSION

Individual products

The times required with various heating methods to bring the average internal temperature of the prepared product to 180°F are shown in Table 1. For all the heating methods compared, frozen mashed potatoes (5 lb) required the longest time while frozen fried fish portions (2 lb heated in one layer) needed the shortest heating time. Overall, the microwave oven heating was the fastest, followed by infrared and steamer methods, and the convection oven heating was the slowest. Frozen fried fish portions were the exception where heating time for all treatments was comparatively short. Tables 2 to 5 present data on vitamin content and retentions of the six products on a dry weight or fat free dry weight basis.

Mashed potatoes. Frozen mashed potatoes reheated in the microwave and infrared ovens were higher in solids content than those otherwise heated. This could be attributed to the more frequent stirrings required for heating in those ovens.

Ascorbic acid appeared to be the most unstable nutrient. Retention of ascorbic acid was as low as 39.6% after holding

for 3 hr and ranged from 24.1–41.4% for frozen prepared product reheated by various methods. Statistical analysis showed that the ascorbic acid content was significantly reduced at each stage when holding warm for 1/2, 1-1/2 and 3 hr.

All of the frozen reheated mashed potatoes contained less ascorbic acid than the freshly prepared product held warm for 1-1/2 hr. A slightly higher content of ascorbic acid was found in steamer-reheated product but not significantly higher than in the convection-reheated product or the product held for 3 hr. The infrared and microwave oven reheating methods were least favorable to the retention of ascorbic acid. This was again probably due to the more frequent stirring required. The losses of ascorbic acid in the frozen and thawed samples occurred during the slow freezing and thawing of the product.

There was no significant reduction in riboflavin content of mashed potatoes although slightly lower amounts were observed after steamer and infrared reheating.

Table 1–Heating times (min) of various bulk-packed prepared products[a]

No.	Treatment	Mashed potatoes	Pot roast & Gravy[b]	Peas/onions	Beans/franks	Diced carrots	Fried fish portions
3	Freshly prepared, no holding	–	30–35	22–24	33–39	25–30[c]	13–14
4	Freshly prepared, held 1/2 hr	–	30–35	22–24[b]	33–39	25–30[c]	13–14
5	Freshly prepared, held 1-1/2 hr	–	27–30	22–24[b]	33–39	25–30[c]	13–14
6	Freshly prepared, held 3 hr	–	28–32	22–24[b]	33–39	25–30[c]	13–14
8	Frozen-heated in convection oven, held 1/2 hr	57–61[c]	50–60	56–65[b]	47–57[b]	60–68[c]	13–14
9	Frozen-heated in infrared oven, held 1/2 hr	58–70[d]	30–40[c]	25–28[c]	25–35[c]	26–33[d]	9–10
10	Frozen heated in steamer, held 1/2 hr	44–56	30–35	26–31	42–54	23–30	13–15
11	Frozen-heated in microwave oven, held 1/2 hr	30–37[e]	21–25[e]	31–33[e]	25–27[e]	17–20[e]	9–10[e]

[a] Time to reach average internal temp. of 180°F (82.2°C)
[b] Precooking time of beef was 3-1/2–5-1/2 hr
[c] Heating was interrupted once for stirring
[d] Heating was interrupted several times for stirring
[e] Includes heating and standing time: Products were stirred during standing periods.

Table 2–Vitamin content and percentage retention in mashed potatoes subjected to various heating treatments

No.	Treatment	(mg/100g)[a] Ascorbic acid	Riboflavin	Thiamine	Retention (%)[b] Ascorbic acid	Riboflavin	Thiamine
Raw materials							
1A	Instant potato flakes	20.81	0.076	0.217	–	–	–
1B	Nonfat dry milk	13.30	1.619	0.264	–	–	–
1C	Margarine	–	0.034	0.007	–	–	–
1	Mixed raw materials (calculated value)	14.37	0.255	0.166	196.16	100.00	101.68
Conventional institutional handling							
3	Freshly prepared, no holding	7.47 ± 0.59[c]	0.255 ± 0.008	0.163 ± 0.001	100.00	100.00a	100.00a
4	Freshly prepared, held 1/2 hr	4.94 ± 0.53	0.249 ± 0.010	0.158 ± 0.002	66.13	97.65a	96.73
5	Freshly prepared, held 1-1/2 hr	3.81 ± 0.76	0.250 ± 0.006	0.148 ± 0.003	51.00	98.04a	90.80bc
6	Freshly prepared, held 3 hr	2.96 ± 0.74	0.248 ± 0.008	0.133 ± 0.005	39.62a	97.25a	81.60
Convenience food system handling							
7	Frozen-thawed	6.96 ± 0.22	0.251 ± 0.001	0.166 ± 0.004	93.17	98.43a	101.84a
8	Frozen-reheated in convection oven, held 1/2 hr	2.67 ± 0.50	0.246 ± 0.006	0.144 ± 0.005	35.74a	96.47ab	88.34bd
9	Frozen-reheated in infrared oven, held 1/2 hr	1.75 ± 0.53	0.239 ± 0.009	0.145 ± 0.004	23.83b	93.73bc	88.96bd
10	Frozen-reheated in steamer, held 1/2 hr	3.09 ± 0.41	0.236 ± 0.008	0.141 ± 0.002	41.36a	92.55c	86.50d
11	Frozen-reheated in microwave oven, held 1/2 hr	1.80 ± 0.32	0.246 ± 0.010	0.150 ± 0.003	24.10b	96.47ab	92.02c

[a] Dry weight basis; average of three determinations for raw materials
[b] Mean percent retention in relation to freshly prepared product (No. 3); means followed by the same letters are not significantly different at the 5% level. Treatment 1 is not included in computation.
[c] Mean and standard deviation of six preparations

With respect to the thaimine content, appreciable losses were found when freshly prepared products were held warm for up to 3 hr. The retention of thiamine was comparable for convection, infrared and steamer treatments (86.5–88.3%) and was significantly higher than for product held for 3 hr (81.6%). Microwave heating resulted in the greatest retention of thiamine of all reconstitution methods used but the retention was slightly lower than for freshly prepared product and held for 1/2 hr. The shorter heating time required by the microwave technique was apparently an important factor in retaining thiamine.

Peas and onions. Table 3 shows the vitamin content and retentions in vegetable items. The ascorbic acid content of peas and onions is not reported here due to the instability of this nutrient when samples were partially thawed during a freezer breakdown. The retention of riboflavin was greater than 90% for all the heat treatments. The vegetables heated in the steamer were very watery, and the liquid was subsequently discarded before analyzing. Relatively lower amounts of riboflavin were retained in steamer heating as compared to the dry-heating methods.

The infrared oven heating appeared to retain the highest amount of thiamine among all of the high speed heating methods, although the difference between infrared and microwave ovens was not significant. The retention of thiamine after infrared heating was comparable to that of the aluminum pan-steamer heated and held up to 1-1/2 hr. The thiamine content of the convection oven heated products was between that of the products held 1-1/2 and 3 hr, but not significantly different from either one. The pouch steamer heating method resulted in the lowest retention of thiamine (80.15%) followed by the conventional pan-steamer heating followed by 3 hr holding (88.24%).

Carrots. Heat-treated carrots were analyzed for thiamine and carotene and the results are shown in Table 3. Microwave oven heated carrots retained the highest level of thiamine among all of the treatments, although it was not significantly different from the freshly prepared or the infrared heated products. These higher retentions by the two high speed heating methods seemed to be due both to the shorter heating time and to the absence of any free liquid. The relatively dry heating convection oven method resulted in retention of amounts of thiamine comparable to steamer heating in aluminum pans followed by 1-1/2 hr holding or to steamer heating using pouches. Similarly to peas with onions, carrots were very watery after steam cooking. Approximately 3–4 oz of liquid were collected per pan. Results show that the retention of thiamine in vegetables is affected both by heat and by leaching. The total carotene content was similar among treatments. Although the data presented in Table 3 are based on only single or duplicate determinations, high retentions of the carotene are obvious. Sweeney et al. (1959, 1960) and Chapman et al. (1960) also reported that there were no significant losses of carotene in fresh and frozen broccoli cooked by different methods, including boiling, steaming and microwave heating.

Pot roast with gravy. The vitamin content of this product is expressed on a fat-free moisture-free basis to provide an accurate comparison of vitamin retentions. The results of analysis on the raw materials, the cooked beef and the prepared pot roast with gravy are shown in Table 4. Riboflavin appeared to be very stable during the pre-cooking process while thiamine showed significant losses. Retentions of riboflavin were not

Table 3—Vitamin content and percentage retention in vegetables subjected to various heating treatments

No.	Treatment	Peas and onions (mg/100g)[a] Riboflavin	Peas and onions (mg/100g)[a] Thiamine	Peas and onions Retention (%)[b] Riboflavin	Peas and onions Retention (%)[b] Thiamine	Carrots Thiamine (mg/100g)[a]	Carrots Retention (%)[b]	Carrots Total beta-carotene (mcg/g)[c]	Carrots Retention[b] (%)
Raw materials									
1A	Frozen peas	0.635	1.426	–	–				
1B	Frozen onions	0.152	0.227	–	–				
1	Mixed peas and onions or frozen diced carrots	0.601	1.358	100.00	100.00	0.407	100.00	1157	100.00
Conventional institutional handling									
3	Heated in steamer, no holding	0.566 ± 0.026[d]	1.32 ± 0.04	94.18abc	97.06a	0.395 ± 0.016	97.05abc	1099	94.99
4	Heated in steamer, held 1/2 hr	0.556 ± 0.015	1.28 ± 0.02	92.51abc	94.12e	0.382 ± 0.016	93.86ce	1067	92.22
5	Heated in steamer, held 1-1/2 hr	0.545 ± 0.026	1.25 ± 0.04	90.68a	91.91cde	0.360 ± 0.011	88.45d	1018	87.99
6	Heated in steamer, held 3 hr	0.550 ± 0.025	1.20 ± 0.03	91.51abc	88.24b	0.339 ± 0.012	83.29	1062	91.79
Convenience food system handling									
7	Thawed	0.577 ± 0.028	1.36 ± 0.01	96.01c	100.00a	0.405 ± 0.013	99.51a	1046	90.41
8	Heated in convection oven, held 1/2 hr	0.565 ± 0.009	1.22 ± 0.04	94.01abc	89.71bc	0.373 ± 0.013	91.65de	1069	92.39
9	Heated in infrared oven, held 1/2 hr	0.569 ± 0.032	1.27 ± 0.05	94.68bc	93.38de	0.389 ± 0.006	95.58 bc	1104	95.42
10	Heated in steamer (in pouches) held 1/2 hr	0.545 ± 0.016	1.09 ± 0.03	90.85ab	80.15	0.363 ± 0.015	89.19d	1075	92.91
11	Heated in microwave oven, held 1/2 hr	0.560 ± 0.013	1.23 ± 0.02	93.18abc	90.00cd	0.396 ± 0.004	97.30ab	1091	94.32

[a] Dry weight basis; average of three determinations for raw materials
[b] Mean percent retention in relation to fresh frozen vegetables (No. 1); means followed by the same letters are not significantly different at the 5% level. Treatment 1 is not included in computations.
[c] Data based on single or duplicate analyses
[d] Mean and standard deviation of six preparations

Table 4—Vitamin content and percentage retention in meat items subjected to various heating treatments

No.	Treatment	Pot roast with gravy (mg/100g)[a] Riboflavin	Pot roast with gravy (mg/100g)[a] Thiamine	Pot roast with gravy Retention (%)[b] Riboflavin	Pot roast with gravy Retention (%)[b] Thiamine	Beans and frankfurters (mg/100g)[c] Riboflavin	Beans and frankfurters (mg/100g)[c] Thiamine	Beans and frankfurters Retention (%)[b] Riboflavin	Beans and frankfurters Retention (%)[b] Thiamine
Raw materials									
1A	Fresh beef or frozen frankfurters	0.819	0.251	–	–	0.339	0.247	–	–
1B	Gravy or beans	1.317	0.154	–	–	0.173	0.078	–	–
1	Mixed frankfurters with beans (calculated value)			–	–	0.228	0.120	100.00	100.00
2A	Cooked beef	0.821	0.137						
2	Pot roast with gravy	0.903	0.145	100.00	100.00				
Conventional institutional handling									
3	Freshly prepared, no holding	0.810 ± 0.062	0.144 ± 0.009	89.70abc	99.31a	0.221 ± 0.006	0.393 ± 0.009	96.93a	95.56a
4	Freshly prepared, held 1/2 hr	0.800 ± 0.053	0.135 ± 0.006	88.59abc	93.10ab	0.224 ± 0.011	0.380 ± 0.012	98.25a	93.37bc
5	Freshly prepared, held 1-1/2 hr	0.822 ± 0.072	0.133 ± 0.008	91.03abc	91.72ab	0.212 ± 0.020	0.350 ± 0.016	92.98a	86.00d
6	Freshly prepared, held 3 hr	0.745 ± 0.084	0.121 ± 0.013	82.50b	83.45c	0.216 ± 0.013	0.333 ± 0.005	94.74a	81.82
Convenience food system handling									
7	Frozen and thawed	0.848 ± 0.088	0.137 ± 0.005	93.90ac	94.48ab	0.216 ± 0.012	0.395 ± 0.006	94.74a	97.05a
8	Frozen-reheated in convection oven, held 1/2 hr	0.826 ± 0.080	0.126 ± 0.013	91.47ac	86.90c	0.221 ± 0.013	0.377 ± 0.018	96.93a	92.63bc
9	Frozen-reheated in infrared oven, held 1/2 hr	0.838 ± 0.037	0.126 ± 0.008	92.80ac	86.90bc	0.227 ± 0.019	0.383 ± 0.011	96.56a	94.10ab
10	Frozen-reheated in steamer, held 1/2 hr	0.774 ± 0.055	0.121 ± 0.010	85.71bc	83.45c	0.211 ± 0.003	0.357 ± 0.006	92.54a	87.81de
11	Frozen-reheated in microwave oven, held 1/2 hr	0.801 ± 0.014	0.127 ± 0.005	88.70abc	87.59bc	0.226 ± 0.008	0.367 ± 0.018	99.12a	90.17ce

[a] Fat-free dry weight basis; average of three determinations for raw materials
[b] Mean percent retention in relation to freshly mixed products (No. 1 for frankfurter and beans and No. 2 for pot roast with gravy). Means followed by the same letters are not significantly different at the 5% level. Treatment 1 and 2 are not included in computation.
[c] Dry weight basis
[d] Mean and standard deviation of six preparations

significantly different among treatments, except that the steamer-reheated product retained significantly lower amounts of riboflavin than the frozen thawed product. The 3 hr held product had lower riboflavin content than the frozen products reheated in the infrared and convection ovens. Losses of thiamine were significant when the product was either held at 180°F for 3 hr, or was frozen and reheated by different methods as compared to the freshly prepared product. Reductions of thiamine in freshly prepared pot roast held for 1/2–1-1/2 hr and in the frozen thawed product were not significantly different from product freshly prepared or frozen and reheated in the convection, infrared and microwave ovens. The freshly prepared product held for 3 hr and the frozen steamer reheated product retained the least amount of thiamine.

Since the half-size disposable aluminum pans were very flexible and it was difficult to handle 5 lb of product containing gravy, a larger size disposable aluminum pan was used to support the half-size steam table pan during heating in the convection and infrared ovens and holding in the food warmer. The use of this additional pan might have prolonged the heating time (Table 1), but it had no effect on the holding temperature in the food warmer and this practice also prevented scorching of products in the bottom of the pan during infrared heating. The reheating time for the meat with gravy was shorter for the infrared, steamer and microwave methods (Table 1) than for the convection oven. However, the gravy heated more rapidly by the first three methods, causing it to boil while the meat was still cold. The boiling of the gravy was probably the major factor decreasing the retention of thiamine.

Beans with frankfurters. The results of analysis of beans with frankfurters are also shown in Table 4. The riboflavin retentions in the heated product were 92.54–99.56% relative to the starting raw materials. No significant differences were found among all the treatments. The losses of thiamine in this product were more serious. The hot-holding of prepared product for up to 3 hr retained significantly lower amounts of thiamine as compared to the freshly prepared product. Relatively higher levels (90–94%) of thiamine were retained by the three high speed reconstitution methods, i.e., infrared, convection and microwave heating and the retentions were comparable to the freshly prepared and 30 min held product.

Fish. Table 5 shows the thiamine content and percent retentions in the raw materials and the frozen fried fish product subjected to various heating treatments. Weight measurements during processing in the fish plant indicated that the weight gain from the frozen raw fish portions to the battered-breaded portions was about 30.3%, while the net weight gain from battered-breaded portions to fried fish was about 3.4%. The total solids content of fish increased from 27.4 to 33.1% and the fat content from 0.99 to 7.18%. Thiamine content of this product is expressed on a fat-free dry weight basis. The 3 hr held product contained the lowest and the convection oven heated product before holding contained the highest amounts of thiamine. Other treatments in decreasing order of thiamine content were the convection oven heating–1/2 hr holding; infrared or microwave heating–1/2 hr holding; convection heating–1-1/2 hr holding; steamer heating–1/2 hr holding; and the convection oven heating–3 hr holding. Average retentions of thiamine in heated products were from 77.18–103.73% relative to the frozen fried fish product. Comparatively large variations were observed within each treatment. Statistical analysis showed no significant differences among the convection, infrared and microwave heated and the thawed frozen fried fish. The variations within each treatment could be due to the differences in thickness of fish portions, and such differences would result in different final internal temperatures among and within fish portions.

Comparison of nutrient retentions among products

Thiamine. The actual retention values of thiamine by the same treatment varied from product to product. A general pattern indicated that the freshly prepared product not subjected tc hot-holding retained the greatest amount of thiamine followed by the product subjected to hot-holding for 30 min. An inverse relationship was found to exist between the holding time and thiamine content. The average retention in all six products was 82.6% after 3 hr of hot-holding. This value is

Table 5—Vitamin content and percentage retention of frozen fried fish portions subjected to various heating treatments

		Thiamine	
No.	Treatment	(mg/100g)[a]	Retention[b] (%)
Raw materials			
1A	Frozen raw fish	0.299	–
1B	Batter mix (dry)	0.118[c]	–
1C	Breading mix	0.226[c]	–
2	Frozen breaded raw fish portions	0.250	–
Conventional institutional handling			
3	Heated in convection oven, no holding	0.250 ± 0.017[d]	103.73a
4	Heated in convection oven, held 1/2 hr	0.241 ± 0.011	100.00ab
5	Heated in convection oven, held 1-1/2 hr	0.220 ± 0.020	91.29bc
6	Heated in convection oven, held 3 hr	0.186 ± 0.028	77.18
Convenience food system handling			
7	Frozen fried fish portions, thawed	0.241 ± 0.019	100.00ab
8	(Same as No. 4)	–	–
9	Heated in infrared oven, held 1/2 hr	0.231 ± 0.014	95.85abc
10	Heated in steamer, held 1/2 hr	0.215 ± 0.021	89.21c
11	Heated in microwave oven, held 1/2 hr	0.231 ± 0.026	95.8abc

[a] Fat-free, dry weight basis; average of three determinations for raw materials
[b] Mean percent retention in relation to frozen fried fish portions (No. 7); means followed by the same letters are not significantly different at the 5% level.
[c] Dry weight basis
[d] Means and standard deviation of six preparations

somewhat higher than those reported by Harris and Von Loesecke (1960) for some vegetables and by Kahn and Livingston (1970) for entree mixtures in individual portions. There was no significant loss of thiamine during frozen storage or thawing. The prepared frozen foods reconstituted by any of the high speed heating methods and held for 30 min were found to be more favorable to nutrient retention than the conventionally prepared and held for 3 hr, except in the case of steam-pouch-heating of frozen peas/onions or carrots where appreciable amounts of nutrients were lost.

Among the four reconstitution methods studied, the high pressure steamer method appeared to be the least favorable; however, in some instances, there was no significant difference between the steamer and convection oven (for mashed potatoes, pot roast/gravy and carrots), between the steamer and infrared oven (for mashed potatoes, pot roast/gravy, fish portions) and between the steamer and microwave oven (for pot roast/gravy, beans/frankfurters and fish portions). Less thiamine was retained by convection oven-heating in mashed potatoes and carrots compared to the infrared and microwave oven heatings, but retention levels were comparable in the four other products. The microwave and infrared methods were comparable in most instances except for mashed potatoes, where infrared heating resulted in a lower retention of thiamine and in beans with frankfurters, where it resulted in a higher retention. The two methods were equivalent to or slightly better than the method of conventional preparation and hot holding for 1-1/2 hr. The average retention of thiamine in all six bulk products was 92.5 and 92.2% by infrared and microwave, respectively. These values are comparable with the findings of Kahn and Livingston (1970).

Riboflavin. The retentions of riboflavin in four products were above 90% by any treatment. There were no significant losses when the product was held hot for up to 3 hr. The frozen reconstituted products were equivalent to the conventionally prepared, except that the steam heated vegetables retained less riboflavin. The high percentage retention of riboflavin was also reported by Bowers and Fryer (1972) in their studies of turkey muscles, whereas lower values were reported by Nobel (1970) for cooked variety meats. No direct comparison of the present study can be made with other reported data since the cooking methods, products used and data presentations varied significantly among researches.

CONCLUSIONS

THE RETENTION of nutrients in frozen prepared food products varies with the nature of the nutrient, the nature of the product, the preparation methods, and the heating methods used. Ascorbic acid and thiamine were found unstable to heat, whereas riboflavin and carotene were more stable. The freshly prepared products were of the highest nutritive quality and the 3 hr hot-holding practice tended to reduce thiamine and ascorbic acid content to an appreciable extent. Microwave oven heating which required shorter heating times, tended to retain higher amounts of heat-labile nutrients. In heating bulk packed foods, however, frequent stirring was required as were resting periods to avoid scorching. This practice was evidently detrimental to ascorbic acid retention. Infrared heating (which required longer heating times but fewer interruptions) had a similar effect on nutrient retention as microwave heating in most cases. Both of these methods retained equal or greater amounts of nutrients as compared to fresh preparation followed by 1-1/2 hr hot-holding. Convection oven heating required the longest heating times for all of the bulk packed frozen foods. Retentions of heat-labile nutrients, such as thiamine, in the convection oven were sometimes lower (mashed potatoes and carrots) and sometimes comparable to infrared and/or microwave heating. For the heating of single layered fish portions (2 lb per pan) the convection oven heating time was greatly reduced and the method was found to be slightly more favorable than the microwave or the infrared oven method with respect to thiamine retention.

High pressure steamer heating in all instances resulted in substantially lower levels of thiamine and riboflavin than other reconstitution methods. However, steamer heating did have the advantage of preserving ascorbic acid in mashed potatoes and in most instances, this method was better than holding hot for 3 hr after preparation.

REFERENCES

Ang, C.Y.W. and Livingston, G.E. 1974. Nutritive losses in the home storage and preparation of raw fruits and vegetables. In "Nutritional Qualities of Fresh Fruits and Vegetables," Ed. White, P.L. and Silvey, N., p. 51. Futura Publishing Co., Inc., Mount Kisco, N.Y.

Ang, C.Y.W., Co, D.Y.C.L., Frey, A.E. and Livingston, G.E. 1975. Development of methods for achieving desired end product temperatures in bulk packed prepared frozen foods heated by various means. (Submitted to J. Food Sci.)

Armed Forces Recipe Service. 1969. Beef Pot Roast (Oven Method) L. Meat, Fish and Poultry. No. 9 (2) United States Army TM10-412.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911.

Bodwell, C.E. and Womack, M. 1974. Effects of heating methods on protein nutritional value of five fresh or frozen food products. Presented at the 34th Annual Meeting of the Institute of Food Technologists, May 12—15, New Orleans, La.

Bowers, J.A. and Fryer, B.A. 1972. Thiamine and riboflavin in cooked and frozen, reheated turkey: Gas vs. microwave ovens. J. Am. Diet. Assn. 60: 395.

Catering Research Unit, University of Leeds, U.K. 1970. An Experiment in Hospital Catering Using the Cook/Freeze System.

Causey, K. and Fenton, F. 1951a. Effect of reheating methods on palatability, nutritive value, and bacterial count of frozen cooked foods. 1. Vegetables. J. Am. Diet. Assn. 27: 390.

Causey, K. and Fenton, F. 1951b. Effect of reheating methods on palatability, nutritive value, and bacterial count of frozen cooked foods. 2. Meat dishes. J. Am. Diet. Assn. 27: 491.

Chapman, V.J., Pritz, J.O., Gilpin, G.L., Sweeney, J.P. and Eisen, J.N. 1960. Electron cooking of fresh and frozen broccoli. J. Home Economics 52: 161.

Co, D.Y.C.L. and Livingston, G.E. 1969. Heat reconstitution equipment for prepared frozen foods. Food Technol. 23: 72.

Eheart, M.S. 1969. Variety, fresh storage, blanching solution and packing effects on ascorbic acid, total acids, pH and chlorophylls in broccoli. Food Technol. 23: 238.

Goldblith, S.A., Tannenbaum, S.R. and Wang, D.I.C. 1968. Thermal and 2450 MHz microwave energy effect on the destruction of thiamine. Food Technol. 22: 1266.

Harris, R.S. and Von Loesecke, H. 1960. "Nutritional Evaluation of Food Processing." John Wiley & Sons, N.Y.

Kahn, L.N. and Livingston, G.E. 1970. Effect of heating methods on thiamine retention in fresh or frozen prepared foods. J. Food Sci. 35: 349.

Livingston, G.E., Ang, C.Y.W. and Chang, C.M. 1973. Effects of food service handling. Food Technol. 27: 28.

Nobel, I. 1970. Thiamine and riboflavin retention in cooked variety meats. J. Am. Diet. Assn. 56: 225.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics," p. 128. McGraw-Hill Book Co., Inc., New York.

Sweeney, J.P., Gilpin, G.L., Martin, M.E. and Dawson, E.H. 1960. Effect of cooking time, method and storage on the palatability and nutritive value of frozen broccoli. J. Am. Diet. Assn. 36: 122.

Sweeney, J.P., Gilpin, G.L., Statey, M.G. and Martin, M.E. 1959. Effect of cooking methods on broccoli-L. Ascorbic acid and carotene. J. Am. Diet. Assn. 35: 354.

Sweeney, J.P. and Marsh, A.C. 1970. Separation of carotene stereoisomers in vegetables. J. of A.O.A.C. 53: 937.

U.S.D.A. Agriculture Handbook No. 8. 1963. "Composition of Foods—Raw, Processed and Prepared," Ed. Watt, B.K. and Merrill, A.L. Consumer and Food Economics Research Div., ARS, USDA, Washington, D.C.

Van Zante, H.J. and Johnson, S.K. 1970. Effect of electronic cookery on thiamine and riboflavin in buffered solutions. J. Am. Diet. Assn. 56: 133.

Vitamin Chemists Association. 1966. "Methods of Vitamin Assay," Ed. Freed, M. Interscience Publishers Inc., New York, N.Y.

Ms received 4/7/74; revised 5/13/75; accepted 5/20/75.

The research reported upon herein, was conducted by the authors while affiliated with the Institute of Human Nutrition, Columbia University, New York, N.Y.

Project ACTION sponsors were the Campbell Soup Co., Hoffman-La Roche, National Restaurant Association, Reynolds Metals Co. and Young-Block Associates. The specific research reported here was supported in part by the USDA Human Nutrition Research Div., Agricultural Research Service, Beltsville, Md. through cooperative agreement HN 09-04-09(CA).

The authors also gratefully acknowledge the technical guidance received from the American Dietetic Association, the U.S. Dept. of Agriculture (Human Nutrition Research Lab.) and the Surgeon General of the United States Army through the participation of their representatives on the Project ACTION Advisory Committee. Gratitude is also extended to Mrs. Anne Marsh (USDA) for the carotene assays on carrot samples.

DIANNE ODLAND[1] and MARY S. EHEART
College of Humam Ecology and Agricultural Experiment Station
University of Maryland, College Park, MD 20742

ASCORBIC ACID, MINERAL AND QUALITY RETENTION IN FROZEN BROCCOLI BLANCHED IN WATER, STEAM AND AMMONIA-STEAM

INTRODUCTION

SINCE the early days of freezing preservation the necessity of blanching vegetables prior to freezing has been recognized as essential for enzyme inactivation. The relative merits of steam and water blanching have been widely studied. Excellent reviews of these methods (Feaster, 1960; Joslyn, 1966; Lee, 1958) have been published. Although some disagreement exists among published reports, the concensus seems to be that retention of soluble nutrients is higher in steam-blanched than in water-blanched vegetables (Feaster, 1960). There is some evidence, however, that steaming results in greater destruction of chlorophyll in green vegetables than water heating. Adverse changes in both color and flavor were found by Fisher and Van Duyne (1952) when steam under pressure was used to blanch several green vegetables. Gordon and Noble (1959) reported better retention of color in green vegetables cooked in water than those steamed at atmospheric pressure.

In assessing the advantages and disadvantages of water and steam blanching, pollution is another factor to be considered. Annually, the canned and frozen fruit and vegetable industry discharges about 83 billion gallons of waste water, generates 800 million pounds of BOD, 392 million pounds of suspended solids and 8 million tons of solid residuals (National Canners Association, 1971). Although the effluent from blanching is small in volume compared to other processing steps, e.g., washing, it contributes a significant portion to the total pollution load. Blanching methods which maximize water conservation and minimize waste discharge must be sought. The benefits of steam over water blanching to achieve this end are obvious.

As previously mentioned, steam blanching has usually been found to conserve more soluble nutrients than blanching in water but there is some indication that color of green vegetables is adversely affected by steam. The possible presence of volatile acids in the enclosed steam in addition to nonvolatile acids in the vegetable tissue would appear to be a factor in the degradation of chlorophyll to pheophytin. The recognition that volatile acids are liberated when vegetables are cooked was proposed as early as 1920 (Charley, 1972). Since solutions of ammonium bicarbonate produce NH_3 gas when heated, this salt becomes of interest as a possible means of neutralizing acids and thus improving color retention during steam blanching of green vegetables. The use of ammonium compounds to increase chlorophyll retention during blanching and cooking green vegetables in water has been reported previously (Eheart and Odland, 1973a, b).

The present study investigates the effects of using ammonium bicarbonate in the steam blanching of broccoli. This treatment was compared to conventional steam and water blanches. Ascorbic acid and mineral retention, color and sensory quality of the vegetable, as well as effluent composition, were the major factors considered.

[1] Present address: Consumer and Food Economics Institute, ARS, USDA, Beltsville, MD 20705. Formerly with the Maryland Agricultural Experiment Station.

EXPERIMENTAL

BLUE OCEAN VARIETY of broccoli grown in Exmore, Va. was transported by truck to the laboratory where it was refrigerated over night at 4°C (mean temperature) until used in the experiment.

Broccoli was divided into five replicate lots. Lots 1–3 were blanched the first day and lots 4 and 5 on the following day. Immediately prior to blanching, broccoli from each lot was washed, dried, trimmed to a 5-inch length, split lengthwise into portions having heads about 1.5 inches in diameter, mixed and then divided into three sublots for the three blanching methods.

Blanch methods

Water blanch. Tap water (1.5 gal) was brought to boil in an aluminum pot (9 in. ht × 10 in. diam) equipped with an overall-perforated aluminum blanching basket. The broccoli (700g) was added and blanched uncovered for 4 min.

Steam blanch. Tap water (500 ml) was placed in a closely fitted two piece home-style canner (12 in. × 12 in. diam) and was heated to a full boil (measured by thermocouple) to fill the canner with steam. The top of the canner was removed and the broccoli placed inside in an elevated perforated aluminum blanching basket similar to that used for water blanching. The top was replaced and the broccoli blanched for 6 min at atmospheric pressure.

NH_3–steam blanch. Broccoli was blanched for 6 min identically as in the steam blanch except that 1g NH_4HCO_3 was dissolved in the 500 ml of tap water. This amount was determined in preliminary study to give broccoli having optimum sensory qualities. Preliminary distillation studies demonstrated that NH_3 gas was evolved at a fairly constant rate for at least 10 min after the NH_4HCO_3 solution was brought to a boil.

Blanch times for each method had been predetermined by negative peroxidase tests (Masure and Campbell, 1944). For each replication, the sequence of the three blanching methods was rotated. Samples for each method were held under refrigeration until blanching.

For each method and each replicate, two lots of 700g each were blanched separately in fresh solutions. Samples were cooled in ice water (changed between blanch methods) and drained. Broccoli from the two blanches was then mixed, weighed, packed in freezer bags and boxes, quick-frozen at −23°C and freezer stored at −18°C. From the original 1400g of blanched broccoli, four 300-g samples were packaged for use in chemical and physical tests and one 200-g sample for use in sensory evaluation.

Effluent volumes were measured after each blanch and aliquots (5% for the water blanch and 50% for the steam blanches) from the two blanches were combined for analyses.

Cooking methods

Cooking times for frozen broccoli blanched by each of the three methods were subjectively determined in preliminary study to give similar degrees of doneness. Salted tap water (0.63g NaCl/150 ml water/300g broccoli or 0.42g NaCl/100 ml water/200g broccoli) was brought to boil in a pyrex saucepan, the vegetable added, the pan covered and doneness timed from addition of the vegetable to the pan. Cooking times were 10.5, 11 and 8.5 min, respectively, for water-blanched, steam-blanched and steam-NH_3-blanched broccoli.

Chemical and physical tests

Blanch effluents. Blanch effluents were analyzed for total solids, total ash, P, K, Na, Mn, Ca, Mg and Cu.

Total solids were determined by reducing the volume of each aliquot in a beaker on a hot plate and drying to constant weight in a forced-draft oven at 65°C.

These residues were quantitatively transferred to porcelain crucibles and ashed overnight at 525°C in a muffle furnace. Ash weights were recorded. Ashed samples were then dissolved in 10 ml 1:1 HCl, the solutions heated to boiling, filtered through Whatman #2 filter paper, washed with hot water and after cooling, brought to a 100 ml volume with water. These solutions were analyzed colorimetrically for P (AOAC, 1965). Mg, Cu, Ca and Mn were determined by atomic absorption (Perkin-Elmer, 1971) and Na and K by flame photometry using a Beckman (Model DU) spectrophotometer equipped with a flame attachment.

On each day of blanching, tap water samples were drawn prior to the first blanch and again after the last blanch. Equal volumes of the two samples were combined for mineral analyses. These analyses were used to correct the concentration of minerals in the effluents to give the amounts leached from the broccoli.

Vegetables. After 1 wk frozen storage, uncooked samples of broccoli were analyzed for total solids, pH, titratable acidity, color difference (Gardner a/b), dehydro- and reduced ascorbic acids (DAA and RAA, respectively) according to previously described methods (Eheart and Odland, 1973a, b) and for total ash, P, K, Na, Mn, Ca, Mg and Cu.

For ash and mineral analyses, oven-dried plant material was ground in a Wiley mill fitted with a 20-mesh screen. Samples of 2g were taken for ashing. The procedures for total ash and mineral analyses have been described above for blanch effluents. Raw samples of broccoli were similarly analyzed.

At 6 mo frozen storage both uncooked and cooked samples of broccoli were analyzed for the same parameters with the exception of total ash and minerals. In addition, firmness of cooked samples was determined by shear press measurement (Kramer and Twigg, 1970). These values were obtained using a 1600 lb ring with a 30 sec stroke at 50% range and were expressed as the average peak height of duplicate 100-g samples.

Sensory evaluation

After 5½ mo of frozen storage, a sensory panel of five trained members evaluated cooked broccoli samples for both intensity and acceptability of flavor, texture (firmness and sloughing) and color and also for overall acceptability. Flavor and texture were judged under blue light.

Three samples were served to each judge at each of five sessions. Water-blanched samples were labelled "control." The other two blanching treatments were coded and were presented in random order. Panel members compared the coded samples to the control and for each quality factor rated the two samples either "the same as, more than, or less than the control." These judgements were scored 3, more than 3 or less than 3 points, respectively, on a 5-point rating scale.

Statistical analyses

Data were statistically analyzed by variance and by Newman-Keul's Studentized range (Sokal and Rohlf, 1969).

RESULTS & DISCUSSION

Total solids and minerals

The effects of blanching method on total solids and minerals in broccoli and in the effluents from blanching are reported in Table 1. Water-blanched broccoli was significantly lower ($p < 0.01$) in total solids, total ash, P and K than steam-blanched, either with or without the addition of NH_4HCO_3. Retention of all other minerals analyzed, i.e., Na, Mn, Ca, Mg and Cu, was not affected by blanch method.

Effluents from water blanching contained significantly more ($p < 0.01$) solids, ash and all of the minerals analyzed than did the effluents from the two steaming methods; the latter two methods did not differ.

In general, the concentrations of solids, ash and the several minerals in the effluent from water blanching ranged from 9–16 times as high as those from the steam-blanch methods. These differences in effluent concentration from water and steam blanching could greatly affect stream pollution from processing plants.

It is obvious that analyses of solids and minerals in the effluents revealed more significant differences between water and steam blanches then did analyses of the blanched vegetables. Loss of solids and minerals to the cooling water was thought to be the probable cause of this finding. Since the cooling waters were not analyzed, the total amounts of minerals accounted for in the vegetable and in the blanch water were calculated as a percentage of the raw vegetable (Table 1). Significant differences were found for total solids, ash, P, K and Na. In all cases, these totals were higher for water than for steam blanching. This would indicate that losses of these components to the cooling water were higher for steam- than water-blanched broccoli. Losses of Mn, Mg and Ca to the cooling water were greater for the steam blanches than for the water blanch but these differences were not significant. Retzer and co-workers (1945) found that steam-blanched cauliflower retained more ascorbic acid than water-blanched during the

Table 1—Total solids, ash and mineral content of blanched broccoli and blanch effluents from three methods of blanchings[a]

Blanching method[b]	Total solids	Total ash	P	K	Na	Mn	Ca	Mg	Cu
				In 100g[d] broccoli, wet basis					
Raw[c]	9.24	0.95	61.52	330.0	10.69	0.33	56.91	28.87	23.55
Water	7.48[y]	0.54[y]	47.62[y]	174.0[y]	5.17	0.22	53.02	22.27	11.91
Steam	8.08[z]	0.68[z]	51.50[z]	232.0[z]	5.57	0.28	57.71	25.11	16.61
Steam-NH_3	9.02[z]	0.67[z]	53.29[z]	230.0[z]	6.31	0.28	53.07	25.01	12.95
				In blanch effluent (from 100g[d] raw broccoli)					
Water	1.24[z]	0.26[z]	3.33[z]	90.4[z]	4.14[z]	0.09[z]	13.21[z]	6.35[z]	0.06[z]
Steam	0.10[y]	0.02[y]	0.66[y]	6.9[y]	0.36[y]	0.00[y]	1.62[y]	0.57[y]	0.00[y]
Steam-NH_3	0.09[y]	0.02[y]	0.41[y]	5.9[y]	0.32[y]	0.00[y]	1.46[y]	0.46[y]	0.00[y]
				Total in broccoli and effluent (% of raw)					
Water	94.3[z]	83.7[z]	90.9[z]	78.9[z]	87.9[z]*	95.2	114.5	99.2	57.1
Steam	88.4[y]	73.4[y]	84.7[y]	72.2[y]	56.4[y]	84.8	102.0	89.0	75.0
Steam-NH_3	87.7[y]	72.6[y]	87.3[yz]	71.0[y]	63.2[y]	85.4	93.8	88.3	61.7

[a] Different superscripts indicate significance at the 1% level unless marked * (5% level)
[b] Mean of five replications
[c] Means for the raw vegetable (eight samples) are reported for comparison purposes
[d] Reported in g for total solids and total ash, in μg for Cu and in mg for all other minerals

blanching process but the steam-blanched lost a larger amount of the vitamin in the cooling water. In the present study, calculations indicated that Ca was absorbed from the cooling water, at least in the case of the water-blanched broccoli. Absorption of Ca from blanching water by vegetables has been reported previously (Bengtsson, 1969).

A recent study (Wedler, 1971) reported higher retentions (data were not statistically analyzed) of K, Na, Ca, Mg and P in steam-blanched than in water-blanched vegetables, with the greatest difference being in K. In the present study only K and P were found to be significantly higher in steam-blanched than in water-blanched broccoli. The steam-blanched broccoli was higher than water-blanched in Na, Mn, Ca, Mg and Cu, but the differences were not statistically significant. The fact that Na was not significantly higher in the steam-blanched vegetable was evidently due to the higher loss ($p < 0.05$) from the steam-blanched vegetables to the cooling water. Thus loss of minerals during blanching and cooling of broccoli (Table 1) varied with the mineral analyzed.

Ascorbic acid, color and pH

Broccoli blanched by the three methods differed significantly in pH (Table 2). Steam-blanched broccoli was lowest in pH and steam-NH_3 blanched was highest. Total acids were also higher in the steam-blanched vegetable than in those blanched by the other two methods. The pH of vegetable tissue is related to chlorophyll degradation and therefore to the color of green vegetables. Gardner a/b values were significantly lower ($p < 0.01$) for steam-blanched than for water-blanched or steam-NH_3-blanched broccoli. The latter two methods did not differ in color.

Steam-blanched broccoli was found to be firmer, as measured by the shear press, than that which had been water blanched.

Total ascorbic acid (TAA) was significantly higher ($p < 0.01$) in both types of steam-blanched broccoli than in the water-blanched. A number of earlier studies, reviewed by Feaster (1960), have reported similar results although in many cases blanching times in steam and water had not been based on enzyme inactivation.

Storage of frozen broccoli for 6 mo resulted in a significant decrease in pH. Cooking did not change the pH further but greenness was decreased considerably. Ascorbic acid, both RAA and TAA, was decreased ($p < 0.01$) by 6 mo storage and further decreased during cooking. DAA increased significantly during storage, a change which was expected. The stored, cooked broccoli contained approximately 45% of the original TAA in the raw broccoli.

Table 3—Blanch method X storage/cooking interaction for greenness (Gardner)[a]

Blanch method	Gardner values (a/b)		
	1 wk uncooked	6 mo uncooked	6 mo cooked
Water	-0.82^{yz}	-0.90^{z}	-0.75^{y}
Steam	-0.81^{yz}	-0.85^{z}	-0.66^{x}
Steam-NH_3	-0.85^{z}	-0.89^{z}	-0.83^{yz}

[a] Means of five samples; different superscripts indicate significance at the 1% level

The only significant interaction between storage/cooking and blanching method was found for Gardner a/b values (Table 3). Gardner a/b values were not decreased by 6 mo storage (−18°C) of uncooked broccoli blanched by any of the three methods. Cooking the frozen stored samples, however, revealed differences in chlorophyll stability among the three blanching methods. In contrast to steam- and water-blanched samples, NH_3-steam blanched broccoli did not decrease in a/b value during cooking. Thus, the use of NH_4HCO_3 in steam blanching evidently stabilized the chlorophyll during freezer storage and subsequent cooking.

Panel scores

Panel scores for cooked broccoli which had been freezer stored for 6 mo are given in Table 4. Judges found the flavor of steam-blanched broccoli to be more intense ($p < 0.05$) than water-blanched but there was no difference in flavor acceptability. The texture of steam-blanched broccoli was preferred over that of the water-blanched. Although the steam blanches resulted in broccoli which was more firm and less sloughed than water blanched the differences were not statistically significant. Color scores of broccoli for the three blanches differed significantly ($p < 0.01$) for both intensity and acceptability of color with the steam—NH_3 blanched receiving the highest score and the steam blanched the lowest. For overall acceptability, the steam-NH_3 blanched samples were rated the highest of the three blanches.

CONCLUSIONS

THE RESULTS of this study indicated that the use of NH_4HCO_3 in steam-blanching broccoli gave an excellent prod-

Table 2—Effects of blanch method and storage (−18°C)/cooking on pH, color (Gardner a/b) and ascorbic acid retention of frozen broccoli[a]

Factor effect	Dry matter (%)	pH	Titratable acidity[b] (meq/100g)	Gardner (a/b)	Firmness (lb)	Ascorbic acid, mg/100g		
						Reduced	Dehydro-	Total
Raw[b]	9.24	6.64	3.49			94.0	4.2	98.2
Blanch method[c]								
Water	7.96^{y}	6.56^{y}	1.47^{y}	-0.82^{z}	421^{y}	45.3^{y*}	5.7^{y*}	51.0^{y}
Steam	8.60^{z}	6.50^{x}	1.97^{z}	-0.77^{y}	499^{z}	48.8^{yz}	7.4^{z}	56.2^{z}
Steam-NH_3	8.40^{z}	6.86^{z}	1.60^{y}	-0.85^{z}	446^{yz}	51.2^{z}	6.3^{y}	57.5^{z}
Storage/cooking[c]								
1 wk/uncooked	7.86^{y}	6.72^{z}	1.77^{z*}	-0.83^{y}	–	56.5^{z}	7.0^{y}	63.5^{z}
6 mo/uncooked	8.07^{y}	6.61^{y}	1.69^{yz}	-0.88^{z}	–	48.5^{y}	8.8^{z}	57.3^{y}
6 mo/cooked	9.03^{z}	6.59^{y}	1.58^{y}	-0.74^{x}	–	40.4^{x}	3.5^{x}	43.9^{x}

[a] Different superscripts indicate significance at the 1% level unless marked * (5% level); data are reported on the wet basis.
[b] Means for the raw vegetable (eight samples) are reported for comparison purposes
[c] Means of 15 samples except five samples for firmness

Table 4–Panel scores[a,b,c] for broccoli cooked after 6 months storage at −18°C

Blanch method	Intensity				Acceptability			
	Flavor	Firmness	Sloughing	Color	Flavor	Texture	Color	Overall
Water	3.0^{y*}	3.0	3.0	3.0^{y}	3.0	3.0^{y*}	3.0^{y}	3.0^{y}
Steam	3.5^{z}	3.5	2.6	2.4^{x}	3.2	3.3^{z}	2.5^{x}	3.2^{y}
Steam-NH_3	3.2^{yz}	3.4	2.7	3.8^{z}	3.1	3.2^{yz}	3.8^{z}	3.6^{z}

[a] Controls (water-blanched samples) were assigned a score of 3.0. Judges rated experimental samples "greater than" or "less than" control on a 5-point scale

[b] Means of 25 judgments (5 judges X 5 replications)

[c] Different superscripts indicate significance at the 1% level unless marked *(5%)

uct. Greenness, as indicated by Gardner color difference values, was greatly improved over that which was conventionally steam blanched. Ascorbic acid, total solids, total ash, P and K contents were significantly higher in broccoli steam-NH_3 blanched than in samples conventionally blanched in water. A panel of trained judges rated steam-NH_3-blanched broccoli superior in color and in overall acceptability to broccoli blanched by either of the two conventional methods. Thus, the use of NH_4HCO_3 in steam blanching was successful in producing broccoli as green as water blanched and as high in nutrients as steam blanched.

REFERENCES

AOAC. 1965. "Official Methods of Analysis," 10th ed. Association of Official Agricultural Chemists, Washington, D.C.

Bengtsson, B.L. 1969. Effect of blanching on mineral and oxalate content of spinach. J. Food Technol. 4: 141.

Brown, H.D. 1941. Freezing preservation of fruits and vegetables. Quick Frozen Foods 3(3): 14.

Charley, H. 1972. Fruits and vegetables. In "Food Theory and Applications," Ed. Paul, P.C. and Palmer, H.H., p. 268. John Wiley and Sons, Inc., New York.

Eheart, M.S. and Odland, D. 1973a. Use of ammonium compounds for chlorophyll retention in frozen green vegetables. J. Food Sci. 38: 202.

Eheart, M.S. and Odland, D. 1973b. Quality of frozen green vegetables blanched in four concentrations of ammonium bicarbonate. J. Food Sci. 38: 954.

Feaster, J.F. 1960. Effects of commercial processing on the composition of fruits and vegetables. A. Washing, trimming and blanching. In "Nutritional Evaluation of Food Processing," Ed. Harris, R.S. and Von Loesecke, H., p. 109. John Wiley and Sons, Inc., New York.

Fisher, W.B. and Van Duyne, F.O. 1952. Effect of variations in blanching on quality of frozen broccoli, snap beans and spinach. Food Res. 17: 315.

Gordon, J. and Noble, I. 1959. Effect of cooking method on vegetables. Ascorbic acid retention and color differences. J. Am. Diet. Assoc. 35: 578.

Joslyn, M.A. 1966. The freezing of fruits and vegetables. In "Cryobiology," Ed. Meryman, H.T., p. 565. Academic Press, New York.

Kramer, A. and Twigg, B.A. 1970. "Quality Control for the Food Industry," Vol 1. Fundamentals; 3rd ed., p. 94. Avi Publishing Co., Inc., Westport, Conn.

Lee, F.A. 1958. The blanching process. In "Advances in Food Research," Ed. Mrak, E.M. and Stewart, G.F., Vol 8, p. 63. Academic Press, New York.

Masure, M.P. and Campbell, H. 1944. Rapid estimation of peroxidase in vegetable extracts—An index of blanching adequacy for frozen vegetables. Fruit Prod. J. & Am. Food Mfg. 23: 369.

National Canners Association. 1971. Liquid wastes from canning and freezing fruits and vegetables. Western Regional Research Laboratory, Berkeley, Calif.

Perkin-Elmer. 1971. "Analytical Methods for Atomic Absorption Spectrophotometry," Norwalk, Conn.

Retzer, J.L., Van Duyne, F.O., Chase, J.T. and Simpson, J.I. 1945. Effect of steam and hot-water blanching on ascorbic acid content of snap beans and cauliflower. Food Res. 10: 518.

Sokal, R.R. and Rohlf, F.J. 1969. "Biometry. The Principles and Practice of Statistics in Biological Research," p. 239. W.H. Freeman and Co., San Francisco.

Wedler, A. 1971. Results of experiments on the change in contents of nutritional compounds through processing and preparation of vegetables (German). Qual. Plant. Mater. Veg. 21: 79.

Ms received 2/6/75; revised 4/19/75; accepted 4/22/75.

The authors express their gratitude to the Department of Horticulture for help in obtaining the broccoli and for the use of facilities for mineral analyses.

ANNE M. KYLEN[1] and ROLLAND M. McCREADY
USDA Western Regional Research Lab., ARS, Berkeley, CA 94710

NUTRIENTS IN SEEDS AND SPROUTS OF ALFALFA, LENTILS, MUNG BEANS AND SOYBEANS

INTRODUCTION

THE ANCIENT PRACTICE of sprouting seeds for food use is increasing in popularity. Several kinds of sprouts are available in Western supermarkets and increasing amounts are being consumed in raw or cooked form. Sunset (Anon., 1974) test-sprouted 31 different kinds of seeds and judged 10 as palatable and easy for home-sprouting. In 2–7 days anyone can make sprouts by soaking viable dried seeds.

The nutrient value of cooked mung bean sprouts is reported in Home & Garden Bull. No. 72 (USDA, 1971) but chemical analyses of the nutrients of popular sprouts is incomplete. This study reports proximate mineral and selected vitamin analyses on alfalfa seeds, lentils, mung and soybeans, and on their sprouts both raw and cooked.

EXPERIMENTAL

Preparation of samples

Alfalfa seeds, dry lentils, mung and soybeans purchased from a health food store were ground to a powder in a Udy Cyclone Sample Mill for analyses. Seed powders were weighed directly for analysis.

Sprouting was done in the dark in gallon jars with cheesecloth covers held in place by rubber bands (Anon., 1974; Whyte, 1973). Seeds were soaked overnight in three times their weight of tap water, then during the 3-day growing period they were rinsed with tap water at 4-hr intervals during the day. Alfalfa sprouts were placed in the light the third day to allow them to develop green color. Loose seed coats were discarded.

Sprouts were cooked for 2 min by the stir-fry technique in a preheated covered pan treated with a nonstick agent and stirred after 1/2, 1-1/2 and 2 min during cooking.

200g of the sprouts were weighed into a blender, and equal weight of absolute ethyl alcohol was added and the sample was blended to form a slurry (Randall et al., 1975). The alcohol slurries were stored in capped glass containers at about 0°C until subsamples were withdrawn for analyses.

Proximate analysis

Total solids. 20g of the sprout slurry were measured into a weighed glass evaporating dish, partly dried on a steam bath, and drying completed in vacuo at 70°C for 16 hr. 2g of seed powder were dried in vacuo as described.

Protein. 20g of slurry were measured into a weighed evaporating dish, evaporated nearly to dryness on a steam bath and nitrogen determined by the usual Kjeldahl procedure (AOAC, 1970). Protein is N × 6.25. 1g of seed powder was taken for nitrogen analysis.

Crude-fiber. 20g of slurry were taken to apparent dryness on a steam bath and crude-fiber determined in the usual way (AOAC, 1970). 2g of seed powder were taken for crude-fiber analysis.

Ash. 20g of slurry were weighed into a Vycor crucible, evaporated to dryness on a steam bath, carbonized under an infrared heater, heated in a muffle furnace at 550°C overnight, cooled and weighed (AOAC, 1970). 2g of seed powder was ashed as described.

Fat. 20g of slurry were weighed into a porous thimble and placed into a Soxhlet extractor, dried in a forced draft oven at 70°C, and the fat extracted with ether (AOAC, 1970). 0.5g of seed powder was extracted with ether directly.

Metal determinations

Solids (300 mg) from determination of solids content of sprout samples was pelletized and metals determined by energy dispersive X-ray fluorescence spectroscopy (Reuter and Raynolds, 1974). Dry seed powders (300 mg) were pelletized and analyzed directly.

Vitamin analysis

Vitamin C, total. Total ascorbic acid was determined on a 5% metaphosphoric acid extract in the bromine-oxidized filtrate by the 2,4-dinitrophenylhydrazine procedure as described by Freed (1966).

Thiamine (Vitamin B_1). Thiamine was determined by the thiochrome method as described by Freed (1966) as modified by Pippen and Potter (1975).

Riboflavin (Vitamin B_2). Riboflavin was determined by microbiolgocial assay by the method of Johnson (1948) using *L. casei*. The Technicon AutoAnalyzer was used to measure the turbidity and vitamin concentrations related to appropriate standards.

Niacin. Microbiological assay was conducted by the method of Sarett et al. (1945) using *L. plantarum*.

Sugar analyses

Sugar mixtures from mung beans and dried sprouts were converted to their trimethylsilyl ethers (Delente and Ladenburg, 1972; Becker et al., 1974) and analyzed by gas chromatography.

RESULTS & DISCUSSION

VALUES IN TABLE 1 are on the basis of fresh weight as consumed, for ease in estimating nutritional value and in comparing values in Handbook 8 (Watt and Merrill, 1963). Proximate and mineral analyses are single analyses, while the vitamin analyses are averages of replicates. Calories were calculated as 4/g of carbohydrate and protein and 9/g of fat. When calculated on the solids basis (data not presented here because solids can be calculated from Table 1) the protein content of all of the sprouts was higher than that of the seeds (from 106.6% of the original in lentils to 119% in mung beans) due in part to a loss of leachable sugars and seed coats during the sprouting procedure and partly due to protein synthesis. Evidence of protein synthesis was presented earlier by Klein (1955) who reported a rise in the amino acid content of lettuce seeds during germination. Young and Varner (1959) concluded that the protein in the enzymes of germinated peas was newly formed from seed nitrogen compounds. Seed nitrates are not analyzed in the usual Kjeldahl nitrogen method but when reduced to plant proteins or ammonium compounds they are measured. Fat decreased upon sprouting in all tests.

B-Carotene was not determined on either the seeds or sprouts because they are not significant sources of vitamin A.

Changes in thiamine content during sprouting were small. Riboflavin content of the sprouts on the dry basis increased to three times the original in the alfalfa seeds and about 2.5 times the original content in mung and soybeans, but was the same in lentil sprouts. The niacin content of all the sprouts was greater than the seeds while alfalfa and mung beans showed larger increases than the lentils and soybeans. Ascorbic acid content of mung and soybeans was too low to measure; however, soybean sprouts contained measurable amounts, and the other three kinds of sprouts contained much more ascorbic acid than the seeds. Mineral contents were usually the same

[1] Dept. of Food Science & Nutrition, Colorado State University, Fort Collins, CO 80523

Table 1—Nutrients per hundred grams of seeds or sprouts

Sample	Water (%)	Food energy (Cal.)	Protein (g)	Fat (g)	Fiber (g)	Ash (g)	Calcium (mg)	Iron (mg)	Zinc (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Ascorbic acid (mg)
Alfalfa													
Seed	7.4	389	35.1	12.6	7.9	3.1	136	12.9	6.9	1.08	0.58	1.8	26
Sprouts													
Raw	88.3	41	5.1	0.6	1.7	0.4	28.0	1.4	1.0	0.14	0.21	1.6	16
Cooked	87.5		5.1	–	1.7	0.4	28.3	1.4	1.0	0.12	0.20	0.8	11
Lentils													
Seed	9.6	340	26.1	1.6	4.6	2.6	33.0	12.8	4.6	0.72	0.29	3.2	7
Sprouts													
Raw	72.7	104	8.4	0.3	1.1	0.8	12.0	3.0	1.5	0.21	0.09	1.1	24
Cooked	68.7		8.8	–	1.1	0.8	13.7	3.1	1.6	0.22	0.09	1.2	24
Mung beans													
Seed	10.1	334	22.9	1.4	4.9	3.4	83	11.6	3.8	0.70	0.47	1.8	–
Sprouts													
Raw	85.9	53	4.3	0.2	0.6	0.6	13.0	1.9	0.9	0.14	0.18	1.1	20
Cooked	84.3		4.3	–	0.7	0.6	13.1	1.9	0.9	0.14	0.18	1.2	16
Soybeans													
Seed	8.4	428	38.2	20.1	5.1	4.6	220	1.5	6.2	1.19	0.23	3.0	–
Sprouts													
Raw	73.2	105	12.0	2.6	2.3	3.0	75.0	0.4	1.6	0.32	0.16	1.1	12
Cooked	67.2		13.1	–	2.5	3.2	81.7	0.4	2.1	0.42	0.19	1.1	12

except for slight absorption of calcium from tap water.

Nutrient losses due to cooking were small except for the loss of niacin and ascorbic acid in alfalfa sprouts. The cooking time was short and stir-fry methods used for sprouts are not destructive of nutrients.

Dry mung beans have a low glucose (0.4%), fructose (0.04%) and sucrose (1.6%) content and significant amounts of galactose-containing sugars (3.9%). Upon sprouting, glucose and fructose increased tenfold, sucrose doubled, and the galactose-containing sugars disappeared.

The analytical values of the sprouts reported here suggest that they are a nutritive addition to the human diet and compare well with their fresh vegetable counterparts.

REFERENCES

Anon. 1974. Sprouts in your Kitchen. "Sunset," Central ed. 152(2): 64 (Lane Magazine & Book Co., Menlo Park, Calif.).

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, D.C.

Becker, R., Olson, A.C., Frederick, D.P., Kon, S., Gumbmann, M.R. and Wagner, J.R. 1974. Conditions for the Autolysis of alpha-galactosides and phytic acid in California small white beans. J. Food Sci. 39: 766.

Delente, J. and Ladenburg, K. 1972. Quantitative determination of the oligosaccharides in defatted soybean meal by gas-liquid chromatography. J. Food Sci. 37: 372.

Freed, M. 1966. "Methods of Vitamin Assay," 3rd ed, p. 320; p. 127. Interscience Publishers, New York.

Johnson, B.C. 1948. "Methods of Vitamin Determination," p. 58. Burgess Publishing Co., Minneapolis, Minn.

Klein, S. 1955. Ph.D. thesis, Jerusalem. Quoted in Mayer, A.M. and Poljakoff-Mayber, A. 1963. "The Germination of Seeds." The Macmillan Co., New York.

Pippen, E.L. and Potter, A.L. 1975. Quantitating the recovery of thiamine (vitamin B_1) from Decalso in the thiochrome method for the determination of thiamine. J. Ag. & Food Chem. In press.

Randall, V.G., Pippen, E.L., Potter, A.L. and McCready, R.M. 1975. Determination of total ascorbic acid in vegetables from alcohol slurries. J. Food Sci. 40:894.

Reuter, F.W. III and Raynolds, W.L. 1974. Proceedings of a symposium 'Protein-Metal Interactions.' In "Adv. in Experimental Medicine & Biology," Ed Friedman, M. Plenum Press, New York.

Sarett, H.P., Pederson, R.L. and Cheldelin, V.H. 1945. Microbiological assay methods for nicotinic acid. Arch. Biochem. 7: 77.

USDA. 1971. Nutrient values of foods. Home & Garden Bull. No. 72. USDA, Washington, D.C.

Watt, B.K. and Merrill, A.L. 1963. "Composition of Foods," Agricultural Handbook No. 8, U.S. Dept. of Agriculture, Washington, D.C.

Whyte, K.C. 1973. "The Complete Book of Sprouting." Troubador Press, San Francisco, Calif.

Young, J.L. and Varner, J.E. 1959. Enzyme synthesis in the cotyledons of germinating seeds. Arch. Biochem. Biophys. 84: 71.

Ms received 1/24/75; revised 3/17/75; accepted 3/22/75.

We thank members of the Chemical Analysis Research Unit, Western Regional Research Center, Albany, Calif., for aiding in chemical, vitamin and mineral analyses.

Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

ANTOINETTE A. BETSCHART
USDA Western Regional Research Center, ARS, Berkeley, CA 94710

FACTORS INFLUENCING THE EXTRACTABILITY OF SAFFLOWER PROTEIN (Carthamus tinctorius L.)

INTRODUCTION

THE LIMITED SUPPLY and disproportionate distribution of food throughout the world has been one of the major problems of the twentieth century. Less reliance upon animal protein and increased consumption of plant protein by humans has been proposed as a partial solution (Anonymous, 1974). Spiraling costs have directed the attention of both developed and developing nations toward plant protein. One such protein source which has not been fully explored is safflower (*Carthamus tinctorius* L.).

The world production of safflower increased sharply in the early 1960's as a result of the increased use of the highly unsaturated oil as an edible, rather than an industrial, oil. Mexico and the United States are currently the leading producers with total world production estimated at over 500,000 tons for 1974. With safflower seeds containing approximately 15% protein, this represents a potential of some 75,000 tons of protein.

The utilization of safflower protein has been reviewed (Betschart et al., 1975). Processes have been patented which describe the preparation of protein concentrates and flour from safflower seeds or meal (Kopas and Kneeland, 1966; Goodban and Kohler, 1970). These methods involve either cracking or milling the seeds, followed by classification on the basis of particle size.

Safflower meal, a by-product of the oil industry, is generally separated into high fiber and high protein fractions. These fractions are currently marketed as animal feeds. The high protein fraction is not suitable for human consumption because of the presence of glycosides which have been reported to be responsible for the bitter flavor and cathartic activity of the meal (Palter et al., 1972). The high protein fraction may also contain as much as 17% fiber. Thus, the extraction and preparation of a protein isolate from this meal is one method of preparing safflower protein for human consumption. The extracted residue could either be used for animal feed as such, or marketed in combination with the high fiber fraction.

In earlier work, Van Etten et al. (1963) described the extraction of nitrogen from safflower kernel meal. The objective of this study was to examine the influence of various factors, including heat treatment of the meal, upon extractability of safflower protein.

EXPERIMENTAL

Materials

Safflower seeds were obtained from a commercial source (Pacific Vegetable Oil International, Inc. (PVO), Richmond, CA). Three experimental meals were prepared from these seeds which differed in heat treatment. First, the unheated control meal was prepared in the laboratory by twice extracting seeds ground in a Morehouse Mill with hexane at 25°C and air drying the defatted meal. Second, commercial expeller press cake (PVO), i.e., the residue after seeds have been mechanically prepressed prior to final solvent extraction, was used. The press cake, which reaches temperatures of from 85–93°C in the expeller, was twice extracted with hexane at 25°C and air dried in the laboratory. Third, a commercial meal (PVO) was used, which had been pre-pressed, extracted, heated and desolventized in a desolventizer-toaster at temperatures of 107–110°C. Each of the meals was passed through a 40 mesh sieve to remove most of the fibrous fraction.

Methods

Fractionation. A method similar to the classical fractionation procedure of Osborne and Mendel (1914) was used to fractionate safflower protein. Duplicate 10g samples of the unheated, control meal were twice extracted successively for 2 hr with 100 ml of double distilled water, 1N NaCl, 70% aqueous alcohol, and 0.1N NaOH. The extracts with each solvent were pooled, centrifuged at 2,000 × G for 30 min, and filtered through Whatman No. 1 paper. Each of the pooled extracts were then dialyzed through a cellulose acetate membrane against 10 volumes of double distilled water with four changes for 48 hr. All operations were performed at 4°C. Extracts were freeze dried at shelf temperatures of ≤15°C and milled to pass through a 20 mesh sieve.

Extraction of nitrogen. Safflower meals were extracted according to a previously described method (Betschart, 1974) with minor modifications. Extracts were centrifuged at 4,000 × G, 25°C for 15 min, filtered through Whatman No. 1 paper, and Kjeldahl N was determined on an aliquot of the supernatant. Percent nitrogen (N) extracted was calculated as mg N in the supernatant/mg N in the meal × 100. The influence of such parameters as extractant, extraction time (15–120 min), extraction temperature (10–70°C), concentration of the meal (1–10% w/v), and ionic strength was investigated using the control meal. After conditions for extraction were established, the three experimental meals were extracted at pH 2 to 10.

Precipitation of proteins. Portions (7.5g) of each of the meals were extracted with 150 ml of water adjusted to pH 8. The centrifuged, filtered extract was adjusted to pH 1.5–10.5, held for 30 min, and centrifuged at 4,000 × G for 15 min. The quantity of N precipitated, as a function of pH, was determined indirectly by Kjeldahl analysis of N remaining soluble in the supernatant.

All experiments were duplicated and all values were plotted.

Analyses. Proximate analyses were conducted according to AOAC procedures (1970). Amino acids analyses were determined by the method of Kohler and Palter (1967). Protein is expressed as N × 6.25 (crude protein) and N × 5.3 (FAO, 1970).

RESULTS & DISCUSSION

Composition

The distribution of protein, crude fat and crude fiber within the safflower seeds used in this study is shown in Table 1. The kernel is high in oil and protein, whereas the hull contains most of the fiber. After the oil has been extracted and the major portion of the fiber removed by screening, a high protein meal remains which contains 7.2–8.9% N (Table 1). The high fiber fraction of the meal typically consists of approximately 3% N, 2% crude fat and 50% crude fiber. This fraction serves as a source of fiber in feed formulations.

Fractionation of protein

The classical fractionation of the unheated, control safflower meal protein into general classes, on the basis of solubility, provided information for further extraction studies. The major protein fractions were soluble in salt or dilute alkali (Fig. 1). Since all fractions were dialyzed, they should be free of low molecular weight, N-containing compounds. On the basis of extracted, nondialyzable N, 18.1% was water soluble, 41.5% salt soluble and 39.1% alkali soluble. Although 70%

Table 1–Composition of safflower constituents and meal (Percent-moisture free basis)

Sample	Nitrogen	Protein (N X 6.25)	Protein (N X 5.3)	Crude fat	Crude fiber	Ash
Safflower seed	2.63	16.44	13.94	44.23	21.94	2.68
Kernel	3.58	22.38	18.97	65.15	2.74	3.13
Hull	1.08	6.75	5.72	13.03	49.38	1.96
Meal						
Unheated, control	7.54	47.13	39.96	6.29	13.01	7.74
Extracted expeller press cake	8.88	55.50	47.06	1.74	9.95	8.82
Commercial, desolventized	7.21	45.06	38.21	1.76	17.33	7.47

aqueous alcohol extracted nearly 18% of the total solids of the meal, the N content of this fraction was less than 0.2%. The salt and alkali soluble fractions contained more than 17% N, whereas the N content of the water soluble fraction was 11.7% (Table 2). When compared with values in the literature, safflower protein contained less of the water and salt soluble, and more of the alkali soluble fractions than did sunflower meal (Gheyasuddin et al., 1970).

Differences in the amino acid composition of the protein fractions are shown in Table 2. As compared with the original meal, the water soluble fraction contained equivalent or higher quantities of seven of the essential amino acids; the proteins soluble in water contained markedly higher quantities of lysine, threonine, phenylalanine and tyrosine. The alkali soluble fraction was considerably higher in isoleucine, methionine, cystine and the aromatic amino acids than was the salt soluble fraction. The reported amino acid composition of safflower protein isolate (Van Etten et al., 1963) was intermediate between that of the salt and alkali soluble fractions.

From these data it appears that a fraction of safflower protein, higher in lysine than the original meal, could be initially extracted with water. A second extraction at an alkaline pH would extract most of the remaining protein.

Extractability

Experiments designed to establish optimum conditions for protein extraction were conducted on unheated, control meal at pH 8.5 and 25°C unless otherwise specified. Data from experiments in which N was precipitated by either 1N HCl or 10% (w/v) trichloroacetic acid showed that from 88–92% of extracted safflower N was precipitated. Thus, from 8–12% of the extractable N would not be recovered if acid were used to isolate the protein.

Time. Using a meal concentration of 5%, N extractability was maximized within 45 min (Fig. 2). Approximately 80% of the safflower meal N was removed with extraction times of from 45–120 min; slightly smaller amounts were extracted at 15 and 30 min. Time was also reported to have a minimal effect upon the extraction of N from sunflower meal (Gheyasuddin et al., 1970).

Temperature. The heat stability of 5% solutions of safflower protein extracted at pH 8.5 was established in preliminary experiments. From 96–100% of the extracted N remained in solution at temperatures of from 10–80°C. At 90°C and above, however, the protein began to precipitate with from 60–65% remaining in solution at 95°C. Subsequently, extraction temperatures of from 10–70°C were examined.

When safflower meal was extracted at various temperatures, the least effective extraction temperature was 10°C, whereas approximately 80% of the N was extracted at from 20–40°C (Fig. 3). Since extractability increased only slightly at 60 and 70°C, an ambient temperature of 25°C was selected for extraction. The observed temperature effects were similar to

Table 2–Essential amino acid composition of safflower meal and protein fractions

Fractions	FAO provisional pattern	Meal	Fractions: Water soluble	1N NaCl soluble	0.1N NaOH soluble	Extracted residue
		g amino acid/16g nitrogen				
Amino acid						
Isoleucine	4.00	3.81	4.21	3.21	4.35	3.35
Leucine	7.04	6.42	7.08	5.91	6.84	5.62
Lysine	5.44	2.83	4.55	2.17	1.97	2.62
Methionine + Cystine	3.52	3.57	4.20	1.80	4.43	1.77
Phenylalanine + Tyrosine	6.08	6.27	7.67	6.42	9.21	5.80
Threonine	4.00	3.17	4.88	2.75	3.07	2.75
Tryptophan	0.96	–	–	–	–	–
Valine	4.96	5.88	6.46	5.03	5.35	4.63
% Nitrogen	–	9.31	11.71	17.16	17.45	1.41
% Nitrogen recovered	–	92.5	93.8	90.9	89.6	83.8

those reported for sunflower meal in which N extractability increased at temperatures up to 45°C, and began to decrease at 75°C (Gheyasuddin et al., 1970).

Concentration. The extractability of safflower meal N was examined at pH 1.8 through 10.2 and at concentrations of 1, 5 and 10% (w/v). Varying the solvent to meal ratios had negligible effects upon N extractability (Fig. 4). To avoid the use of excessive quantities of solvent, future laboratory experiments were conducted using a meal concentration of 5%.

Other extractants. Various solvents were investigated in an attempt to enhance N extractability. Increasing the ionic strength with NaCl at pH 8.3 and 10.0 diminished the extractability of N. Reducing agents, such as 0.1M mercaptoethanol or 0.001 or 0.1M sodium bisulfite in a sodium borate buffer at pH 8.0, did not significantly increase N extraction. Thus, experimental conditions for extraction were a 5% concentration of meal at 25°C for 60 min. These conditions were used to evaluate the influence of various heat treatments upon the N extractability of safflower meal.

Effect of heat treatment of meal on nitrogen extractability

The influence of pH upon N extractability of safflower meal exposed to various heat treatments is apparent in Figure 5. pH was adjusted with either 0.1 or 1.0N HCl or NaOH. The commercially prepared desolventized meal, which was exposed to the highest temperatures (107–110°C) during processing in

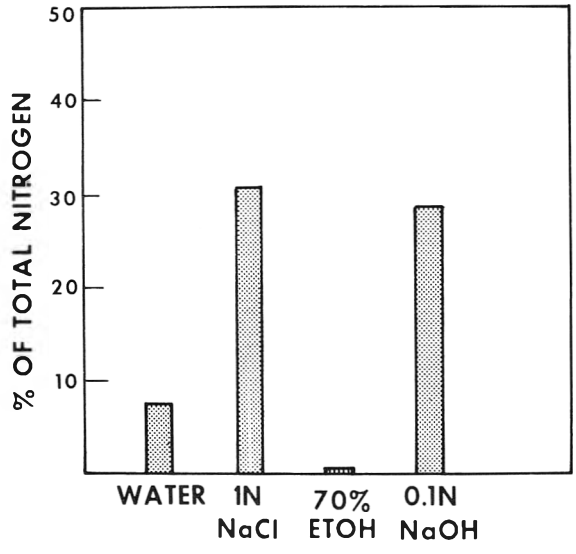


Fig. 1–Fractions of safflower meal protein soluble upon successive extraction of the unheated, control meal with various solvents and subsequent dialysis of the extracts.

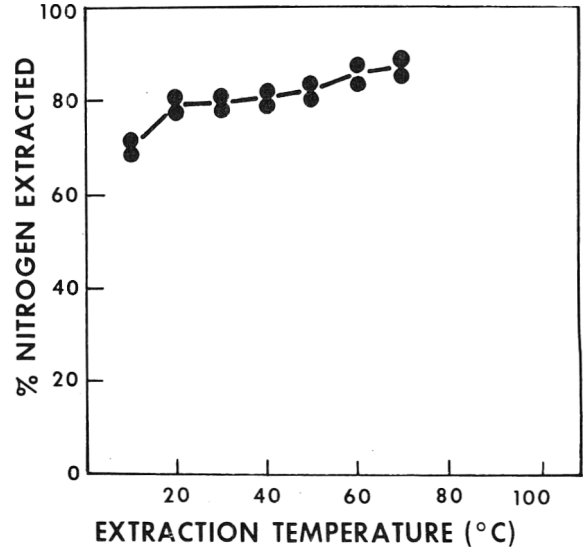


Fig. 3–Influence of extraction temperature upon extractability of nitrogen from unheated, control safflower meal at 5% concentration, pH 8.5, for 60 min.

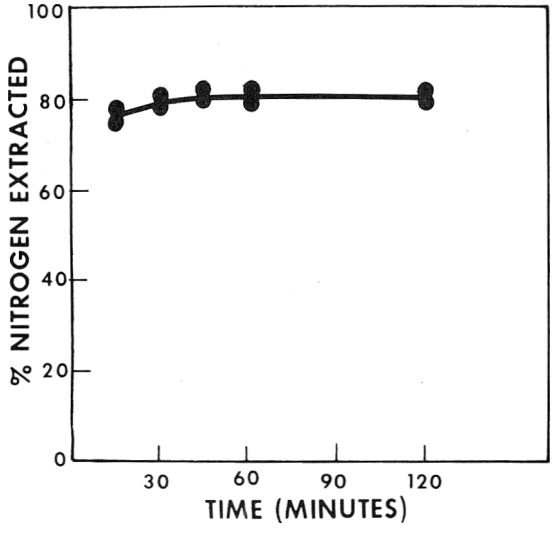


Fig. 2–Influence of extraction time upon extractability of nitrogen from unheated, control safflower meal at 5% concentration, pH 8.5 and 25°C.

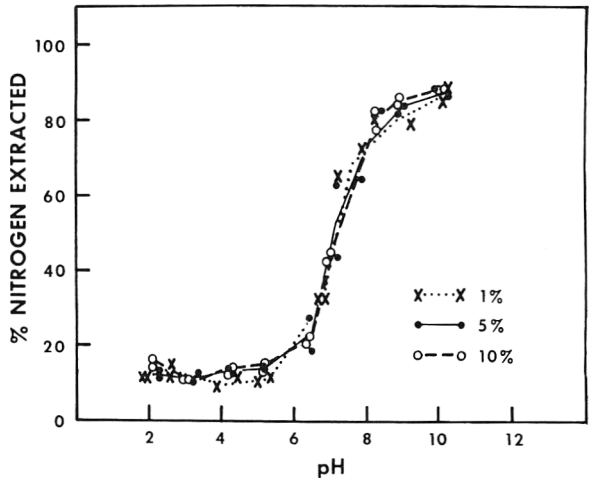


Fig. 4–Influence of concentration of meal (w/v) upon extractability of nitrogen from unheated, control safflower meal at pH 1.8–10.2, and 25°C for 60 min.

the desolventizer-toaster, exhibited the poorest N extractability. The percent N extracted from the desolventized meal at pH 8, 9 and 10 was 60, 68 and 73%, respectively. The N extractability of the unheated, control meal at these same three pH values was 75, 83 and 88%, respectively. The extractability of the expeller cake was similar to that of the control meal at most pH values. At pH 9, 68, 80 and 83% of the nitrogen was extracted from the desolventized meal, the expeller cake and the control meal, respectively.

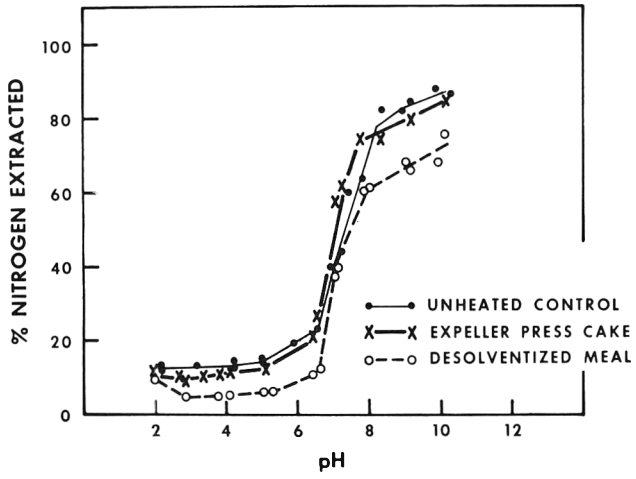


Fig. 5—Influence of heat treatment of safflower meal upon extractability of nitrogen as a function of pH. Meals included the unheated, control in which oil was extracted at 25°C; the expeller press cake heated to from 85–93°C; and the commercial, desolventized meal heated to from 107–110°C. Extraction conditions: 5% concentration, and 25°C for 60 min.

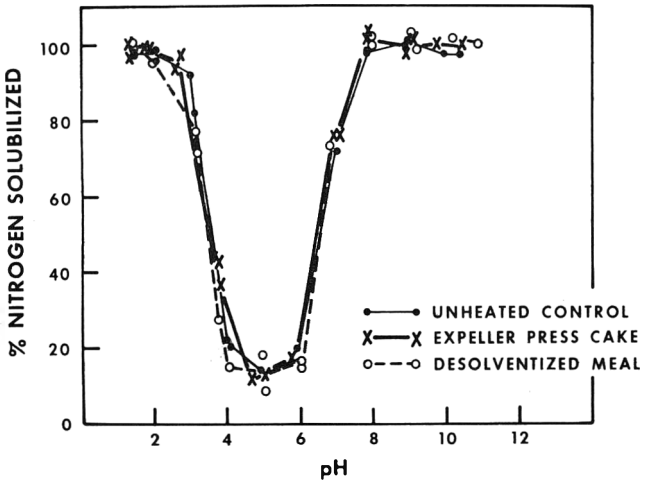


Fig. 6—Precipitation of extracted nitrogen from various safflower meals as a function of pH.

Nitrogen solubility is often taken as a general indication of protein denaturation. The protein of the desolventized meal, which was exposed to the highest temperatures, appears to be the most denatured. It may be inferred from these data that the extractability of safflower protein is not markedly influenced by temperature of 85–93°C, i.e., the temperature of the expeller press cake. The higher temperatures of 107–110°C attained in the desolventizer-toaster, however, are sufficient to impair extractability.

Precipitation of extracted nitrogen

The precipitation of extracted N as a function of pH indicates the quantity of protein N present, as well as the point of maximum precipitation for protein recovery. The precipitation curves of N extracted from the heat treated and control safflower meals were similar (Fig. 6). From 95–100% of the extracted N remains soluble below pH 2, and above pH 8. Minimal solubility occurred at pH 4 through 6, with pH 5 being the point of minimum solubility and/or maximum precipitation. It is apparent that 10–12% of the N extracted from the three meals was not precipitated at pH 5. This is in agreement with the nature of N precipitation by 10% (w/v) trichloroacetic acid, as discussed previously. Although heat treatment affects the quantity of protein which is extracted, that fraction which is extracted from meals exposed to various heat treatments appears to precipitate similarly as a function of pH.

In summary, properties of various protein fractions, and conditions which influence extraction and precipitation of safflower meal protein, have been described. Studies are in progress to evaluate the composition, nutritional value, functional properties and yield of safflower protein isolates prepared under selected conditions.

REFERENCES

Anonymous. 1974. "Western Hemisphere Nutrition Congress IV." Nutrition Today 9(7): 28.

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Agricultural Chemists, Washington, D.C.

Betschart, A.A. 1974. Nitrogen solubility of alfalfa protein concentrates as influenced by various factors. J. Food Sci. 39: 1110.

Betschart, A.A., Lyon, C.K. and Kohler, G.O. 1975. Sunflower, safflower, sesame, and castor protein. In "International Biological Programme Handbook, Protein Syntheses," Ed. Pirie, N.W. (In press).

FAO. 1970. Amino acid content of foods and biological data on proteins. Food and Agriculture Organization Nutritional Studies No. 24. Rome.

Gheyassudin, S., Cater, C.M. and Mattil, K.F. 1970. Effects of several variables on the extractability of sunflower seed proteins. J. Food Sci. 35: 453.

Goodban, A.E. and Kohler, G.O. 1970. U.S. Patent #3,542,559. Nov. 24, 1970.

Kohler, G.O. and Palter, R. 1967. Studies on methods for amino acid analysis of wheat products. Cereal Chem. 44: 512.

Kopas, G.A. and Kneeland, J.A. 1966. U.S. Patent #3,271,160. Sept. 6, 1966.

Osborne, T.B. and Mendel, L.B. 1914. Nutritive properties of proteins of the maize kernel. J. Biol. Chem. 18: 1.

Palter, R., Lundin, R.E. and Haddon, W.F. 1972. A cathartic lignan glycoside isolated from Carthamus tinctorus. Phytochem. 11: 2871.

Van Etten, C.H., Rackis, J.J., Miller, R.W. and Smith, A.K. 1963. Amino acid composition of safflower kernels, kernal proteins and hulls, and solubility of kernel nitrogen. J. Agr. Food Chem. 11: 137.

Ms received 2/23/75; revised 5/13/75; accepted 5/20/75.

The author sincerely thanks Dee E. Spanevello for technical assistance, Marian E. Allis for amino acid analyses, and Linus V. Richards for nitrogen analyses.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Dept. of Agriculture to the exclusion of others that may be suitable.

MARIAN F. CHASTAIN, STELLA JIA-SHIOW SHEEN and TOMMIE JO COOPER
Dept. of Nutrition & Foods, Auburn University, Auburn, AL 36830
and D. R. STRENGTH
Dept. of Animal & Dairy Science, Agricultural Experiment Station, Auburn University, Auburn, AL 36830

COCONUT BREAD AS A MEANS OF IMPROVING PROTEIN NUTRITION

INTRODUCTION

THE PROBLEM of protein malnutrition, so prevalent in many parts of the world, makes the development of acceptable, economical protein rich foods imperative. Supplementation of bread would be a convenient means for increasing the protein value of diets to improve the nutritional status of populations. The protein in wheat is low in nutritional quality, for it is limited in certain amino acids, especially lysine (Block and Mandl, 1958; Bressani et al., 1960). The nutritional value of wheat flour can be improved by the addition of either the limiting amino acids or, more economically, by combination of the flour with another protein source. Soy, cottonseed, peanut and chickpea flours, fish protein concentrate, and certain other protein sources have been used to produce bread with higher protein quality as well as greater total protein content (Harris, 1967; Hart et al., 1970; Mathews et al., 1970; Mizrahi et al., 1967; Pomeranz et al., 1969; Shehata and Fryer, 1970; Sidwell and Hammerle, 1970; Tsen and Hoover, 1973).

Studies by Strength (1970) have shown the protein efficiency ratio (PER) in rats of coconut flour (CF) to be 3.26 as compared with 3.48 for casein. Incorporation of CF into bread should result in a product of higher protein quality than that of conventional wheat bread. However, no matter how nutritionally valuable a new food may be, it is not always readily accepted by a population with well-established food habits. Coconut is grown in many developing countries and thus is familiar to the inhabitants. A product containing coconut might therefore be more acceptable than one containing a completely unknown food.

The study reported here was done in two stages. The purpose of the first part was to develop an organoleptically acceptable coconut bread product. Bread produced in this part of the study was held in freezer storage before undertaking the second part, the purpose of which was to evaluate the nutritional quality of the coconut bread.

EXPERIMENTAL

CF WAS PREPARED from commercial dried ground coconut meat from coconuts grown in Quezon Province, Philippines (Durkee Famous Foods) by the procedure of Strength (1970). The dried ground coconut meat was milled and mixed with n-hexane to extract the fat. Averages of about 65% oil and 35% flour were recovered from ground dried coconut meat. This treatment resulted in flour containing less than 10% fat and approximately 20% protein. The composition of ground coconut meat, CF, and wheat flour (WF) are compared in Table 1.

Preparation of bread

Bread containing eight different levels of CF were prepared by replacing 10, 13, 15, 18, 20, 30 and 50% of all-purpose WF with CF in a standard yeast bread formula. The bread formula is shown in Table 2. Ingredients other than flour remained constant in each variation. Mixing was done by a conventional straight dough method (Bennion and Hughes, 1970).

Volume measurement

Loaf volume of the baked breads was determined by cutting each loaf in half crosswise and measuring the height in centimeters at the outer edges, at the center, and at points one-half the distance from the center to the edges. The mean of these five measurements was then recorded as index of volume (Funk et al., 1969).

Taste panel evaluation

A six member taste panel consisting of three Americans and three Chinese was selected by pretesting through triangle and ranking tests. The bread was scored for color, grain, texture, flavor and general acceptability by the panel. The score card was based on a 7-point scale in which a score of "7" was given to an "Excellent" product and "1" to a "Very Poor" product.

The freshly sliced bread samples were cut to uniform size and presented to the judges on white plates with each sample numbered. Judges were seated at individual tables and each was provided with a score sheet, a cup of room-temperature water and the bread samples.

A control bread (no CF) sample was compared with other samples each time. Each level of coconut bread was judged at least twice and if there was not close agreement among judges on a given sample, it was judged a third time. The scores were averaged and the means recorded in Table 3.

Chemical analysis of breads

After freezer storage, breads containing coconut flour at levels of 13, 15 and 20% were broken by hand into pieces approximately 1 inch in diameter and were dried in a hot air oven at 56°C for 24–48 hr to stabilize the moisture content. The dried bread samples were ground in a Wiley Mill and blended at ambient temperature and moisture. Mois-

Table 1—Composition of ground coconut meat, coconut flour and wheat flour[a]

Ingredient	Crude protein (N X 6.25) (%)	Moisture (%)	Fat (ether extraction) (%)
Coconut meat	8.02	2.41	63.66
Coconut flour	20.93	6.12	9.19
Commercial all-purpose wheat flour	11.91	9.56	0.74

[a] Figures represent averages of three analyses

Table 2—Basic bread formula

Commercial all-purpose wheat flour	330.0g
Milk	118.0 ml
Sugar	19.5g
Salt	7.5g
Commercial hydrogenated vegetable shortening	12.0g
Yeast, dry	3.5g
Water	118.0 ml

ture was determined after heating the ground blended samples at 100°C for 24 hr. Nitrogen determinations were done on the dried samples by the macro-Kjeldahl method (AOAC, 1955) and protein estimated by the formula N × 6.25.

Preparation of diets

Dried ground portions of 13, 15 and 20% CF bread were incorporated into rat diets which were formulated to contain 10% protein (Strength, 1970) from casein or from bread samples. Diets were mixed in quantities of 1 kg and stored under refrigeration at 4°C until used. Diet composition is shown in Table 4.

Salt mixture #8 (Salmon, 1964) fed at 50 g/kg of diet supplied the following in g/kg of diet: calcium, 7.33; copper, 0.016; iodine, 0.022; iron, 0.20; magnesium, 0.81; manganese, 0.04; phosphorus, 5.68; potassium, 3.15; sodium, 1.91; zinc, 0.056; sulfur, 1.06.

Vitamin mixture #6 (Salmon, 1964) fed at 20 g/kg of diet supplied the following in mg/kg of diet: vitamin A (retinol), 30.0; vitamin E (α-tocopherol), 55; vitamin K (vitamin K_1 equivalent), 50; niacin, 100; calcium pantothenate, 50; riboflavin, 20; thiamine hydrochloride, 20; pyridoxine hydrochloride, 24; inositol, 200. Fed in separate solutions were: choline chloride, 3 g/kg of diet; vitamin D_2 (calciferol), 600 IU/kg of diet; vitamin B_{12}, 50 μg/kg of diet; biotin, 500 μg/kg of diet; folic acid, 2 mg/kg of diet; calciferol was added dissolved in 0.2 ml hexane for each amount added to 1 kg of diet.

Animals and feeding

21-day old weanling male albino rats of the Charles River CD strain were used. The rats were randomly placed in six treatment groups with eight animals per group. They were housed in individual wire-bottom cages in a temperature-controlled room. For the first 3 days, the animals were maintained on commercial ration and water to stabilize them. At the end of this stabilization period one group was sacrificed. Carcasses were wrapped in aluminum foil and frozen for later total nitrogen determinations. The remaining five groups were fed the respective experimental diets and were similarly sacrificed at the end of the experimental period.

The rats were fed immediately after being placed in the individual cages and daily thereafter. Glass distilled water was supplied ad libitum. Animals were weighed at approximately the same time daily at which time the feed jars were removed, weighed and replenished.

Feed consumption and weights were recorded daily for 2 wk. At this time rats in the group receiving the 13% CF bread were killed and the carcasses were frozen. The rats of the remaining groups were fed for an additional week before they were killed and frozen. Feeding was terminated at the times indicated because of the limited supply of the bread. Strength (1970) observed that the PER for the first 2 wk was equal to the PER for the second 2-wk period of a 4-wk study.

Protein efficiency ratio

The PER was calculated as weight gain in grams for each gram of protein (N× 6.25) consumed. The individual PER values were determined for the animals at 2- and 3-wk periods, and group means were calculated from individual PER values.

Total carcass nitrogen determinations

The frozen carcasses of animals in each test group were allowed to thaw at room temperature. Fissures were made in the head and abdomen to facilitate drying which was done at 100°C for 48 hr after which the carcasses were ground. Moisture determinations were done on pooled samples from each group. Nitrogen content was determined on aliquots of the pooled samples by the macro-Kjeldahl method (AOAC, 1955).

Table 3–Effect of coconut flour on loaf volume and organoleptic qualities[a]

Percentage of coconut flour in bread	Index of volume[b] (cm)	Color	Grain	Texture	Flavor	General acceptability
0	8.9	5.8	6.1	6.2	5.1	5.5
10	8.0	5.2	5.1	5.2	5.1	5.3
13	7.8	5.3	5.1	4.8	5.6	5.3
15	7.6	5.2	4.6	4.9	5.6	5.1
18	7.3	5.1	4.5	4.6	5.5	5.0
20	6.9	4.5	4.4	4.2	5.3	4.4

[a] Optimum score = 7. Scores of control bread (with no coconut flour) are means of five judgments. Scores of 10 and 13% coconut bread are means of two judgments. Scores of 15–20% coconut bread are means of three judgments.
[b] Average measurements taken at five locations across center of vertical surface of loaf.

Table 4–Composition of diets

Diet	I Casein (g)	II 0% CF bread (g)	III 13% CF bread (g)	IV 15% CF bread (g)	V 20% CF bread (g)
Protein source	115	875	798	785	790
Cornstarch	650	–	77	90	85
Sugar	100	–	–	–	–
Vitamin mixture #6[a]	20	20	20	20	20
Salt mixture #8[a]	50	50	50	50	50
Lard	50	50	50	50	50
Ascorbic acid	1.0	1.0	1.0	1.0	1.0
Agar	10	–	–	–	–
Penicillin G	0.1	0.1	0.1	0.1	0.1
Alphacel	20	20	20	20	20
Water	24	24	24	24	24

[a] Composition given in Experimental section.

Table 5–Protein content of experimental breads

Percentage of coconut flour in bread	Crude protein[a] (N X 6.25) (%)	Protein in bread ingredients[b] WF (%)	CF (%)	Milk (%)	Yeast (%)
0	11.39	9.82	–	1.20	0.37
13	12.53	8.56	2.39	1.21	0.37
15	12.74	8.39	2.77	1.21	0.37
20	12.67	7.50	3.55	1.18	0.35

[a] Figures represent averages of three analyses done on a moisture-free basis
[b] Calculated values based upon proportion of protein in ingredient added to dough mix

RESULTS & DISCUSSION

Acceptability of CF bread

It was found possible to bake acceptable bread with up to 20% CF. Bread containing 30, 40 and 50% levels of CF were not acceptable and did not taste like bread. The dough lacked consistency and elasticity; it broke apart easily and failed to rise adequately. Neither volume measurements nor taste panel evaluations were done on these products.

In all cases, inclusion of CF resulted in a more compact loaf of lower volume. The effect of CF on loaf volume is shown in Table 3.

The crust of the coconut breads was golden brown and free from cracks. The crumb was darker and coarser than that of wheat bread, and the relatively large size of the CF particles gave the product a grainy mouthfeel.

The taste panel scores on desirability of bread (Table 3) indicated that bread containing up to 18% CF was as acceptable as the control product. Although the general acceptability of 20% coconut bread was lower, it was still considered to be desirable. The flavor of coconut bread was considered very acceptable by most of the panel members and also by other individuals who sampled the bread. A sweeter taste was ob-

Table 6—Essential amino acids[a] in wheat flour, coconut flour and in experimental breads

			Breads[c]			
	WF[b]	CF[b]	100% WF	13% CF 87% WF	15% CF 85% WF	20% CF 80% WF
Isoleucine	4617	3710	549	579	584	590
Leucine	7711	8370	913	1015	1030	1059
Lysine	2285	3590	348	405	413	431
Methionine	1285	1630	163	185	189	196
Phenylalanine	5522	5110	619	671	678	683
Threonine	2904	3650	361	411	420	433
Trytophan	1190	1180	137	151	153	158
Valine	4284	5510	531	609	624	628

[a] Calculated from published data (Church and Church, 1970; Strength, 1970).
[b] mg Amino acid/100g protein
[c] mg Amino acid/100g bread

served with increasing amounts of CF. Flavor of the bread apparently had the greatest overall effect on acceptability. The decrease in scores for general acceptability with higher levels of CF was not as great as the decrease in scores for color, grain and texture.

Freshness may be a very important factor in determining acceptability. During selection and practice trials of the taste panel, bread samples that had been frozen for several weeks were thawed and served. The bread was not as acceptable as was freshly baked coconut bread. However, it was found that panel members tended to score the coconut bread higher with successive judgements on the same samples. It seemed that the coconut-flavored bread became more acceptable as judges became accustomed to it. Taste panel data were not treated statistically because of the drift toward higher acceptability with successive judgements, and because each individual panel member had his own range of values.

Chemical analysis and nutritional quality

Table 5 shows the results of the chemical analyses for protein content of the bread along with protein values for the bread components, as calculated from published values (Church and Church, 1970; Strength, 1970). As the figures indicate, CF breads contained a higher percentage of crude protein than did 100% WF bread due to the higher protein content of CF as compared to WF. Milk and yeast also contributed small amounts of protein.

In the 100% WF bread, 86.2% of the total protein was from WF; in the 20% CF bread, only 60.0% of the total protein was from WF. As might be expected, a change in amino acid proportions resulted. Calculated data for eight essential amino acids are shown in Table 6. The levels of all amino acids were increased. This is particularly important in the case of lysine, the most limiting amino acid in wheat protein and may be important for methionine which is also present in wheat in low amounts.

Means for weight gain, protein consumption, and PER values are shown in Table 7. As expected, the response on casein diets in each of these was much greater than that on any of the bread diets at both 2 and 3 wk. The casein PER value 3.31 compares favorably with PER's of 3.08 reported by Shehata and Fryer (1970) and 3.48 reported by Strength (1970). The differences in means of weight gain, protein consumption and PER's between treatment groups were tested for significance by Duncan's Multiple Range Test (Steel and Torrie, 1960).

There was no pattern in weight gain for the diets containing different levels of CF at 2 wk. However, weight gain after 3 wk was significantly greater ($p \leq 0.01$) with an increase in percentage CF added to the diet. The animals on Diet IV (15% CF) did not eat and gain as well as others initially but in the third week they registered comparable weight gains.

At 2 wk there was no consistent change in protein consumption with increases in CF. The protein consumption at 3 wk was significantly ($p < 0.05$) greater on Diet V (20% CF) than on Diet IV (15% CF); however, protein consumption on Diet II (100% WF) was not significantly different at 2 or 3 wk ($p \geq 0.05$).

The PER for Diet IV appears to be out of line at 2 wk but analysis indicates a significant ($p \leq 0.05$) increase in PER as the percentage of CF increased. At 3 wk there was a significant ($p \leq 0.01$) linear response to the addition of CF. The PER for

Table 8—Effect of diet on body nitrogen content

Group	Total group dry body wt (g)	Total nitrogen/ group (g)[a]	Mean gN/100g[b]
Base[c]	188.5	19.0	10.1
Casein	516.4	33.7	6.5
100% WF	306.0	18.6	6.1
13% CF[d]	284.0	18.8	6.6
15% CF	302.0	22.2	7.4
20% CF	325.0	23.1	7.1

[a] Means of seven determinations
[b] Grams nitrogen per 100 gram dry weight of tissue
[c] Base—Not fed experimental diet; killed at beginning of experimental period.
[d] This group was fed the experimental diet for 2 wk while other groups received diets for 3 wk

Table 7—Performance of rats on coconut bread diets[a]

Diet	Mean wt gain (g)		Mean protein consumption (g)		Mean PER	
	2 wk	3 wk	2 wk	3 wk	2 wk	3 wk
Diet I: Casein	64.0	105.9	18.2	31.8	3.5	3.3
Diet II: 100% WF, 0% CF	15.0a	23.8a	11.7a	19.1ab	1.3a	1.3a
Diet III: 87% WF, 13% CF	20.8b	–	13.3b	–	1.6b	–
Diet IV: 85% WF, 15% CF	14.9a	27.8ab	10.5a	17.3a	1.4ab	1.6b
Diet V: 80% WF, 20% CF	18.1ab	32.8b	11.8a	19.9b	1.5b	1.6b

[a]Means followed by different letters (within a column) are different (5% level of significance of Duncan's Multiple Range Test).

Diet II (100% WF) was 1.25, which was only slightly higher than the PER of 1.18 for 100% WF reported by Shehata and Fryer (1970).

Table 8 shows means of the total carcass nitrogen retention of the pooled groups of rats. These data indicate that as supplementation of wheat bread with CF increased, the amount of body nitrogen retained also increased. However, the carcass nitrogen retentions of the animals fed all of the diets containing bread as a protein source was much lower than that of those on casein diets.

CONCLUSIONS

THE FOLLOWING conclusions can be drawn from these studies: (1) it is possible to bake an acceptable loaf of bread with up to 20% coconut flour; (2) the inclusion of coconut flour increases the protein content of bread from 11.39% in the all wheat product to 12.67% in bread in which coconut flour is substituted for 20% of the wheat flour; (3) the inclusion of coconut flour increases the nutritive values of bread for the rat as measured by PER and by total body nitrogen.

REFERENCES

AOAC. 1955. "Official Methods of Analysis," 8th ed. Association of Official Agricultural Chemists, Washington, D.C.
Bennion, M. and Hughes, O. 1970. "Introductory Foods," 5th ed. Macmillan Publishing Co., Inc., New York.
Block, R.J. and Mandl, R.H. 1958. Amino acid composition of bread proteins. J. Amer. Dietet. Assn. 34: 724.
Bressani, R., Wilson, D.L. Behar, M. and Scrimshaw, N.S. 1960. Supplementation of cereal proteins with amino acids. 3. Effect of amino acid supplementation of wheat flour as measured by nitrogen retention of young children. J. Nutr. 70: 176.
Church, C.F. and Church, H.N. 1970. "Food Values of Portions Commonly Used." J.B. Lippincott Company, Philadelphia.
Funk, K., Zabik, M.E. and Elgidaily, D.A. 1969. Objective measurements for baked products. J. Home Ec. 61: 117.
Harris, E.A. 1967. Use of peanut flour as a nutritive supplement in wheat bread. M.S. thesis, Auburn University, Auburn, Ala.
Hart, M.R., Graham, R.P., Gee, M. and Morgan, A.I. Jr. 1970. Bread from sorghum and barley flour. J. Food Sci. 35: 661.
Mathews, R.H., Sharpe, E.J. and Clark, W. 1970. The use of some oilseed flours in bread. Cereal Chem. 47: 181.
Mizrahi, S., Zimmermann, G., Berk, Z. and Cogan, U. 1967. The use of isolated soybean protein in bread. Cereal Chem. 44: 193.
Pomeranz, Y., Shogren, M.D. and Finney, K.F. 1969. Improving bread making properties with glycolipids. 2. Improving various protein enriched products. Cereal Chem. 46: 512.
Salmon, W.D. 1964. Relative potency of diets containing amino acids and proteins for promoting growth of rats. J. Nutr. 82: 76.
Shehata, N.A. and Fryer, B.A. 1970. Effect on protein quality of supplementing wheat flour with chickpea flour. Cereal Chem. 47: 663.
Sidwell, V.D. and Hammerle, O.A. 1970. Changes in physical and sensory characteristics of doughs and of bread containing various amounts of fish protein concentrate and lysine. Cereal Chem. 47: 739.
Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw Hill Book Company, New York.
Strength, D R. 1970. Preparation, characterization, and evaluation of coconut protein. SOS/70 Proceedings, Third International Congress of Food Science and Technology, Washington, D.C.
Tsen, C.C. and Hoover, W.J. 1973. High-protein bread from wheat flour fortified with full-fat soy flour. Cereal Chem. 50: 7.
Ms received 10/24/74; revised 4/23/75; accepted 4/28/75.

Preliminary report presented at a meeting of Southern Agricultural Scientists, Richmond, Va., February, 1972.

R. W. BUESCHER, W. A. SISTRUNK and P. L. BRADY
Dept. of Horticultural Food Science, University of Arkansas, Fayetteville, AR 72701

EFFECTS OF ETHYLENE ON METABOLIC AND QUALITY ATTRIBUTES IN SWEET POTATO ROOTS

INTRODUCTION

PREVIOUS INVESTIGATIONS have shown that ethylene stimulates levels of catecholase (Stahmann et al., 1966), peroxidase (Birecka and Miller, 1974; Gahagan et al., 1968; Imaseki, 1970; Stahmann et al., 1966), phenylalanine ammonia lyase and phenolics (Imaseki et al., 1968) in excised tissues from sweet potato roots. The oxidation of phenolics by peroxidase and catecholse is well known to lead to discoloration of plant products (Arthur and McLemore, 1956; Buescher et al., 1974; Craft and Audia, 1962; Sheen, 1974). However, the effects of ethylene on phenolic oxidase systems and processed quality attributes in intact sweet potato roots have not been reported. Since many organisms emanate ethylene (Abeles, 1973) it is likely that sweet potato roots would be exposed to concentrations of ethylene that are higher than levels produced endogenously during post harvest handling and storage. Furthermore, healthy roots may be in contact with roots with stimulated ethylene production caused by wounding or microbial infection (Imaseki et al., 1968; Stahmann et al., 1966).

In this investigation we determined the effects of ethylene on respiration, phenolic content, peroxidase, catecholase and beta-amylase activities in fresh sweet potato roots as well as associated changes in the quality attributes of flavor, firmness and discoloration in baked roots.

METHODS & MATERIALS

Preparation and treatments

Freshly harvested sweet potato roots (*Ipomoea batatas*), variety Centennial, were obtained from a local processor, washed in water containing 200 ppm chlorine, air dried, and sorted into uniform lots of 10 roots. Each lot represented one of three replicates used for each treatment and sampling time. Each lot was weighed and then placed in 8 liter respiration chambers with air flow adjusted to approximately 200 ml per minute. Treatments consisted of (1) control (air), (2) 10 ppm ethylene during and after cure, (3) 10 ppm ethylene during cure and air after cure, and (4) air during cure and 10 ppm ethylene after cure. Ethylene was mixed in the air stream by the system previously described by Shaw and Kattan (1971). Curing was accomplished at 29.4°C and 90–95% RH for 7 days. After curing the roots were stored at 15.6°C with 85–90% RH (Lutz et al., 1968). Roots were removed for evaluations of fresh and baked attributes after 0, 7, 14 and 21 days in storage.

Respiration

Respiration rates were determined daily from the levels of CO_2 in the air streams. A Fisher-Hamilton Gas Partitioner was used to analyze CO_2 in the gas samples (0.3 ml) taken with a Hamilton teflon tip syringe.

Enzymes and phenolics

Peroxidase, catecholase and beta-amylase were extracted by homogenizing internal tissue storage (cross sectional slices of roots with periderm and vascular cambium removed) with 0.15M NaCl in 0.05M sodium phosphate buffer at pH 6.5 (1g tissue/5 ml buffer), and centrifuging for 10 min at 10,000 rpm. The supernatant was assayed after appropriate dilution for enzymatic activity. Peroxidase activities were determined by the increase in absorbancy at 460 nm of a mixture containing the enzyme extract, hydrogen peroxide and 0-dianisidine at pH 6.5 and 20°C. Catecholase activities were measured by the increase in absorbancy at 420 nm of a solution at 20°C containing 0.06M catechol in 0.05M phosphate buffer (pH 6.5) and a portion of the enzyme extract (Buescher et al., 1974). Beta-amylase activities were assayed by measuring the increase in reducing units (Nelson, 1944) of a mixture containing 1% soluble starch in 0.016M acetate buffer (pH 4.6) and the enzyme extract incubated for 5 minutes at 30°C.

Phenolics were extracted by homogenizing internal storage tissue with 80% hot ethanol and repeated washing of the pellet after centrifugation. The total phenolic content was assayed by the method described by Swain and Hillis (1959) using Folin-Ciocalteau reagent.

Baking and quality

Sweet potato roots were baked for 75 min at 168°C and then frozen until all treatments could be evaluated at the same time. Sensory evaluations were conducted by a five member panel on a scale of 1 to 10 for flavor, firmness and discoloration.

RESULTS & DISCUSSION

RESPIRATION as determined by CO_2 evolution was enhanced by the presence of ethylene during and after curing (Fig. 1). Ethylene stimulated respiration after 6 hr at 29.4°C while maximum stimulation was observed after 24 hr. Respiration of the control during curing was similar to that reported by Appleman et al. (1943). The magnitude of CO_2 stimulation by ethylene after curing was about the same for roots which

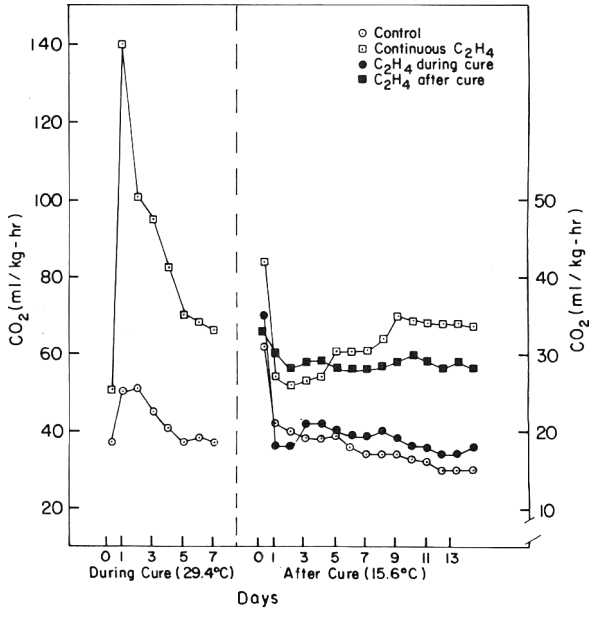


Fig. 1—Effect of ethylene on respiration of sweet potato roots during and after curing.

had been treated during curing or only after curing. Furthermore, no residual effects on respiration were evident after ethylene was removed. Imaseki et al. (1968) have reported similar findings for the stimulation of oxygen consumption by ethylene in excised root tissues, i.e., the physical presence of ethylene was required for increased respiratory activity.

Figure 2 illustrates the close relationships between peroxidase and catecholase activities, and phenolic content in fresh tissue and discoloration in baked roots. These parameters were enhanced by the presence of ethylene. Ethylene induced stimulation was less rapid when ethylene was applied after curing (15.6°C) than during the cure process (29.4°C) which was probably due to the temp differential. When ethylene was removed, peroxidase activity declined but remained significantly higher than the activity in roots not exposed to ethylene. Catecholase activity and discoloration were not significantly affected by the removal of ethylene although phenolic levels did continue to increase. Discoloration of fresh roots was not immediately observed after slicing which indicates that the discoloration of ethylene treated roots occurred during baking.

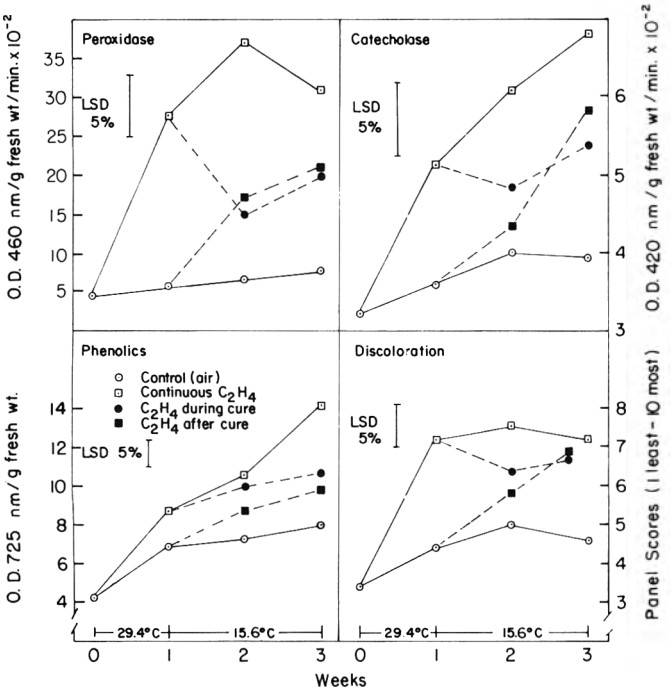


Fig. 2–Effects of ethylene on peroxidase and catecholase activities and phenolic content in sweet potato roots during and after curing and associated changes in discoloration of the baked product.

Flavor evaluation of baked sweet potatoes treated with ethylene appeared to be inversely related to the levels of peroxidase, phenolase, phenolics, and discoloration (Fig. 3). Ethylene induced enhancement of phenolic content and phenolic oxidizing enzymes in fresh roots not only enhanced discoloration but also appeared to manifest adverse flavors in baked roots. These observations are substantiated by other investigations which have shown that polymerized phenolics produce off-flavors especially astringency and bitterness to food products (Joslyn and Goldstein, 1964; Van Buren et al., 1966). In addition, carrot roots treated with ethylene had increased levels of phenolics which were associated with adverse flavor development (Chalutz et al., 1969; Sarker and Ton Phan, 1974).

Roots exposed to ethylene had reduced beta-amylase activity (Fig. 4). Stahmann et al. (1966) have reported that amylase activity was increased in sweet potato tissue treated with ethylene although the species of amylase was not indicated and may have been alpha-amylase rather than beta-amylase. Since a close association has been established between firmness and starch content in canned sweet potatoes (Kattan and Lttrell, 1963), the decline in firmness (Fig. 5) which we observed in baked roots treated with ethylene, might have been caused by a stimulation of alpha-amylase activity. However, the effect of beta-amylase on firmness should not be disregarded since its activity might have been reduced when in contact with phenolic compounds during extraction (Young, 1965). In fact, a close inverse relationship is observed between beta-amylase activity and phenolic levels.

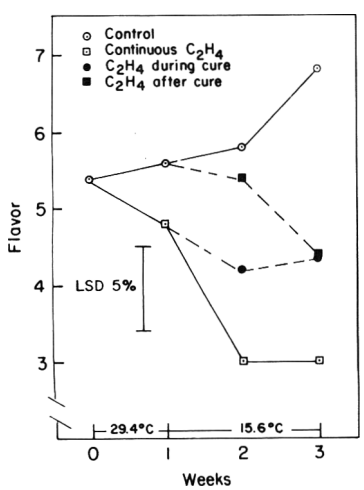


Fig. 3–Flavor of baked sweet potato roots as influenced by ethylene treatments during and after curing of the fresh roots.

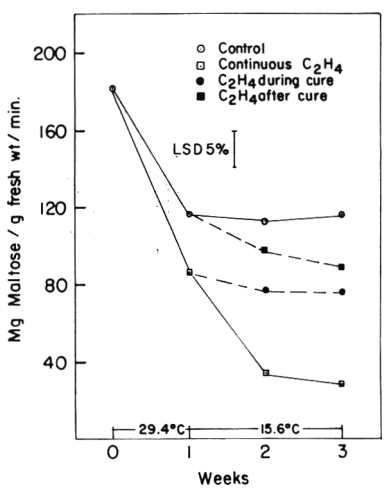


Fig. 4–Effect of ethylene on beta-amylase activities in sweet potato roots during and after curing.

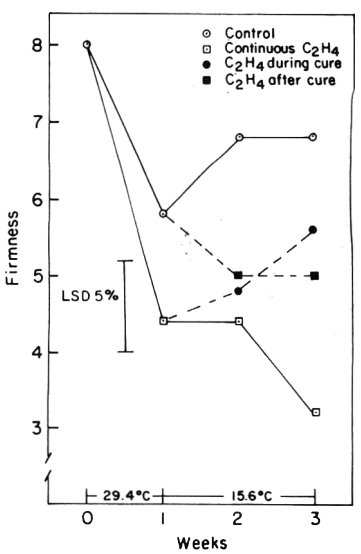


Fig. 5–Firmness of baked sweet potato roots as influenced by ethylene treatments during and after curing of the fresh roots.

In conclusion, sweet potato roots which were exposed to ethylene during or after curing had enhanced levels of peroxidase, catecholase and phenolics. Discoloration and adverse flavor development in baked roots were associated with the stimulation of phenolic levels and phenolic oxidizing enzymes. Therefore, exposure of sweet potato roots to ethylene should be avoided during storage and handling processes.

REFERENCES

Abeles, F.B. 1973. "Ethylene in Plant Biology." Academic Press, N.Y.

Appleman, C.O., Shirk, H.G., Heinze, P.H. and Brown, R.G. 1943. Curing and storage of Maryland Golden sweet potatoes. Maryland Agric. Exp. Sta. Bull. A22: 325.

Arthur, J.C. and McLemore, T.A. 1956. Properties of polyphenolases causing discoloration of sweet potatoes during processing. J. Agric. Food Chem. 4: 553.

Bernfeld, P. 1951. Enzymes of starch degradation and synthesis. Adv. Enzymol. 12: 379.

Birecka, H. and Miller, A. 1974. Cell wall and protoplast isoperoxidases in relation to injury, indoleacetic acid and ethylene effects. Plant Physiol. 53: 569.

Buescher, R.W., Reitmeier, C. and Sistrunk, W.A. 1974. Association of phenylalanine ammonia lyase, catecholase, peroxidase, and phenolic content with brown-end discoloration of snap bean pods. HortScience 9(6): 585.

Chalutz, E., Devay, J.E. and Maxie, E.C. 1969. Ethylene induced isocoumarin formation in carrot root tissue. Plant Physiol. 44: 235.

Craft, C.C. and Audia, W.V. 1962. Phenolic substances associated with wound barrier formation in vegetables. Bot. Gaz. 123: 211.

Gahagan, H.E., Hohm, R.E. and Abeles, F.B. 1968. Effect of ethylene on peroxidase activity. Physiol. Plant. 21: 1270.

Imaseki, H. 1970. Induction of peroxidase activity by ethylene in sweet potato. Plant Physiol. 46: 172.

Imaseki, H., Uchiyama, M. and Uritani, I. 1968. Effect of ethylene on the inductive increase in metabolic activities in sliced sweet potato roots. Agric. Biol. Chem. 32: 387.

Joslyn, M.A. and Goldstein, J.L. 1964. Astringency of fruits and fruit products in relation to phenolic content. Adv. Food Res. 13: 179.

Kattan, A.A. and Littrell, D.L. 1963. Pre- and post-harvest factors affecting firmness of canned sweet potatoes. Proc. Amer. Soc. Hort. Sci. 83: 641.

Lutz, J.M. and Hardenburg, R.E. 1968. The commercial storage of fruits, vegetables and florist and nursery stocks. USDA, Agr. Handbook No. 66, p. 51.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for determining glusose. J. Biol. Chem. 153: 375.

Sarkar, S.K. and Ton Phan, C. 1974. Effect of ethylene on qualitative and quantitative composition of phenol content of carrot roots. Physiol. Plant. 30: 72.

Shaw, G. and Kattan, A.A. 1971. Development of experimental CA generator. Ark. Farm Res. 20(6).

Sheen, S.J. 1974. Polyphenol oxidation by leaf peroxidases in Nicotiana. Bot. Gaz. 135: 155.

Stahmann, M.A., Clare, B.G. and Woodbury, W. 1966. Increased disease resistance and enzyme activity induced by ethylene and ethylene production by Black Rot infected sweet potato tissue. Plant Physiol. 41: 1505.

Swain, T. and Hillis, W.E. 1959. The phenolic constituents of Prunus domestica 1. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10: 63.

Van Buren, J.P., Senn, G. and Neukom, H. 1966. Proanthocyanins in apple. J. Food Sci. 31: 964.

Young, R.E. 1965. Extraction of enzymes from tannin-bearing tissue. Arch. Biochem. Biophys. 111: 174.

Ms received 1/27/75; revised 3/18/75; accepted 3/22/75.

The authors express gratitude to Steele Canning Co., Div. of Pioneer Foods, Springdale, Arkansas, for furnishing sweet potato roots for this study.

S. C. SHARMA and R. R. WOLFE
Dept. of Biological & Agricultural Engineering
Rutgers University, New Brunswick, NJ 08903
and N. F. HAARD
Dept. of Food Science, Rutgers University, New Brunswick, NJ 08903

EVALUATION OF METHODS FOR MEASURING ASPARAGUS TEXTURE

INTRODUCTION

NUMEROUS METHODS have been proposed for the evaluation of the textural quality of asparagus. These include a number of chemical and physical methods similar to those used on other food materials, as well as histochemical lignin staining since it has long been recognized that the toughness is due in large part to the lignification of fibrovascular bundles (Bittings, 1917; Isherwood, 1955). Although the general validity of some of these methods has been demonstrated, there is a need for further understanding of their interrelationships and the sensitivity of each of them to post-harvest textural changes. Since these methods differ considerably in principle, it is possible that their relative usefulness may depend on the post-harvest history of the material up to the time of evaluation.

The principal methods that have been applied to asparagus may be classified as follows: (1) chemical separation of elements responsible for toughness; (2) microscopic examination of stained sections; (3) mechanical separation of fibrous elements; (4) "shear" and pressure tests; and (5) organoleptic ratings.

The chemical analyses for crude fiber and lignin have been discussed by Joslyn (1970). He reported that recovery of cellulose varies from 60–80% and lignin recovery from 4–67%. The inconsistency in recovery of lignin, which is largely responsible for imparting toughness to the cell wall, suggests that this method would have limited value in measuring asparagus texture.

Alcohol insoluble solids (AIS) content was suggested by Lee (1943) to be a reliable indicator of asparagus stringiness. However, Kramer et al. (1960) reported that both the tender tips and tough basal segments gave high AIS readings compared to the middle portions of the spears, indicating that this method is not suitable for asparagus.

Microscopic examination of spear cross-sections stained for lignin by various chemicals, usually a combination of phloroglucinol and HCl, has been used for many years to follow fiber changes in asparagus (Bittings, 1917; Bisson et al., 1926; Lipton, 1957). Examination of both cross and longitudinal sections are of value for several reasons: they permit observations of the relative quantities and types of fibrous material present, observations on the distribution and compactness of the tough elements, and most important, an estimation of the degree of lignification as indicated by differences in staining intensity. Isherwood (1960) reported, however, that the reaction of lignin to a given stain may vary somewhat according to the age, variety and cultural conditions of the plant material used in the investigation.

A mechanical method of fiber determination was devised by Smith and Kramer (1947) which involves macerating the asparagus sample and separating the fibrous material from the parenchymatous tissues on a standard sieve. This method is rapid, when compared to chemical analyses, and was found by Kramer et al. (1949) to correlate well with organoleptic ratings for toughness. If it is recognized that the toughening process involves primarily the cementing together of true cellulose fibers by lignin (Isherwood, 1963; Northcote, 1972) then this method may be justified, since the individual cellulose fibers should pass through the sieve while the lignified structural elements are retained.

The shearing and pressure methods and associated instrumentation are reviewed extensively by Voisey (1971) and Szczesniak (1973). Various mechanical devices, including the tenderometer (Jenkins and Lee, 1940; Carolius et al., 1953) and the fibrometer (Wilder, 1948) have been developed for asparagus. Application of the so-called shear press to testing asparagus was first reported by Wiley et al. (1956). Subsequently other studies using the shear press on raw and canned asparagus were reported by Backinger et al. (1957) and Werner et al. (1963). Peak cutting forces have been found by Wiley et al. (1956) to be highly correlated (R = 0.90) with fiber determinations by the maceration method. It should be pointed out that the use of the term "shear" in this context is really a misnomer. As Szczesniak et al. (1970) suggest, the spear specimens are actually subjected to a combination of two or more types of forces which may include compression, shear and extrusion. Bourne (1966) attempted to quantitatively describe the shear and compression components involved in these methods.

Since the consumer must always be considered the ultimate judge of texture, no empirical test is of much value unless it can be correlated with human sensory evaluations. In order to obtain reliable organoleptic determinations, it is essential that the taste panel be conducted using proven statistical and psychometric procedures to minimize the degree of subjectivity (Kramer and Twigg, 1970).

The objectives of this study were to establish the relationships between each of the indirect methods and organoleptic ratings and to determine if these relationships are dependent on any of the various storage factors and spear characteristics that are known to affect asparagus texture. A further objective was to present a unified conceptual interpretation of these methods consistent with the empirical results obtained in this study.

EXPERIMENTAL

Design of experiment

Consider texture measurements (T_x, T_y) by any two of the methods and assume that their relationship can be described adequately by a simple first order equation as follows allowing for transformation if necessary:

$$T_y = a + b\,T_x \qquad (1)$$

An important question is whether or not a and b are functions of any of the storage factors or spear characteristics (Z_1, Z_2;...Z_i; i = 1,5). A first order model allowing for all of these possible effects is

$$T_y = u_o + v_o T_x + \sum_{i=1}^{5} (u_i + v_i T_x) Z_i \qquad (2)$$

In order to balance the response space and minimize data collection a $\frac{1}{2}(2)^4$ factorial design was used to handle the treatment variables: temperature, storage time, spear length and diameter. Due to the extremely large curvilinear influence of axial position on toughness, the measurements were taken at 1-in. intervals along the spear.

Material and methods

Asparagus (*Asparagus officinalis*) spears of variety Martha Washington were manually harvested early on the test day and transported to the laboratory in an insulated chest maintained at 2–5°C with ice packs. After removing any defective spears, the remainder were trimmed at ground level and classified into three length categories: viz, 7.0–8.5 in.; 9.5–11.0 in.; and others. The first two groups were then graded according to diameter, measured 5 in. from the tip end. Those falling into 0.30–0.40 in. and 0.45–0.55 in. categories were accepted from which appropriate samples of 10 spears were randomly selected for each trial.

The samples were held at 3 and 24°C for durations of 3 and 25 hr in accordance with the experimental design. Relative humidity was maintained constant at 90%. After removal from the chamber, five spears were randomly selected for organoleptic evaluation and the other five were used for the cutting, histochemical and maceration tests.

The spears selected for organoleptic evaluation were cooked in 1% salt water at 100°C for 5 min. After draining, the spears were cooled for 30 min to room temperature and cut into 1-in. segments starting at the butt end and discarding the tip portion. The specimens were placed in covered plates, coded randomly and presented to a panel of five judges which had been previously constituted using a range method similar to that described by Kramer and Twigg (1970). They were asked to rate the samples on a nine-point hedonic scale similar to the one used by Kramer et al. (1949) where 1 = no fiber, 5 = borderline objectionable, and 9 = woody, cannot chew.

From the remaining five spears, 1/16 in. thick cross sections were taken at 1-in. intervals acropetally for histochemical examination. The specimens were immersed in a 1% solution of phloroglucinol in ethanol for 5 min and then transferred to 25% HCl for 10 min (Bisson et al., 1926). Lignified elements of the tissue are known to develop a characteristic red color when treated with phloroglucinol and HCl (Weisner reaction). The degree of lignification can be estimated by the shade of coloration as the slightly lignified fibrovascular bundles appear light pink and those heavily lignified are dark red. Based on microscopic examination of the nature and color intensity of the stained elements, the following scale was devised to evaluate the degree of lignification (Sharma, 1974):

1 - No noticeable color change
2 - Vascular bundles stain light yellow color demarcating them from matrix; no visible coloring of epidermis
3 - Vascular bundles stain light pink; no coloring of epidermis
4 - Vascular bundles stain pink; light yellow coloring of epidermis
5 - Vascular bundles stain pink; epidermis appearing deep yellow or brown
6 - Vascular bundles stain pink-red; epidermis appearing pink with greenish tint
7 - Vascular bundles stain deep pink-red; epidermis appearing pink-red
8 - In addition to (7), pericyclic fibers stain light pink
9 - In addition to (7), pericyclic fibers stain deep pink-red but remain individually separate
10 - In addition to (9), some pericyclic fibers can be seen cemented together
11 - In addition to (10), cells surrounding vascular bundles stain pink
12 - In addition to (11), the cortex and ground tissues are stained giving a pink coloration to entire matrix

Peak cutting forces were determined for the remaining 15/16 in. long spear segments, using a shear cell with a single 0.035 in. thick cutting blade, similar to that reported by Wiley et al. (1956). After measuring the major and minor cross-sectional diameters, the blade was applied at the rate of 1 in./min at the center of the specimens on an individual basis.

The procedure for the maceration method of evaluating fibrousness as outlined by Smith and Kramer (1947) was used with the following modifications:

(a) With regard to position along the spear, corresponding segments from the five spears were pooled together (following the shear test) to provide sufficiently large samples for this method.
(b) Instead of transferring the separated fibrous material to a porous filter or nylon cloth, the residue was left on a U.S. No. 30 sieve for drying and weighing. The samples were dried at 100°C for 48 hr, desiccated for 30 min and weighed on an analytical balance.

RESULTS & DISCUSSION

THE DATA ANALYSIS was carried out with the objective of developing the interrelationships between the three indirect methods and organoleptic evaluation, each being in the form of Eq (2). For each pair of texture indicators, a step-wise regression analysis (Dixon, 1968) was used for selection of significant variables and appropriate logarithmic transforms.

A summary of computed results for the six relationships is presented in Table 1, giving the significant variables, parameter estimates and multiple correlation coefficients. From this table it is evident that (a) all of the textural methods studied are highly correlated; (b) the spear-diameter and axial position show notable influences; and (c) the post-harvest holding temperature and duration do not significantly affect these relationships within the range of experimentation in this study.

An examination of these results suggests that the method of expressing fiber content as determined by the maceration test should be reconsidered As shown in line 1, Table 1, the spear diameter plays a significant role in the f-E relationship. In order to explain this phenomenon, it is helpful to note that during human ingestion, it is primarily the degree of lignification of vascular structures that is sensed as toughness. For cooked asparagus, the nonlignified intervascular parenchyma cells make a relatively small contribution to the mastication forces. Furthermore, it was observed in this study that regardless of diameter, spear cross sections exhibit a similar configuration and quantity of fibrovascular bundles, i.e., approximately six concentric layers holding about 60 bundles. It is evident, therefore, that for spear segments having undergone equivalent condensation and secondary thickening of the cell walls, the amount of nonlignified tissue remaining should be a function of spear diameter.

With these concepts in mind, the significance of spear diameter in using the maceration method can be realized. Fiber content (f) represents the concentration of lignified material within the total spear mass and may be expressed as:

$$f = \frac{\text{weight of dry lignified fibrous material per unit length}}{\text{fresh weight of the spear tissue per unit length}}$$

$$= \frac{\rho_f a_c}{\rho_t A_c} \qquad (3)$$

Where: ρ_f and ρ_t are densities of fibrous material and fresh spear tissue; a_c and A_c are cross-sectional areas of fibrous and fresh spear tissue, respectively. It is clear that the fiber content value (f) as determined above is influenced by the amount of soft, noncontributing intervascular tissue. In order to have a valid indicator of the total tough material per unit length in a spear segment, rather than its concentration, the quantity $\rho_t f A_c$ should be used as per Eq (3). Since ρ_t is nearly the same as that of water and essentially constant, the toughness can be considered proportional to $f A_c$. The results of the log f-E regression analysis given in Table 1 are in complete agreement with this hypothesis since the estimated parameter for log D is highly significant and approximately twice that for log f. The P-f results shown on line 3 follow the same pattern. It should be noted that in practice, if data are available on the length of the spear segments, the appropriate value for toughness by the maceration method ($f A_c$) can be computed simply as the

Table 1–Stepwise regression analysis for developing relations between various texture indicators

No.	Prediction variable (T_y)[a]	Multiple correlation coefficients after each step: 1st Variable into analysis		2nd Variable into analysis		3rd Variable into analysis		Estimates of parameters with all significant variables in analysis: 1st Variable		2nd Variable		3rd Variable	
		Symbol	R	Symbol	R	Symbol	R	β_1	SE	β_3	SE	β_2	SE
1	E	log f	0.931	log D	0.958	X	0.968	1.55	0.193	3.82	0.917	–0.34	0.094
2	E	P	0.960	X	0.969	–	–	0.465	0.051	–0.333	0.090	–	–
3	P	log f	0.924	log D	0.972	–	–	3.115	0.146	10.328	1.203	–	–
4	E	log F	0.949	X	0.977	–	–	3.192	0.064	–0.473	0.086	–	–
5	f	F	0.929	log D	0.952	–	–	0.018	0.009	–0.978	0.229	–	–
6	P	log F	0.954	X	0.970	–	–	5.886	0.560	–0.578	0.111	–	–

[a] See nomenclature for definition of symbols

weight of the dry fibrous material divided by the total length of the spear segments and the fresh spear density.

The subjective scale (P) for the histochemical staining method was designed to be an index of the degree of lignification as shown by the nature and color intensity of the stained elements and not intended to be sensitive to the quantity of intervascular tissue. Thus this method should be generally independent of spear diameter. The results shown in Table 1 (lines 2 and 3) tend to support this contention since P is closely related to the previously accepted indicators of toughness: viz., E and f A_c.

The shear method correlated well with the other toughness indicators. However, the regression results do not show any need to account for diameter changes, as was anticipated since the raw intervascular tissue provides some cutting resistance. This must be attributed to the relatively small forces involved in cutting the nonlignified tissue compared to the total range of forces (up to 95 lb) included in the regression analysis and the experimental error associated with these force determinations. In spite of this complication, the favorable correlations associated with the shear method and the fact that it is the one method that is both objective in nature and can be applied to raw asparagus suggests its potential importance in evaluating asparagus for the fresh market and upon arrival at the processing plant.

At this point, it may be useful to discuss the effect of axial position along the spear, which has a significant influence on most of the relationships. The position factor, X, makes its greatest contribution in accounting for unexplained variation in those relationships involving the shear method (see lines 4 and 6, Table 1). This may be due to a distinct difference in the cutting action that takes place between the tip and butt ends of the spear. Towards the tip end, a simple smooth cutting action was observed whereas near the butt end, the heavily lignified tissue tended to rupture under compressive and bending forces due to failure of the weak interstitial matrix between the lignified fibrovascular bundles. Also the effects of X are common to those relationships involving organoleptic evaluation. This may be due to a difference in the degree of cooking experienced by the larger butt end specimens as compared to the tip segments which could result in a differential in the softness of the intervascular tissue. The panelists may be associating this cooking effect with the toughness and thus overscoring the segments toward the butt end of the spears. Although the position effect as described above is significant, it was decided to exclude it from the final equations. It is believed that in practical application, the simplification thus gained would far outweigh the precision lost by omitting this factor.

The six final prediction equations relating the texture methods are presented in Table 2 with corresponding correlation coefficients and standard error of estimates. All of the correlation coefficients are high, ranging from 0.950–0.970. Note that an improved correlation between the maceration and shear methods was found subsequent to the original step-wise regression analysis, by incorporating the newly validated fA_c term into a linear model. The standard errors for all of the estimated parameters are less than 5% of their respective estimates, indicating favorable confidence levels.

Subsequent to the completion of the above studies, it was decided to give special consideration to the asparagus fibrometer (Wilder, 1948) because of its extensive usage by processors. The fibrometer could not be incorporated into the previous experiment because it is simply a "GO-NO GO" device which determines the critical cut-off point along the spear dividing it into tender and tough portions. In order to find a relationship between the fibrometer and the shear method, random samples of ten spears were subjected to the same $\frac{1}{2}(2)^4$ factorial design treatments as previously used. In this case, five spears were subjected to the shear test as described earlier and the other five were evaluated with the fibrometer after processing in No. 303 sealed cans for 25 min at 115°C. For each sample, the mean peak cutting forces taken at one inch intervals were plotted against a normalized position variable designated as position modulus which is the ratio of distance along spear axis from ground level to the total spear length. By superimposing the mean "cut-off" position modulus determined by the fibrometer on this curve, a peak cutting force (F_{EQ}) for raw asparagus equivalent to the three pound fibrometer load as applied to cooked asparagus was determined for each treatment.

Regression analysis showed that, out of the four treatment

Table 2–Summary of relationships between the various texture methods

Equation	Corr coeff	SE(T_y)	SE(β_1)
E = 8.229 + 2.191 log (fA_c)	0.957	0.574	0.0965
E = 1.10 + 0.33 P	0.960	0.557	0.0269
P = 11.08 + 3.36 log (fA_c)	0.966	0.788	0.132
E = –1.628 + 4.922 log F	0.950	0.630	0.240
fA_c = –0.0346 + 0.004 F	0.970	0.0267	0.00017
P = –4.02 + 7.52 log F	0.954	0.907	0.345

variables, only the spear diameter (D) significantly influences this equivalent cutting force. This effect (R = 0.88) is defined by Eq (4).

$$F_{EQ} = 1.25 + 22.5\ D \quad (4)$$

The substantial diameter effect is not surprising since F_{EQ} includes the cutting resistance of the raw nonlignified intervascular tissue wich does not contribute to toughness as sensed by the fibrometer and consumers because it becomes very soft during the cooking process. As explained earlier, the quantity of nonlignified tissue is a function of spear diameter thus explaining the diameter effect observed here.

Considering the 9-point organoleptic scale and assuming a rating of 4 (some fiber, but not objectionable) can be taken as the critical "cut-off" level for acceptable quality, then the newly developed log F-E relationship (Table 2) indicates that spear segments registering peak cutting forces greater than 14 lb should be deemed unacceptable. In this study the spear diameters at the fibrometer cutoff ranged from 0.34–0.64 in. which if applied to Eq (4) gives an equivalent critical cutting force range of 8.9–14.4 lb. Thus the critical cutting force based on organoleptic ratings compares favorably with the fibrometer (F_{EQ}) for large diameter spears. However, with smaller spears, the fibrometer tends to be more conservative in textural evaluation.

In conclusion, it appears that a unified and consistent conceptual interpretation of these methods is possible. Secondly, precise quantitative relationships are presented for use in asparagus quality control.

NOMENCLATURE

A_c	area of spear cross section at X (sq in.)
a_c	area of the lignified fibrous tissue at X (sq in.)
D	diameter of spear cross section at X (in.)
E	organoleptic evaluation rating (1 = tender, 9 = tough)
F	peak cutting force (lb)
F_{EQ}	peak cutting force equivalent for fibrometer
f	fiber concentration (lb dry fiber/lb fresh weight)
P	histochemical rating (1 = no staining, 12 = deep pink-red staining)
R	coefficient of correlation
SE	standard error of estimate
T_x	texture measurement by method x
T_y	texture measurement by method y
X	axial position along the spear measured from ground level at time of harvest (in.)
Z	storage factor or spear characteristic
β	coefficient of regression
ρ_f	density of dry lignified fibrous tissue
ρ_t	density of fresh spear tissue

REFERENCES

Backinger, G.T., Kramer, A., Decker, R.W. and Sidwell, A.P. 1957. Application of work measurement to the determination of fibrousness in asparagus. Food Technol. 9: 585.

Bisson, C.S., Jones, H.A. and Robins, H.W. 1926. Factors influencing the quality of fresh asparagus after it is harvested. Bull. 410, California Agricultural Experiment Station.

Bittings, D.G. 1917. Deterioration in asparagus. Bull. 11, National Canners Association, San Francisco, Calif.

Bourne, M.C. 1966. Measure of shear and compression components of puncture tests. J. Food Sci. 31(2): 282.

Carolius, R.L., Lipton, W.J. and Apple, S.P. 1953. Effect of packaging on quality and market acceptability of asparagus. Bull. 35. Michigan Agricultural Experiment Station. p. 330.

Dixon, W.J. 1968. Biomedical Computer Programs, University of California Press, Berkeley, p. 218.

Goldstein, L.O., Jennings, P.H. and Marsh, H.V. 1971. L-phenylalanine ammonia-lyase activity in asparagus spears: Effect of excision and incubation. Plant and Cell Physicl. 13: 783.

Isherwood, F.A. 1955. Texture in fruits and vegetables. Food Mfg. 30: 399.

Isherwood, F.A. 1960. The formation of lignin in plant tissues. In "Phenolics in Plants in Health and Diseases," Ed. Pridham. J.B. Pergamon Press, Ltd.

Isherwood, F.A. 1963. Lignin. In "Recent Advances in Food Science 3. Biochemistry and Biophysics," Ed. Leitch, J.M. and Rhodes, D.N. Butterworth, London.

Jenkins, R.R. and Lee, F.A. 1940. Tenderness readings as an index of quality of fresh asparagus. Preliminary paper. Food Res. 5: 161.

Joslyn, M.A. 1970. "Methods in Food Analysis," Academic Press, New York.

Kramer, A., Haut, I.C., Scott, L.E. and Ide, L.E. 1949. Objective method for measuring quality factors of raw canned and frozen asparagus. Proc. Am. Soc. of Hort. Sci. 53: 411.

Kramer, A. and Twigg, B.A. 1970. "Quality Control for Food Industry," Vol 1. Avi Publishing Co., Inc., Westport.

Kramer, A., Wiley, R.C., Twigg, B.A., Decker, R.W. and Sidwell, A.P. 1960. The measurement of fibrousness of asparagus. Proc. Am. Soc. Hort. Sci. 76: 382.

Lee, F.A. 1943. Determination of toughness of frozen asparagus. Food Res. 8: 249.

Lipton, W.J. 1957. Physiological changes in harvested asparagus as related to temperature of holding. Ph.D. thesis, University of California, Berkeley, Calif.

Northcote, D.H. 1972. Chemistry of plant cell wall. Annual Rev. Plant Physiol. 23: 113.

Scott, H.R. and Kramer, A. 1947. Fiber content of canned green asparagus. Food Packer 28(6): 38.

Sharma, S.C. 1974. Mathematical modeling of the post-harvest textural behavior of asparagus. Ph.D. thesis, Rutgers University, New Brunswick, N.J.

Smith, H.R. and Kramer, A. 1947. The fiber content of canned green asparagus. The Canner 104: 14.

Szczesniak, A.S. Humbaugh, P.R. and Block, H.W. 1970. Behavior of different foods in the standard shear compression cell and effect of sample weight on peak area and maximum force. J. Texture Stud. 1: 35.

Szczesniak, A.S. 1973. Instrumental methods of texture measurements. In "Texture Measurements of Foods," Ed. Kramer, A. and Szczesniak, A.S. D. Reidel Publishing Co., Boston.

Voisey, P.W. 1971. Modernization of texture instrumentation. J. Texture Stud. 2: 129.

Werner, G., Meschter, E.E., Lacey, H. and Kramer, A. 1963. Use of shear press in determining fibrousness of raw and canned green asparagus. Food Technol. 15: 81.

Wilder, H.K. 1948. Instruction for use of the fibrometer in measuring the fiber content in canned asparagus. Res. Lab. Report No. 12313-C. National Canners Association, San Francisco.

Wiley, R.C., Elhwany, N., Kramer, A. and Hager, F.J. 1956. The shear press—An instrument of measuring the quality of foods. 4. Application to asparagus. Food Technol. 8: 439.

Ms received 1/8/75; revised 3/18/75; accepted 3/22/75.

Paper of the Journal Series, New Jersey Agricultural Experiment Station, Cook College, Rutgers University, New Brunswick, NJ 08903.

The authors gratefully acknowledge the assistance of the Center for Computer and Information Services at Rutgers University.

ELAINE SCHRUMPF and HELEN CHARLEY
Foods & Nutrition Dept., School of Home Economics, Oregon State University, Corvallis, OR 97331

TEXTURE OF BROCCOLI AND CARROTS COOKED BY MICROWAVE ENERGY

INTRODUCTION

WITH INCREASING USE of the microwave oven, recognition and identification of the possible causes of reported undesirable characteristics in some foods become important. For most vegetables, neither cooking by microwaves nor conventional boiling yielded a consistently superior product (Kylen et al., 1961), but two, broccoli and cauliflower, did score significantly lower in overall palatability when cooked by microwaves. Both vegetables had much less acceptable texture, while for cauliflower other aspects of palatability were involved also. Vegetables cooked by microwaves tended to dehydrate unless covered by water and an outer skin formed which became progressively thicker as cooking increased (Bollman et al., 1948; Bowman et al., 1971).

This study attempted to verify and account for these reported differences in texture, using microwave energy and conventional boiling methods as they are commonly practiced by the homemaker. Two vegetables, fresh broccoli and carrots, cooked by both methods were evaluated for texture by a sensory panel and analyzed for changes in content of pectic substances, weight and cellular structure.

EXPERIMENTAL

Preparation of the vegetables

For each replication (six per vegetable) five carrots or five stalks of broccoli were used. Stalks of broccoli were washed, trimmed of woody tissue and leaves and separated from the flower heads. Carrots were washed, scraped and trimmed to a 4-in. central section of the root. Each piece of vegetable was cut in half lengthwise; one-half was assigned to the lot to be cooked by microwaves and the other to the lot cooked conventionally. Four sets of paired halves were used for sensory evaluation and the fifth set provided both raw and cooked subsamples for histological observations and pectic analysis.

Cooking

Lots to be cooked by microwaves (1600 watts, 2450 MHz) were added to 500 ml boiling water in a covered 2-qt pyrex baking dish, the size of which allowed the pieces to lie in a single layer. Lots cooked conventionally were added to 500 ml boiling water in a 2-qt aluminum saucepan. This amount of water was adequate to keep the vegetables covered throughout the cooking period. The saucepan was uncovered for the first 5 min when broccoli was cooked but was covered the entire cooking period for carrots. Cooking times (Table 1) were predetermined to insure that the degree of doneness for both methods was as nearly equal as possible. Vegetables were removed from the cooking water as soon as they were done, placed cut-side down on absorbant towels, and covered with plastic wrap to avoid excessive evaporation during a 30-min cooling period. Cooling did not interfere with the sensory evaluation of texture and was necessary to eliminate the rapid change in weight as the subsamples were weighed.

Analytical methods

A trained panel of four members evaluated the contour of the paired halves of each vegetable by matching their shapes with sketches on a score card. In addition, the judges ranked the carrots for tenderness of outer cylinder and inner core and broccoli for tenderness of inner flesh and outer layer.

Fractions of pectic substances with solubility characteristics comparable to those in situ were obtained by cold extraction procedure (Dietz and Rouse, 1953; Ruiz, 1958; Mackey et al., 1973). After purification with alcohol, pectic substances were fractionated according to their solubility in water, 0.4% sodium hexametaphosphate, or 0.05N sodium hydroxide. The concentration of pectic substances in each fraction was determined by a uronic acid-carbazole method as modified by Bitter and Muir (1962) and reported as anhydrogalacturonic acid on a fresh weight basis.

Tissue for histological observation was fixed in CRAF II fixative. Periodic acid-Schiff's reaction was used to localize the cell wall polysaccharides (Jensen, 1962).

RESULTS

Contour

Halves of both carrot and broccoli cooked by microwaves had a rather collapsed and shrunken appearance, while those cooked conventionally more nearly resembled the shape of the raw stalk or root. Distortion of shape was observed the length of the piece. Formation, and often separation, of a denser layer on the cut surface of broccoli was observed after cooking by microwaves. Photomicrographs (Fig. 1 and 2) show contours of the raw and cooked vegetables.

Tenderness

Both vegetables were evaluated for tenderness as influenced by two tissue types: (1) parenchymatous tissue which predominates in most vegetables; and (2) supportive/conductive tissues. In carrots, parenchymatous tissue forms the outer layers surrounding the core, while in broccoli it makes up the large central portion of the stalk. Similarly, the core of carrot corresponds in tissue type to the outer layer of supportive/conductive tissue near the skin in broccoli. Halves of carrots cooked by microwaves were judged to have a more spongy outer cylinder and a more fibrous core than did the matching halves cooked conventionally, (Fig. 3). The outer area of broccoli cooked by microwaves was tougher than that of the corresponding halves cooked conventionally but the interior was judged softer. In each case the difference was highly significant ($p < 0.01$).

Weight loss

Loss of weight for each vegetable cooked by microwaves was more than double that of the matching halves cooked conventionally (Table 2), despite enough water to keep the vegetables covered as they cooked. Halves cooked by micro-

Table 1—Raw weights and cooking times of vegetables[a]

	Total wt (g)	Cooking water (ml)	Cooking time (min)
Carrots			
Microwave	81.1	500	8
Conventional	80.8	500	20
Broccoli			
Microwave	67.5	500	5
Conventional	67.5	500	12

[a] Average of six replications

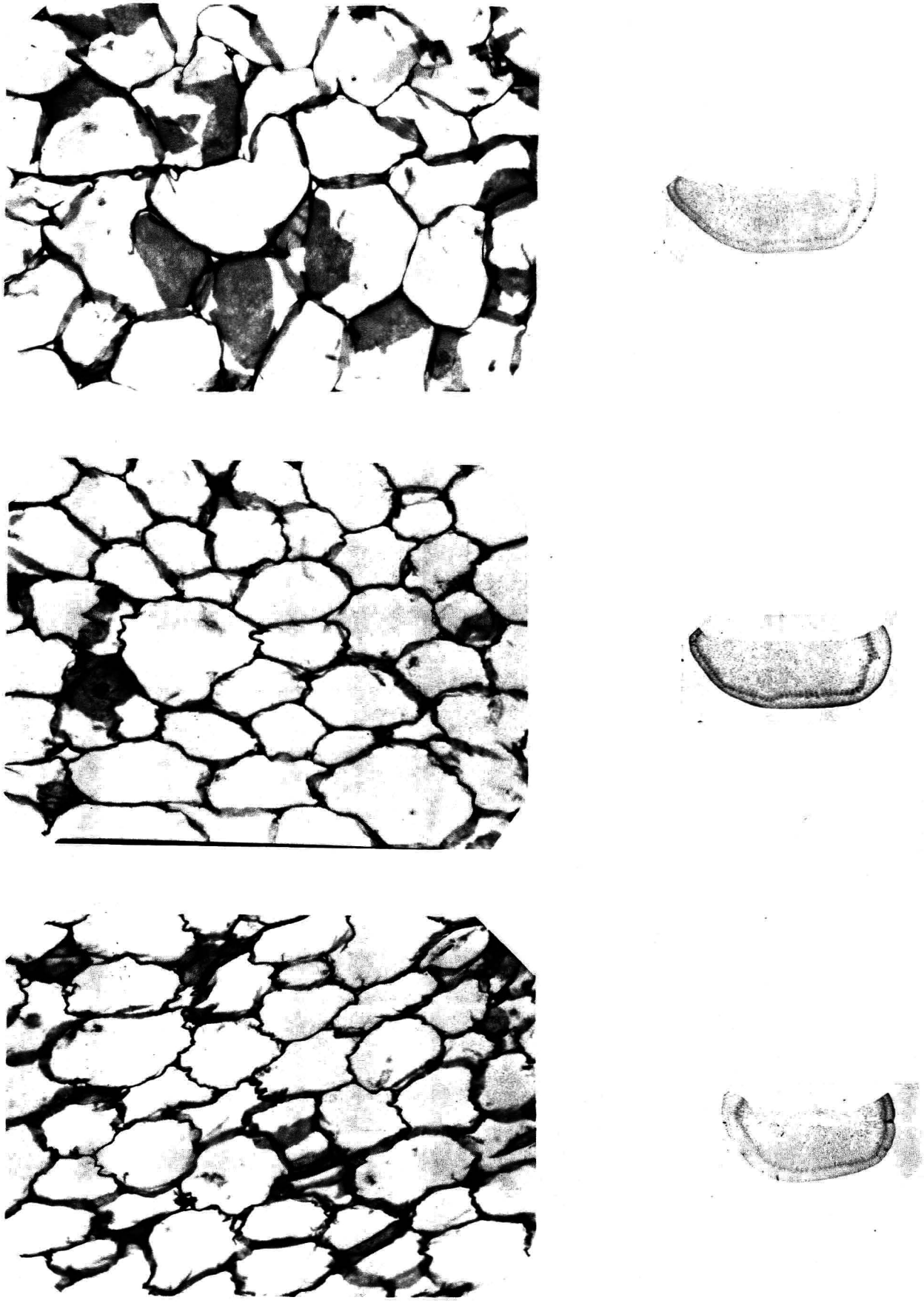


Fig. 1–Cell structure (41X) and cross section (2X) of broccoli. (Top: Raw broccoli; Center: Broccoli cooked conventionally; Bottom: Broccoli cooked by microwaves.)

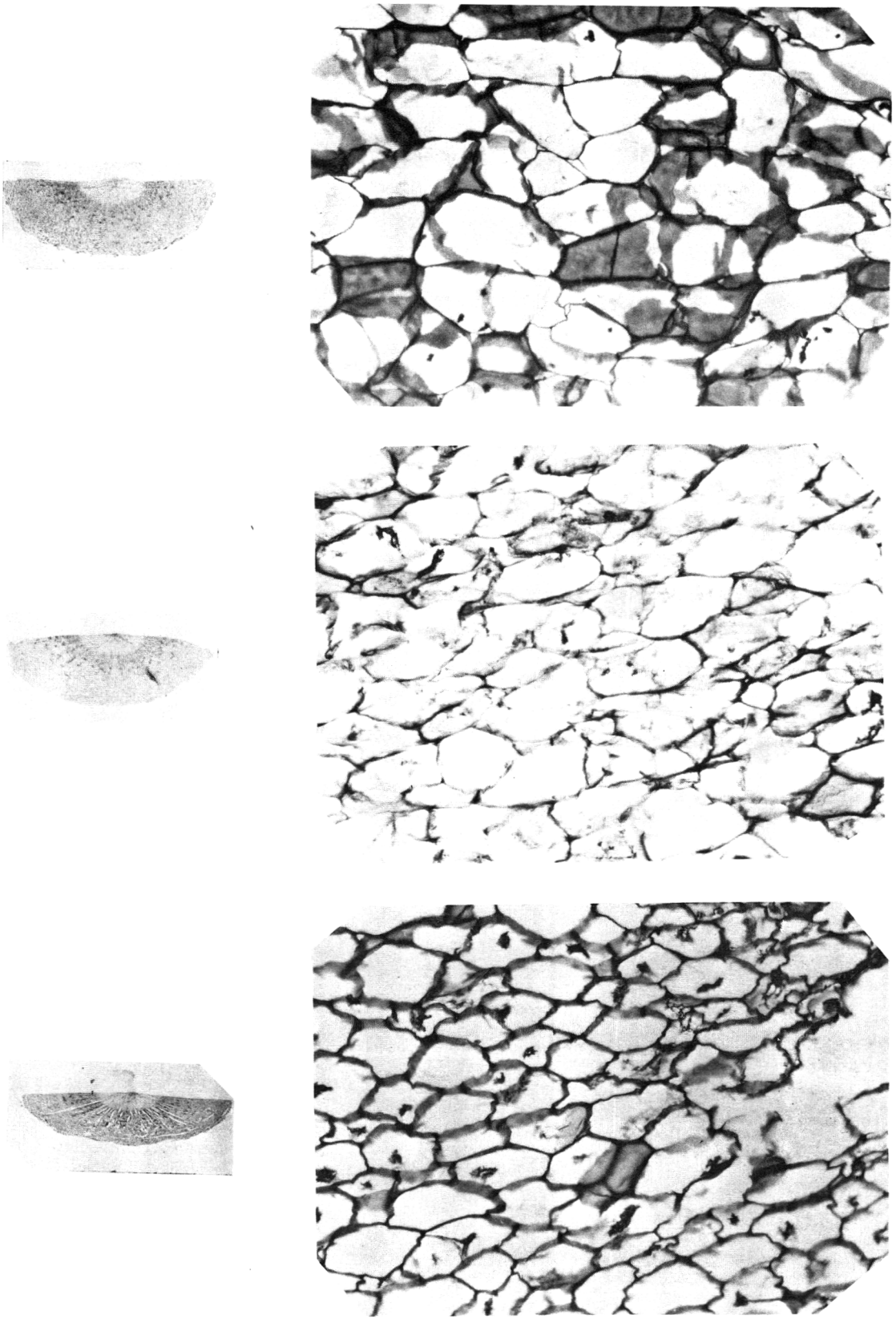


Fig. 2–Cell structure (41X) and cross section (2X) of carrots. (Top: Raw carrots; Center: Carrots cooked conventionally; Bottom: Carrots cooked by microwaves.)

waves floated during the entire cooking period instead of sinking as did those cooked conventionally. The consequent build-up of pressure by rapid and continuous conversion of internal water to steam by microwave energy would not only force steam out of the cells but prevent re-entry of water which could account for the buoyancy of the piece.

Cell structure

Differences due to cooking method were most evident in the predominate parenchyma cells as shown in photomicrographs of raw and cooked broccoli and carrots (Fig. 1 and 2, respectively). Cell walls remained intact after cooking by either method. Cells from tissue cooked conventionally were somewhat diminished in size, as indicated by wrinkling of the cell walls, while those cooked by microwaves were more collapsed, with pronounced folding of the cell walls. Collapse of cells was especially extensive on either side of radial fissures which occurred frequently between cells in broccoli and even more extensively in carrots.

Table 2—Weight loss of cooked vegetable[a] (% of fresh weight)

	Cooking method	
	Microwave	Conventional
Carrots	34.2	15.7
Broccoli	26.8	11.0

[a] Average of six replications

Table 3—Content of anhydrogalacturonic acid in three fractions of pectic substances[a] (% of fresh weight)

	Extractions			
	H_2O	$(NaPO_3)_6$	NaOH	Total
Carrots				
Raw	0.10	0.27	0.34	0.71
Microwave	0.21	0.24	0.12	0.57
Conventional	0.18	0.28	0.08	0.53
Broccoli				
Raw	0.02	0.16	0.24	0.42
Microwave	0.11	0.26	0.18	0.55
Conventional	0.12	0.25	0.17	0.54

[a] Average of two subsamples for each of six replications

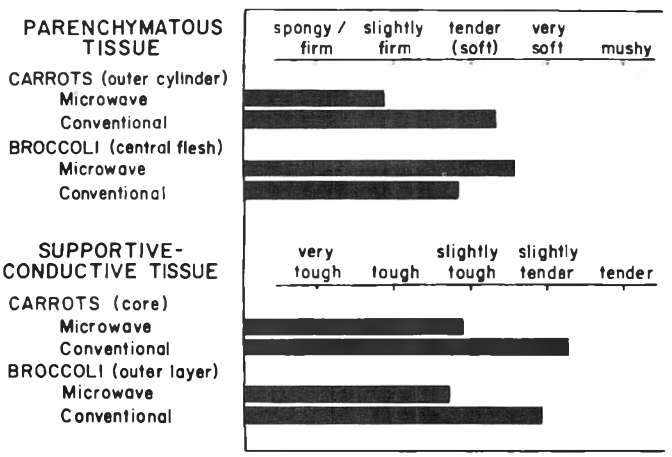


Fig. 3—Average ranks for tenderness of broccoli and carrot tissue.

Content of pectic substances

Concentrations of the three pectic fractions found in carrots and broccoli are shown in Table 3. The sodium hexametaphosphate-soluble fraction consists of low methoxyl calcium and magnesium pectinates and the sodium hydroxide-soluble fraction consists of the remaining calcium and magnesium pectinates and protopectin. Both are insoluble in water and so contribute to cell adhesion and integrity of the cell wall. As anticipated, the firm, raw tissue contained a high proportion of water-insoluble pectic substances and only a small proportion which was water soluble. The cold extraction procedure failed to remove all the pectic substances from the raw broccoli, as indicated by greater total pectic content of the cooked vegetable as compared with the raw. Cooking significantly increased the water soluble fraction ($p < 0.01$) and significantly decreased the sodium hydroxide-soluble fraction ($p < 0.01$), substantiating that cooking increases solubility of pectic substances (Sterling, 1955). Comparing the effects of the two methods, both vegetables cooked by microwaves contained significantly more sodium hydroxide-soluble fraction ($p < 0.01$). Thus the greater toughness of both areas of carrots and the outer layer of broccoli cooked by microwaves accompanied the higher sodium hydroxide-soluble fraction found in these tissues. However, when both insoluble fractions [NaOH and $(NaPO_3)_6$] were combined, there was essentially no difference in the total insoluble pectic substances due to method of cooking. In broccoli, where tenderness of the central portion of the stalk contrasted with toughness of the outer layer, analyzing the tissues separately might have given a clearer picture of the possible role of pectic substances.

DISCUSSION

DATA on the pectic substances are inadequate to account for the difference in texture of the two vegetables due to cooking method. Other observations suggest a more advanced state of dehydration in tissue cooked by microwaves. Loss of weight was more than double that during a longer cooking period by conventional boiling. Contours of the pieces of vegetable and of the parenchyma cells resembled those of vegetables undergoing dehydration (Reeve, 1942, 1943; Van Arsdel, 1963). The photomicrograph of carrot tissue cooked by microwaves (Fig. 2) shows collapse of highly vacuolated cells and pronounced folding of cell walls comparable to that in carrots at early stages of dehydration (Reeve, 1942). Stalks of broccoli cooked by microwaves were shrunken and concave in shape. Lignified xylem cells in the rays of carrots interfered with the formation of a concave curvature, but shrinkage of the outer cylinder was apparent.

While the effects of microwave cooking on tissue indicate that some dehydration has occurred, another effect analogous to that caused by dehydration may be involved. Dehydration increases crystallinity of the carbohydrate gels of the cell wall, thus imparting increased mechanical strength or toughness to tissues. Rapid removal of a considerable amount of water can convert formerly amorphous areas, not only of cellulose but also of hemicelluloses and pectic substances, to the crystalline state (Sterling, 1963). In conventional drying, crystallinity of cellulose increases mainly near the end of the drying period (Shimazu and Sterling, 1967). The gross loss of moisture caused by microwave cooking was not comparable to the stage of conventional dehydration where crystallinity occurs. However, inherent in the microwave method of cooking is the rapid and massive conversion of water in the tissue to steam, which develops such internal vapor pressure as to cause radial fissures in tissue heated by microwaves (Huxsoll and Morgan, 1968). This suggests that water in the tissue is almost entirely in the gaseous rather than liquid state. Rapid removal of a high pro-

portion of the water held in the polysaccharide gel of amorphous regions of cell walls without infusion of liquid water might result in increased crystallinity. Such increased crystallinity could account for the observed stringiness of the core and sponginess of the outer cylinder of carrots and the toughness of the outer layer of broccoli when cooked by microwaves but fails to account for the greater tenderness of the central portion of broccoli. Such difference in response may be a reflection of a difference in the chemical and physical makeup of the cells in the tissue involved.

REFERENCES

Bitter, T. and Muir, H.M. 1962. A modified uronic acid carbazole reaction. Analytical Biochem. 4: 330.

Bollman, M.C., Brenner, S., Gordon, L.E. and Lambert, M.E. 1948. Application of electronic cooking to large-scale feeding. J. Amer. Dietetic Assn. 24: 1041.

Bowman, F., Page, E., Remmenga, E.E. and Trump, D. 1971. Microwave vs conventional cooking of vegetables at high altitude. J. Amer. Dietetic Assn. 58: 427.

Dietz, J.H. and Rouse, A.H. 1953. A rapid method for estimating pectic substances in citrus juices. Food Res. 18: 169.

Huxsoll, C.C. and Morgan, A.I. Jr. 1968. Microwave dehydration of potatoes and apples. Food Technol. 22: 705.

Jensen, W.A. 1962. "Botanical Histochemistry," p. 175. W.H. Freeman and Co., San Francisco.

Kylen, A.M., Charles, V.R., McGrath, B.H., Schleter, J.M., West, L.C. and Van Duyne, F.O. 1961. Microwave cooking of vegetables. Ascorbic acid retention and palatability. J. Amer. Dietetic Assn. 39: 321.

Mackey, A.C., Hard, M.M. and Zaehringer, M.V. 1973. Measuring textural characteristics of fresh fruit and vegetables. Tech. Bull. 123, Oregon State Univ. Agric. Exp. Stn., Corvallis, Or.

Reeve, R.M. 1942. Facts of vegetable dehydration revealed by microscope. Food Ind. 14(12): 51.

Reeve, R.M. 1943. A microscopic study of the physical changes in carrots and potatoes during dehydration. Food Res. 8: 128.

Ruiz, V.T. 1958. Strawberry Pectin Jellies. M.S. thesis, Oregon State College, Corvallis, Or.

Shimazu, F. and Sterling, C. 1967. Changes in crystallinity of cellulose during dehydration. J. Food Sci. 32: 273.

Sterling, C. 1955 Effect of moisture and high temperature on cell walls in plant tissues. Food Res. 20: 474.

Sterling, C. 1963. Texture and cell-wall polysaccharides in foods. In: "Recent Advances in Food Science," Vol 3, p. 259.

Van Arsdel, W.B. 1963. "Food Dehydration," Vol 1, p. 66, Avi Publishing Co., Westport, Conn.

Ms received 3/8/75; revised 5/10/75; accepted 5/14/75.

Material taken from a thesis by the senior author presented in partial fulfillment of the requirements for a M.S. degree.

M. PELEG and L. GÓMEZ BRITO
Instituto Venezolano de Investigaciones Tecnológicas e Industriales, Investi
Apartado 7668, Carmelitas, Caracas 101, Venezuela

ESTIMATION OF THE COMPONENTS OF A PENETRATION FORCE OF SOME TROPICAL FRUITS

INTRODUCTION

THE MAGNESS TAYLOR Fruit Pressure Tester (Magness and Taylor, 1925) has been a useful simple apparatus for the evaluation of texture of many fruits (Bourne, 1966). Although the actual measurements give only one index, the penetration force, it has been shown for pears and peaches that the latter could be correlated with other textural characteristics which can be evaluated by more sophisticated methods (Bourne, 1966, 1974).

One of the problems involved in the interpretation of the results obtained by the Fruit Pressure Tester is the fact that the total penetration force includes components such as compression and shear which are dependent upon the diameter of the penetration plunger. Bourne (1966) showed that the penetration force of foods and models could be represented by the following equation:

$$F = K_c A + K_s P + C$$

where F is the total penetration force; K_c a constant representing the effect of the crossectional area (mainly compression); A the plunger crossectional area; K_s a constant representing the effects of the perimeter (mainly shear); P the plunger perimeter; and C an experimental constant. For circular plungers the equation can be written as a function of the diameter only i.e.,

$$F = K'_c D^2 + K'_s D + C$$

where D is the plunger diameter (Bourne, 1966).

The procedure for the determination of the constants in Bourne's equation was based on two sets of tests in which two sets of flat plungers were used. One set had a constant cross-sectional area and the other a constant perimeter length. For many of the commodities tested by Bourne it has been found that the constant C was very small in magnitude and therefore could be eliminated from the equation without introducing any great inaccuracy (Bourne, 1966). For these commodities, where the constant C is eliminated, Bourne's equation can be written in the form of:

$$F/D = K'_c D + K'_s$$

For an unknown commodity or material, if the latter linear relationship between F/D and D could be proved to exist, the assumption that the constant C is negligible can be considered as correct. The application of this consideration was demonstrated for cucumbers by Su and Humphries (1972), even though they used only three plungers in a fairly large diameter range.

Although Bourne's basic equation has been used for the evaluation of the penetration force in terms of compressive and shear components, it has been reported that some materials might have other kinds of components (De Mann 1969). It has also been reported that other geometrical factors, such as the shape and actual contact area, affected the penetration force as well (Ahmed and Fluck, 1972).

In this study we have evaluated the possibility of using Bourne's equation to estimate the compressive and shear components of a penetration force of some tropical fruits with special reference to the data that can be obtained by the standard Fruit Pressure Tester.

EXPERIMENTAL

Materials

Local pineapple fruits and plantains at different stages of maturity were purchased in a local market and tested on the day of purchase. Mangoes of varieties which do not contain fibers (Manga, Sensation, Kent and Sandersha) were collected from experimental plantations. After being washed and waxed they were left to ripen at room temperature (20–24°C). Papaya fruits collected from commercial plantations at a "green-ripe" stage were left to mature and were tested as previously reported (Peleg, 1974).

Agar gels were prepared using Agar Agar (Merck W. Germany No 1614). The hot solutions were filled into flat containers to about 1.5 cm depth and left overnight before penetration tests were performed.

Test procedure

An Instron Universal Testing Maching (Model TM) was used in a compression arrangement. Freshly cut halves of mangoes and plantains or slices of pineapple and papaya were subjected to the penetration tests. Two sets of five cylindrical metal plungers were employed. For pineapple and papaya the set contained plungers having diameters of 3/16, 5/16, 7/16, 9/16 and 11/16 in. For mangoes and plantains the set contained plungers having diameters of 3/16, 1/4, 5/16, 3/8 and 7/16 in. Three plunger diameters (3/16, 5/16 and 7/16 in.) were used for all fruits. For the Agar gels all the plungers of the two sets were employed. All the plungers had a spherical penetrating end similar in shape to that of the standard Fruit Pressure Tester described by Magness and Taylor (1925). All the samples were penetrated at a speed of 20 cm/min and the plungers were withdrawn after a yield point had been recorded.

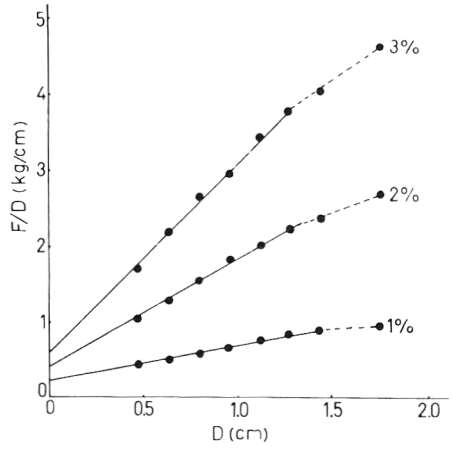


Fig. 1—Force-diameter relationship in penetration of Agar gels with circular plungers.

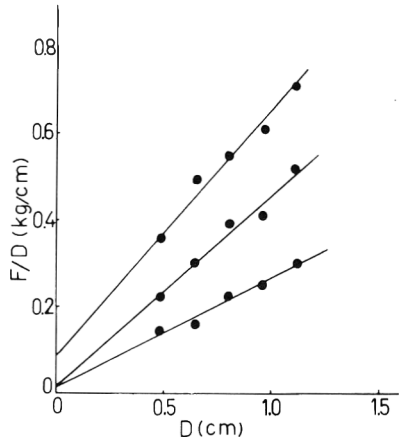


Fig. 2–Force-diameter relationship in penetration tests of mangos.

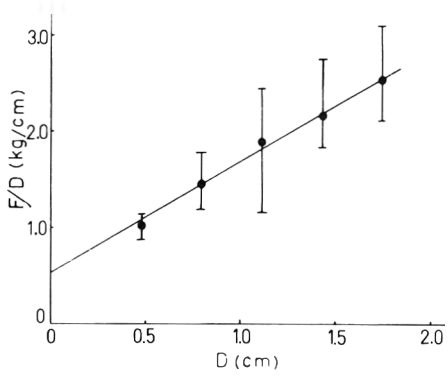


Fig. 4–Force-diameter relationship in penetration tests of pineapples.

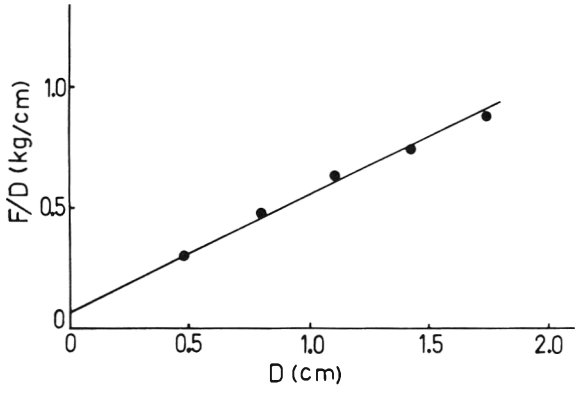


Fig. 3–Force-diameter relationship in penetration tests of papayas.

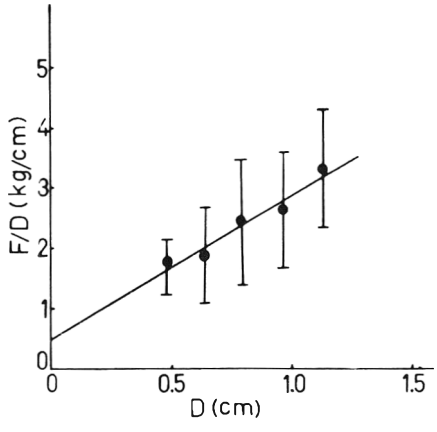


Fig. 5–Force-diameter relationship in penetration tests of plantains.

Between three and eight penetrations were performed with each plunger. The lower number generally represents the two bigger plungers of each set of plungers, for which the available fruit surface was sometimes technically limited.

After the tests, slices of the fruits were peeled and disintegrated by a domestic Osterizer. The total soluble solids content (TSS) of the puree was determined by a refractometer and the results are expressed in °Brix.

RESULTS & DISCUSSION

Agar-agar gels

Results of agar-agar gels penetration in the form of F/D vs. D relationship are given in Figure 1. These showed that for concentration of 1, 2 and 3%, linear relationship could well represent the experimental data for curved plungers ranging in diameter from 3/16–1/2 in. (0.476–1.270 cm). However, for bigger diameters deviations from this linear relationship could be observed.

This suggests that the assumption of linearity for calculation purposes might be justified only under conditions for which the existence of linear relationship has been proved experimentally.

Penetration of mangos, papaya, pineapple and plantains

Penetration results in the form of F/D vs. D are given in Figures 2, 3, 4 and 5. These showed that in the range of plunger diameters between 3/16–7/16 in. (0.476–1.113 cm) for mangoes and plantains and in the range 3/16–11/16 in. (0.476–1.746 cm) for papayas and pineapples, the experimental data could be well represented by a linear relationship between F/D and D. In terms of Bourne's equation it meant that eliminating the experimental constant C was practically justified.

Estimation of the compressive and shear contributions to the penetration force

The possibility of representing the penetration force of agar model gels, mangos, papayas, pineapples and plantains by the reduced form of Bourne's equation and the fact the diameters of the two standard plungers of the Fruit Pressure Tester (5/16 and 7/16 in.) were found to be within the range for which this equation form was valid, enabled a numerical calculation of the equation compression and shear coefficients K'_C and K'_S from results obtained by these two plungers only. In a similar way they enabled the calculation of the compressive and shear contributions to the total force, from the terms $K'_C D^2$ and $K'_S D$, respectively. Formulae for the calculation of the coefficients in Bourne's equation from results obtained by any two plungers and by the two standard (5/16 and 7/16 in.) plungers are given in Table 2. Calculation of the shear and compressive contributions in terms of percentage of the total penetration force is also given in this table. It can be seen that for a given pair of plungers the compressive and shear contributions are a function solely of the ratio between the penetration forces obtained by the two plungers and therefore are independent of the penetration force units.

Table 1—Texture uniformity of some tropical fruits determined by penetration tests[a]

Fruit	TSS (°Brix)	Mean penetration force (kg)	Coef. of variance (%)
Mango (Kent)	23.7	0.576	22.7
	23.1	0.256	19.9
Papaya	8.1	0.529	16.7
	8.8	0.403	15.4
	9.9	0.408	18.8
	10.0	0.514	20.6
Pineapple	14.2	2.69	18.2
	15.2	2.30	25.7
	15.6	2.43	38.9
Plantain	green-unripe	6.54	8.3
	semi-ripe	2.81	10.6
	ripe	1.43	11.0
	ripe	1.23	16.4

[a] Determined by 5/16 in. plunger at a penetration speed of 20 cm/min.

Table 2—Estimation of the penetration force components from results obtained by two plungers

General equation:		$K'_C = \frac{1}{D_1 - D_2} \cdot \left[\frac{F_1}{D_1} - \frac{F_2}{D_2}\right]$
For two plungers:		$K'_S = \frac{1}{D_1 - D_2} \cdot \left[\frac{D_1}{D_2} \cdot F_2 - \frac{D_2}{D_1} \cdot F_1\right]$
Equation for the 5/16 and 7/16 in. standard plungers of the "Fruit Pressure Tester"[a]		$K'_C = 2.834\ F(7/16) - 3.968\ F(5/16)$ $K'_S = 4.409\ F(5/16) - 2.250\ F(7/16)$
% Compression and shear contributions	5/16 in. plunger	$\%\ \text{Comp.} = 178.6 \frac{F(7/16)}{F(5/16)} - 250$ $\%\ \text{Shear} = 350 - 178.6 \frac{F(7/16)}{F(5/16)}$
	7/16 in. plunger	$\%\ \text{Comp.} = 350 - 490 \frac{F(5/16)}{F(7/16)}$ $\%\ \text{Shear} = 490 \frac{F(5/16)}{F(7/16)} - 250$

[a] F in kg, K'_C in kg/cm^2 and K'_S in kg/cm

Table 3—Estimated contributions of the penetration force components of some tropical fruits

Fruit	No. of tests	TSS range (°Brix)	Mean forces ratio	Coef. of variance (%)	Plunger diam (in.)	Contribution Comp. (%)	Contribution Shear (%)
					5/16	94.1	5.9
Mango	25	12.5–24.8	0.519	16.2	7/16	95.7	4.3
					5/16	79.5	20.5
Papaya	19	7.6–11.5	0.542	16.5	7/16	84.4	15.6
					5/16	67.8	32.2
Pineapple	22	12.5–19.2	0.562	11.1	7/16	74.6	25.4
					5/16	85.7	14.3
Plantain	25	–	0.532	10.6	7/16	89.3	10.7

Compressive and shear contributions to the penetration force of mangos, papayas, pineapples and plantains

Individual fruits were subjected to penetration tests with the 5/16 and 7/16 in. plungers. In every test 8–16 penetrations were performed with each plunger and the ratio between the mean forces was calculated for each fruit.

Mean penetration force ratios and their coefficients of variance are given in Table 3. These showed that considerable variations existed within the same group of fruits. It was probably due to the natural textural strength variability within the individual fruits (Table 1) but it might also suggest the possibility that textural differences between fruits are expressed not only by the penetration force magnitude, but by differences in the shear-compression ratio as well.

Results of the calculated contributions of the shear and compressive components based on the mean penetration force ratios are also given in Table 3. These showed that despite individual variations, the fruits as groups differed in their shear-compressive components ratios. It could be seen that while ripe mangoes had an extremely low shear component, in the order of magnitude of 4–6%, pineapples with their fibrous textural structure had a shear component in the order of 25–32%. As could be expected from the definition of the components, shear effects played a larger role in the smaller plunger and the differences between the shear contributions to the penetration forces obtained by the two plungers increased with the increase of the absolute shear contribution magnitude.

REFERENCES

Ahmed, E.M. and Fluck, R.C. 1972. Puncture testing of Tioga strawberries. J. Texture Stud. 3: 165.
Bourne, M.C. 1966. Measures of shear and compression components of puncture tests. J. Food Sci. 31: 282.
Bourne, M.C. 1968. Texture profile of ripening pears. J. Food Sci. 33: 223.
Bourne, M.C. 1974. Textural changes in ripening peaches. Can. Inst. Food Sci. Technol. J. 7(1): 11.
De Man, J.M. 1969. Food texture measurements with the penetration method. J. Texture Stud. 1: 114.
Magness, J.R. and Taylor, G.F. 1925. An improved type of pressure tester for the determination of fruit maturity. Circular No 350. U.S. Dept. of Agriculture.
Peleg, M. 1974. Determination of fresh papaya's texture by penetration tests. J. Food Sci. 39: 156.
Su, C.S. and Humphries, E.G. 1972. Rupture properties of cucumbers. Pickle Pak. Sci. 2(1): 1.

Ms received 11/27/74; revised 3/8/75; accepted 3/10/75.

This study was financed by the Consejo Nacional de Investigaciones Cientificas y Tecnologicas (CONICIT), (National Venezuelan Council of Scientific & Technological Research).

Y. S. HENIG
General Foods Corp., Technical Center, 250 North St., White Plains, NY 10625
and S. G. GILBERT
Dept. of Food Science, Rutgers University, New Brunswick, NJ 08903

COMPUTER ANALYSIS OF THE VARIABLES AFFECTING RESPIRATION AND QUALITY OF PRODUCE PACKAGED IN POLYMERIC FILMS

INTRODUCTION

PACKAGING of produce in polymeric films is a common technique designed to prevent moisture loss, to protect against mechanical damage, and to provide better appearance. Proper selection of packaging films and optimizing package design can favorably alter the gas composition around fruits and vegetables, resulting in an extended shelf life and improved quality. A produce package is a dynamic system in which two main processes, respiration and permeation, are occurring simultaneously. Shortly after hermetic packaging, the rate of produce respiration will be equal to the rate of O_2 permeation into the package and CO_2 permeation out of the package, and the concentration of these gases will be maintained at a constant level (Tomkins, 1962). The lower O_2 level and the elevated CO_2 concentration that prevail during this steady state period will lower the produce respiration rate and consequently extend the produce shelf life. Much of the previous work on produce packaging has been related to the effect of gas composition on produce quality, with little attention to the dynamics of the permeation-respiration interaction (Daun et al., 1973; Hardenburg, 1971). Several workers (Jurin and Karel, 1963; Karel and Go, 1964; Veeraju and Karel, 1966) have studied the permeation-respiration interaction and devised a graphical solution to predict the steady state internal atmosphere in apple and banana packages.

Developed in the present investigation is a computer-aided solution to the mathematical equations representing the changes in respiratory gas concentrations within tomato packages. The computer solution is then validated by comparing it to model packages' results, and is further used to analyze the different packaging system variables' effect on internal package atmosphere. This technique enables the rapid prediction of the O_2 and CO_2 concentrations within produce packages – taking into account all the package variables – and also provides a good tool for package design for any commodity without requiring extensive experimentation or field trials.

MATERIALS & METHODS

Packaging films and their permeability

Plasticized polyvinyl chloride films manufactured by the Borden Chemical Co. (Coded VF-71 and RMF-61) were used in all experiments. These films contained an anti-fogging agent which eliminated any water condensation on the film surface.

The permeability of these films to O_2 and CO_2 was measured by the Gilbert and Pegaz (1969) technique. O_2 and CO_2 concentrations were determined using Aerograph A90-P3 and Beckman GC-5 gas chromatographs, respectively.

Tomato respiration rate measurements

Weighed amounts of tomatoes (typically 4 tomatoes weighing about 400–500g, having a diameter of approximately 2.5 in.) were placed in square aluminum desiccators (4-3/4 in. × 4-3/4 in. × 4-3/4 in., having a free volume of 1280 cc), which served as the respiration chambers. Each chamber was completely sealed by gluing aluminum foil to its edges. At constant time intervals, 0.3-cc samples were drawn from each chamber through a rubber cement sampling port and analyzed for CO_2 and O_2 concentration using the two gas chromatographs mentioned previously. Five chambers were used to measure O_2 consumption rate and CO_2 evolution rate simultaneously, and 5 chambers were used to measure O_2 consumption rate under complete absorption of CO_2 (the CO_2 was absorbed by a KOH solution placed in petri dishes at the bottom of each chamber). The CO_2 and O_2 concentrations were plotted versus time. These curves were divided into linear and curvilinear portions. All points belonging to the curvilinear portion were plotted on a semilogarithmic paper to give a straight line. Regression analysis was carried out on both portions of the curve to determine their regression coefficients and intercept values. Having obtained these coefficients, O_2 consumption rates and CO_2 evolution rates under different O_2 and CO_2 concentrations were calculated.

Model packages of tomatoes

The respiration chambers described above served as the model packages after three windows were opened in each of them, which were then covered by the proper packaging film. A weighed amount of tomatoes was placed in each package, which was then sealed from the top by the proper packaging film and provided with a silicone rubber septum for gas analysis. The film area of each model package was in the range 52.5–53 sq in.; the free volume of an empty model package was 1330 cc. Atmospheric samples (0.3 cc) were taken from the packages at constant time intervals and analyzed for O_2 and CO_2 using gas chromatography.

Mathematical model for the packaging system

In order to find the O_2 and CO_2 concentrations within a produce package at any time between the start of the experiment and the time steady state conditions were achieved, two ordinary first-order differential equations representing the system were solved:

$$\frac{dVoi}{dt} = K_1 \times A \times \left(0.21 - \frac{Voi}{V}\right) - f\left(\frac{Voi}{V}, \frac{Vci}{V}\right) \quad (1)$$

$$\frac{dVci}{dt} = g\left(\frac{Voi}{V}, \frac{Vci}{V}\right) - K_2 \times \frac{Vci}{V} \times A \quad (2)$$

where: Voi = volume of O_2 in the package (cc); Vci = volume of CO_2 in the package (cc); V = total free volume in the package (cc); K_1 = permeability of the film to O_2 (cc/hr × in.^{2}); A = area of the packaging film (in.^{2}); K_2 = permeability of the film to CO_2 (cc/hr × in.^{2}); t = time (min); f = a function representing O_2 consumption rate; and g = a function representing CO_2 evaluation rate.

The functions of f and g were determined from the respiration measurements, and K_1, K_2 from the permeability experiments. Eq. (1) and (2) were solved numerically using an IBM 360 computer at Rutgers Computation Center. The computer print-out provided the O_2 and CO_2 concentrations at 1-hr time intervals until steady state conditions occurred, at which point the analysis was terminated and the final O_2 and CO_2 concentrations were printed out.

RESULTS & DISCUSSION

THE PERMEABILITY VALUES of RMF-61 and VF-71 films to O_2 and CO_2 at 23°C are presented in Table 1. The rates of

O_2 consumption and CO_2 evolution of field tomatoes ("Jet Star" variety) at 23°C as a function of O_2 and CO_2 concentrations are given in Figure 1. The O_2 consumption rate under complete absorption of CO_2 was constant (23.135 cc/kg hr) in the range of 21–11.53% O_2; below 11.53% O_2, a linear decrease in O_2 consumption rate took place to about 4% O_2, with a slope (for the linear portion of the curve) of 2.00 cc/kg hr % O_2.

When CO_2 was accumulating simultaneously with O_2 reduction, the O_2 consumption rate was significantly reduced, but the reduction was surprisingly low. The O_2 consumption rate was constant (21.94 cc/kg hr) in the range 21–12.08% O_2, then decreased linearly with a slope of 1.815 cc/kg hr % O_2. This respiration pattern was observed to be typical of different varieties of tomatoes, at different temperatures, and at different stages of maturity. All tomatoes used in these experiments were of approximately the same size to eliminate any effect that tomato surface area might have on the respiration rate.

The evolution rate of CO_2 was constant up to a CO_2 concentration of 9% in the respiration chamber (18.52 cc/kg hr), above which a step-wise drop in CO_2 evolution rate occurred to a rate of 12.19 cc/kg hr. R.Q. values remained constant at about 0.9 in the range 0–9% CO_2 then a drop to 0.6 was observed, with a further increase after that to 1.4. When O_2 concentrations were less than 4% the R.Q. values rose to 1.5 and higher, indicating that a partial fermentation had begun.

The model packages were designed in order to get a well defined packaging system in which package dimensions could be measured accurately. This was essential in view of the fact that the model packages results had to be compared with the computer calculated values. The model packages consisted of about 0.47 kg of tomatoes (4 tomatoes per package), an area of 53 sq in., and a free volume of 843 cc. In RMF-61 model packages, O_2 concentration decreased to about 8% O_2 concentration in 24 hr, then an equilibrium concentration of O_2 was attained at 6.9% for about 7 days (Fig. 2).

Simultaneously, CO_2 concentration increased to 4% in 12 hr, attained a short equilibrium at that level, and then decreased to a final level of 2% for 7 days. All the RMF-61 model package variables, including surface area, tomato weight, free volume, film permeability and tomato respiration rate under complete absorption of CO_2 were fed to the computer program, which in turn read the change in O_2 and CO_2 concentrations with time until the attainment of equilibrium conditions. The computer calculated results are presented in Figure 2 as compared to the experimental readings of the model package. There is very good agreement between the experimental and computer calculated results.

In VF-71 tomato model packages, O_2 concentration decreased to about 4% in 30 hr and then attained an equilibrium concentration of 3.5% for 7 days (Fig. 3). CO_2 concentration increased to 10% in 16 hr, remained constant for a short period, and decreased to a final equilibrium concentration of 4%. As in the previous example of the RMF-61 package, a good fit between computed and experimental data is observed (Fig. 3).

After establishing the validity of the computer-aided solution, it was used to analyze the effect of each packaging system variable on the change in O_2 and CO_2 concentration with time. A change in the film permeability to O_2 from 2000 to 2600 cc/24 hr X 100 in.^{2} X atm would result in an elevated steady state concentration of O_2 (from 6.95% to 8.20%) for a package having the same dimensions as the model package. Also, a change in O_2 permeability from 600 to 1300 would increase the equilibrium level of O_2 from 2.7 to about 5% for the same package dimensions. A change in the CO_2 film permeability from 4000 to 12000 would result in a decrease of the CO_2 steady state concentration from 3.46% to 2.54%. A change in the weight/free volume ratio from 445/885 to 1000/300 (g/cc) for an RMF-61 model package would result in a decrease of O_2 equilibrium concentration from 7.2% to 4% O_2, as seen from Figure 4. For a package with constant dimensions, an increase in tomato weight would cause a decrease in

Table 1—Permeability of RMF-61 and VF-71 packaging films to O_2 and CO_2 (at 23°C)

Film[c]	O_2 Permeability[a,b] cc 24 hr X 100 in.^{2} X atm	CO_2 Permeability[a,b] cc 24 hr X 100 in.^{2} X atm
RMF-61	2100	10811
VF-71	698	3598

[a] Mean value of at least 3 replicates
[b] Coefficient of variance less than 5%
[c] Thickness of RMF-61, 0.7–0.8 mil; of VF-71, 0.6–0.7 mil

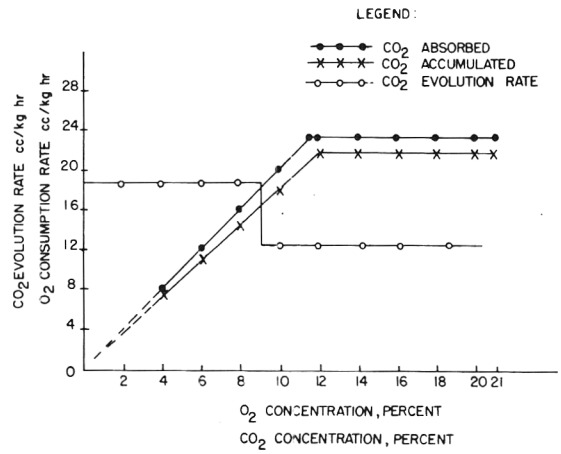


Fig. 1—The effect of O_2 and CO_2 concentrations on O_2 consumption rate and CO_2 evolution rate

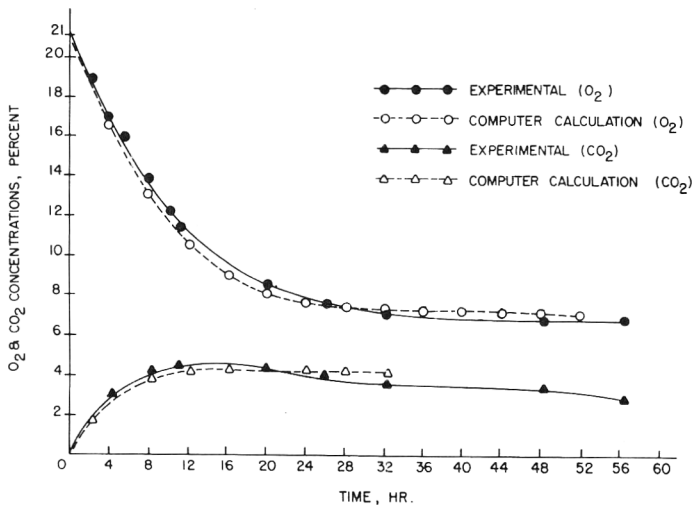


Fig. 2—A comparison between computer calculation and experimental results of the atmosphere change in RMF-61 package

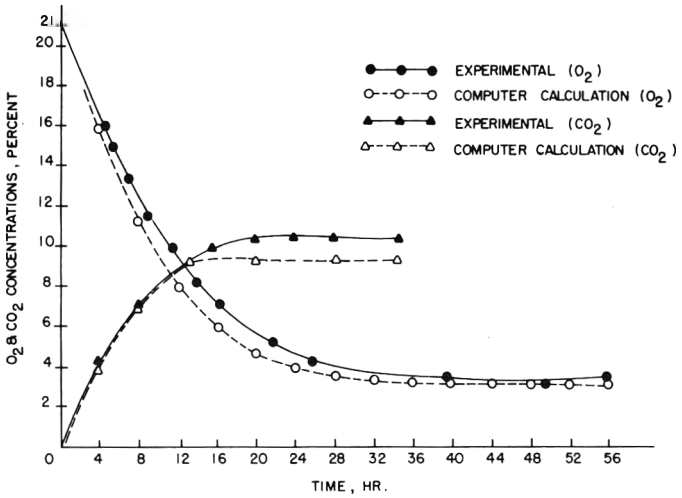


Fig. 3–A comparison between computer calculation and experimental results of the atmosphere change in VF-71 package

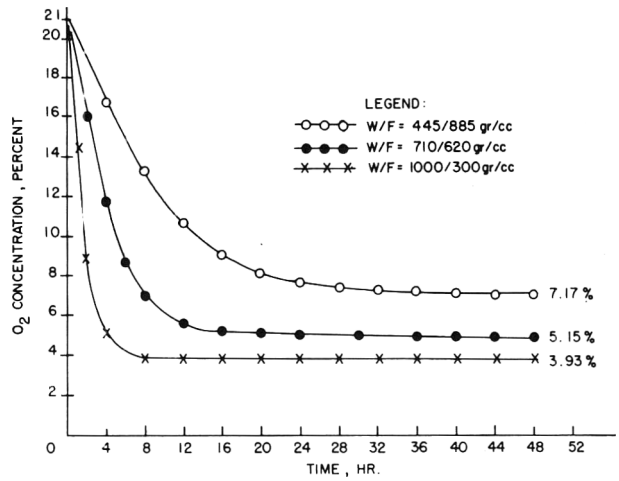


Fig. 4–The effect of weight/free volume ratio on O_2 concentration in RMF-61 package.

O_2 equilibrium concentration and an increase in CO_2 equilibrium concentration. An increase in package free volume when tomato weight was kept constant merely lengthened the time in which the equilibrium conditions were attained. A change in the RMF-61 packaging film area from 40 to 95 sq in. for a commercial package would change the O_2 equilibrium concentration from 5.42% to 9.5% (Fig. 5).

Lowering the temperature from 23°C to 15°C resulted in a reduction of O_2 consumption rate from 23 to 15.5 cc/kg hr, a change in the deflection point from 11.5 to 11.7% O_2, and a change in the slope after deflection from 2.00 to 1.50 cc/kg hr % O_2. This 33% reduction in respiration rate with a decrease in temperature was expected, and agreed well with published literature (Forward, 1960).

The data from model packages demonstrated that while the changes in internal atmosphere composition had been slower to occur at the low temperature, the final O_2 and CO_2 equilibrium concentrations were about the same at 15°C and 23°C for both RMF-61 and VF-71 packages. This phenomenon suggests that temperature changes affect both respiration rate and film permeability rate to the same degree. However, the combined effect of lower temperature, low O_2 concentration, and high CO_2 concentration leads to a further reduction in the respiration rate than the concentration effect itself.

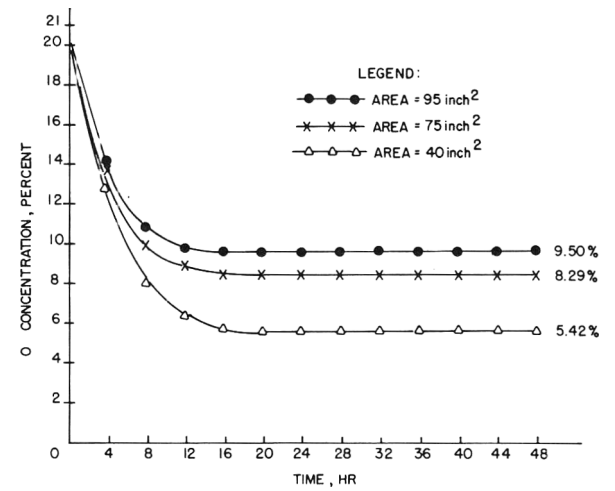


Fig. 5–The effect of area on O_2 concentration in RMF-61 package.

CONCLUSIONS

IN SUMMARY, the two differential equations representing the change in O_2 and CO_2 concentration with time in a model package atmosphere were solved numerically using a computer. Data obtained by the theoretical solution were compared with model packages results with very good agreement. The effect of the packaging system variables on the internal atmosphere composition was then analyzed with the validated computer program. The proposed methods and computer solution provide a rapid and accurate way of predicting equilibrium concentrations of O_2 and CO_2; they also enable the development of a package for any commodity without requiring extensive experimentation or field trials.

REFERENCES

Daun, H., Gilbert, S.G., Ashkenazi, Y. and Henig, Y. 1973. Storage quality of bananas packaged in selected permeability films. J. Food Sci. 38(7): 1247.

Forward, D.F. 1960. Effect of temperature on respiration. Ency. Plant Physiol. 12(2): 234.

Gilbert, S.G. and Pegaz, D. 1969. Finding a new way to measure gas permeability. Package Engr. 14(1): 66.

Hardenburg, R.E. 1971. Effect of in-package environment on keeping quality of fruits and vegetables. HortScience 6(3): 198.

Jurin, V. and Karel, M. 1963. Studies on control of respiration of McIntosh apples by packaging methods. Food Technol. 17(6): 104.

Karel, M. and Go, J. 1964. Control of respiratory gases. Modern Pkg. 37(6): 123.

Tomkins, R.G. 1962. The conditions produced in film packages by fresh fruits and vegetables and the effect of these conditions on storage life. J. Appl. Bact. 25(2): 290.

Veeraju, P. and Karel, M. 1966. Controlling atmosphere in a fresh-fruit package. Modern Pkg. 39(12): 168.

Ms received 3/10/75; revised 5/2/75; accepted 5/6/75.

H. R. BOLIN, VINCENT PETRUCCI[1] and G. FULLER
USDA Western Regional Research Laboratory, ARS, Berkeley, CA 94710

CHARACTERISTICS OF MECHANICALLY HARVESTED RAISINS PRODUCED BY DEHYDRATION AND BY FIELD DRYING

INTRODUCTION

RAISINS are an important world crop, with the largest tonnage produced in the United States. In this country about 200,000 tons are produced annually, returning to the grower over 100 million dollars. Raisin harvesting requires a large amount of hand labor. In the United States, harvesting consists of cutting the grape bunches individually by hand and laying them on paper trays, between the rows. After approximately 2 wk, the raisins on the trays are turned over and the grapes are allowed to dry for another week, or until they contain about 14% moisture. Next, the paper trays are rolled into packets, and later picked up and emptied into wooden bins for delivery to the processing plant. These operations require approximately 35 hr of labor per ton of raisins. An improvement of the whole raisin harvesting operation is desirable if raisins are to continue to be produced at a price that the consumer can afford. This paper evaluates raisins prepared by two different procedures involving mechanical harvesting, an approach to saving labor costs.

One method for mechanically harvesting raisins was investigated by Olmo and Studer (1967) and Studer and Olmo (1974). However, their procedure at this point still has severe limitations that has restricted its widespread usage, especially with respect to critical crop losses due to rains, which sometimes occur during the drying season. Recently another approach to mechanical harvesting has been studied, which consists of using a fatty acid or fatty acid ester treatment to accelerate drying rate.

Petrucci et al. (1974) reported two procedures for mechanical harvesting in conjunction with the preparation of raisins. These harvesting and drying methods circumvent the danger of crop loss by rain. In the first method the grapes are sprayed on the vine with a fatty acid ester emulsion, which modifies the waxy cuticle and allows rapid transpiration of water. When the grapes have dried on the vine they are harvested mechanically. The second method uses mechanically harvested fresh grapes, which are dipped in a 2% ester emulsion and dried in a tunnel by hot air dehydration.

The phase of the study reported in this paper consists of: (1) describing the effectiveness and limitations of various ester emulsions with respect to producing raisins; (2) comparing the effects of mechanical harvesting vs. hand harvesting grapes on some physical and chemical properties of raisins, as well as how oleate treatment influences these properties; and (3) determining what effect the oleate treatment has on texture and water absorption and desorption properties of raisins.

MATERIALS & METHODS

FOR TUNNEL DEHYDRATION experiments the hand harvested grapes were picked as bunches on the day they were dried. For mechanical harvesting, canes were cut and 5 days later the grapes were harvested using a Chisholm-Ryder grape harvester. Both harvest procedures were carried out on the same day, and all of the grapes were then dipped in a 2% aqueous emulsion of fatty ester or acid, which was shown by Stafford et al. (1974) to provide maximum residues of fatty derivatives of about 140 ppm. In preliminary experiments with emulsions ranging from 1–10%, the 2% concentration was picked as the best compromise between drying rate and cost. Since the esters are not water soluble an edible emulsifying agent must be used to keep them in suspension. After dipping, grapes were dried to 11–14% moisture at 65–76°C. Since all mechanically dehydrated raisins are treated in some way to accelerate drying, controls in this case were raisins prepared by the commercial soda-dip process in which the grapes are dipped in hot 0.25% sodium hydroxide before drying. Most dehydrated raisins are "golden bleached" with sulfur dioxide. Samples of the hand-harvested and mechanically harvested raisins were exposed to sulfur dioxide fumes before drying to produce this variety of raisin. Eight pounds of sulfur was used per ton of grapes. In some experiments, several dipping emulsions were tested, including suspensions of methyl oleate, ethyl oleate, butyl oleate, oleic acid, stearic acid, caprylic acid and an ethyl oleate emulsion, "Eemulsoyle" (Victorian Chemical Co.). Both vine-dried and tunnel-dried raisins compared in this study were prepared with an "ethyl oleate" prepared from oleic safflower oil. This product contains approximately 80% ethyl oleate and low percentages of ethyl linoleate, stearate and palmitate.

Raisins were analyzed for moisture and sugar by the AOAC (1970) procedures. Acidity was determined by sodium hydroxide titration, and sulfur dioxide by the method of Ponting and Johnson (1945). Color measurements were made on the whole raisins with a Gardner Automatic Color Difference Meter using a standard tile with L = 24.4, a = +15.7 and b = +4.3 for dark unsulfured raisins and L = 52.2, a = −3.4 and b = +31.6 for the golden sulfured ones; a higher L value indicates a lighter raisin. Texture was determined using an Instron Tensile Tester, Model TTCM7, to measure the force required to force a 0.094-in. rod through a single thickness of raisin skin clamped between two disks. Readings were taken on six raisins at each moisture level. Raisins were hydrated in water at 50°C, followed by centrifugation to remove surface moisture. Desiccation was accomplished in a 17% relative humidity (RH) room.

RESULTS & DISCUSSION

Dips and drying times—dehydration

An oleate emulsion dip is only feasible for use in preparing raisins if it accelerates the drying rate of the grapes, at a residue concentration low enough that it will not affect the flavor of the final product. Since a 2% suspension of the fatty acid esters proved to be the lowest which was consistently effective, this concentration was used throughout the study. Of the dips tested, butyl oleate and stearic acid, either alone or together, accelerate moisture loss the least. Caprylic acid and butyl oleate imparted an objectionable off-flavor to the raisins, and stearic acid-treated products developed an objectionable taste during storage. Methyl oleate, ethyl oleate and oleic acid accelerated drying and did not affect flavor, even after periods of storage. Taste threshold studies will be the subject of another paper. Both sulfured and unsulfured grapes treated with these latter materials dried in about 11 hr at 74–76°C, or 19–20 hr at 65°C. At higher air temperatures heat damage can occur unless the product temperature is carefully monitored and the raisins removed before they become too hot. A safer drying procedure, which still takes advantage of the increased drying rate, consists of drying in two stages. Grapes are first

[1] Dept. of Plant Science, California State University, Fresno, CA 93710

dried at 88°C in a parallel air flow tunnel for 2-1/2 hr, and then placed in a 68°C counter-current airflow for final drying. Soda dipped raisins with no oleate treatment require 2 hr longer to dry than the ethyl or methyl oleate-treated fruit. An advantage of the oleate-treated raisins is that they are free flowing and are not as sticky as soda-dipped raisins. A hot sodium hydroxide dip causes small physical fractures in the grape skin, through which syrup exudation can occur.

Effect of grape mechanical harvesting on tunnel dehydrated raisins

In comparing raisins from mechanically harvested and hand-harvested grapes, there was no statistically significant difference between the color, sugar, or acidity of the dehydrated raisins (Table 1). However, there was a difference in residual sulfur dioxide contents. Raisins produced from mechanically harvested grapes contained a mean of 1330 ppm sulfur dioxide, compared to 770 ppm in hand harvested fruit, which is a statistically significant difference at the 5% level. This difference may possibly arise because the machine-picked grapes are put on the trays as loose berries and are loaded more lightly than hand-picked clusters, thus allowing them to absorb SO_2 more readily.

Table 1–Effect of mechanical harvesting on sulfured and unsulfured processed dehydrated grapes[a]

Harvesting method	Sulfur dioxide (ppm)	Reflectance[b] (L)	Sugar (%)	Acid[c] (%)	Moisture (%)
Hand	–	23.6	78.6	2.19	17.2
Mechanical	–	22.5	80.2	2.06	16.6
Hand	770	32.2	82.6	2.26	17.6
Mechanical	1330	31.3	78.6	2.23	17.1

[a] Average of five values
[b] Used different standard for sulfured and unsulfured (see Materials & Methods)
[c] As tartaric

Table 2–Effect of oleate treatment on processed dehydrated grapes[a]

Treatment	Sulfur dioxide (ppm)	Reflectance[b] (L)	Sugar (%)	Acid[c] (%)	Moisture (%)
None	–	21.0	79.8	2.27	18.0
Oleate	–	24.1	79.8	2.05	16.3
None	2468	31.6	81.0	2.53	17.9
Oleate	1132	31.9	81.2	2.21	17.2

[a] Average of five values
[b] Used different standard for sulfured and unsulfured (see Materials & Methods)
[c] As tartaric

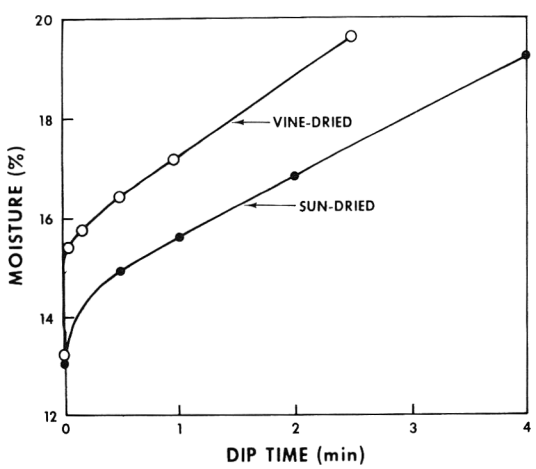


Fig. 1–Hydration rate of unprocessed raisins in 50° C water.

Effect of grape oleate treatment

Oleate treatments also affected the sulfur dioxide absorption and color of raisins. Oleate-treated grapes absorbed and retained 50% less sulfur dioxide than untreated fruit (Table 2), which is statistically significant (α = 0.5). Possibly, the oil coating acts as a barrier and restricts the absorption of sulfur dioxide into the grape. Moreover, oleate treatment causes the grapes to dry faster, allowing less time for enzymatic browning to occur and resulting in a lighter colored unsulfured raisin, (Table 2). A difference in L values of 2 units or more is visually noticeable. Unsulfured oleate-dried raisins, in addition to being lighter in color, had a slight but definite green tint. This color difference, which was noticed only in the vine-dried products, was detected objectively from reflectance hue (a, b) measurements. Dehydrated oleate-treated grapes had an "a" reflectance value of +1.5, compared to –4.1 for the vine-dried products, indicating that the latter contained more green pigment. The enzymatic browning that occurs during regular drying, results in the masking of the green pigmentation of the grapes. The oleate treatment reduces enzymatic browning. There was no shift in the "b" values.

Effect of oleate treatment on raisins

Since fatty acids and their esters modify the skin of the fresh grape to cause faster drying, it would be expected that raisins from oleate-treated grapes would absorb and desorb water differently than regular untreated raisins. The effect of the oleate treatment on moisture absorption and texture was studied on oleate-treated vine-dried raisins. Oleate treatment not only accelerated the drying of the grapes, but it also caused the raisins to absorb moisture more rapidly than raisins from sun-dried untreated grapes, indicating that the raisin surface had undergone a permanent modification. The water absorption characteristics of untreated sun-dried and oleate-treated vine-dried raisins are compared in Figure 1. In the first few seconds, the absorption rate for oleate-treated vine-dried raisins was over 50% faster than for the untreated sun-dried ones. The linear portions of the absorption curves have slopes of 0.66 and 0.50 for the oleate-treated vine-dried and untreated sun-dried raisins, respectively. As these results indicate, the oleate-treated raisins continue to absorb moisture faster during prolonged water contant. This factor will have to be taken into account when processing and cleaning oleate-treated raisins.

Since the oleate treatment accelerated drying of the grapes, it would be suspected that this treatment would also have an effect on the desiccation rate of raisins. The weight loss of regular sun-dried and oleate-treated raisins was followed during storage at 17% RH and 21°C. From these data, equations for moisture loss curves were calculated for both types of 17.5% moisture raisins covering a period of 1–30 days storage. The equation for sun-dried raisins is:

$$y = 17.871x^{-0.198}$$

and the equation for vine-dried raisins is:

$$y = 17.500x^{-0.202}$$

where y is the predicted moisture at x + 1 days, with a correlation of 0.984. The moisture loss differential is greater at higher

Table 3—Texture of field-dried raisins at different moisture levels

Untreated sun-dried		Oleate-treated vine dried	
Moisture (%)	Texture[a] (g)	Moisture (%)	Texture[a] (g)
17.9	304	18.1	290
15.6	299	15.1	237
13.6	301	12.9	235
11.7	320	11.3	270
10.3	345	9.8	303
9.1	360	9.1	307

[a] Grams of force required to rupture the raisin skin

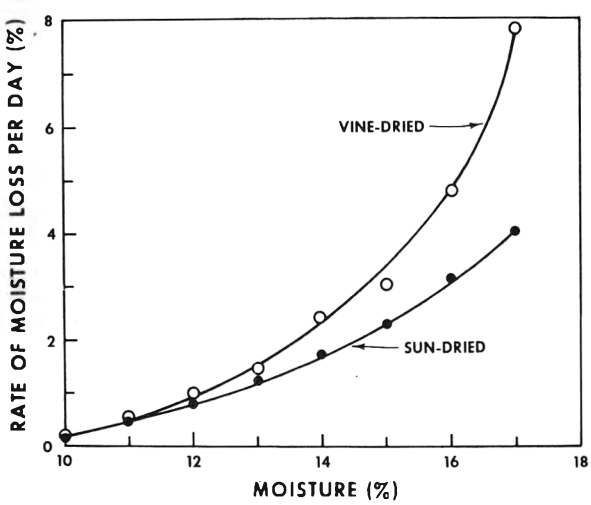


Fig. 2—Moisture loss rate of raisins at 21°C and 17% RH.

moisture levels, as can be readily seen when the rate (slope) of the moisture loss curves are plotted against moisture content (Fig. 2).

Raisin texture was also influenced by oleate treatments, with the oleated-treated vine-dried product being consistently softer than the untreated sun-dried raisins at the same moisture level (Table 3). The rationale behind the physical tenderizing effect oleate has on the raisins skin has not been studied. A comparison of the overall means for the oleate-treated vine-dried and untreated sun-dried raisins, which were 272g and 322g respectively, indicated a statistically significant difference, with $F = 8.36$ and a standard error of 12.3. There was also a significant toughening of the untreated sun-dried raisin at lower moisture levels ($\alpha = 0.5$), but not with oleate-treated vine dried. Textural properties of raisins are important. Raisins are marketed at a lower moisture level than other dried fruits and therefore have a firmer texture; however, this difference is modulated to a degree by their high percentage of invert sugar (approximately 70°).

CONCLUSIONS

METHYL AND ETHYL ester emulsions can be used to accelerate drying of grapes. Ethyl esters are currently approved by FDA for use in food, but methyl esters have not yet received approval. At levels of less than 140 ppm, residues of oleates do not appear to present flavor problems, even after storage at elevated temperatures. Flavor threshold data and storage studies will be the subject of a latter report. Use of ester emulsions to accelerate drying, in conjunction with mechanical harvesting can reduce labor, energy and processing requirements for raisins.

REFERENCES

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, D.C.
Grncarevic, M. 1963. Effect of various dipping treatments on the drying rate of grapes for raisins. Am. J. Enol. & Viticulture 14: 232.
Grncarevic, M. and Radler, F. 1971. A review of the surface lipids of grapes and their importance in the drying process. Am. J. Enol. & Viticulture. 22: 84.
Olmo, H.P. and Studer, H.E. 1967. Mechanical harvesting of Thompson seedless grapes. Wines & Vines 48(2): 25.
Petrucci, V., Canata, N., Bolin, H.R., Fuller, G. and Stafford, A.E. 1974. Use of oleic acid derivatives to accelerate drying of Thompson seedless grapes. J. Am. Oil Chem. Soc. 51(3): 77.
Ponting, J.D. and Johnson, G. 1945. Determination of sulfur dioxide in fruits. Ind. & Eng. Chem. 17: 682.
Ponting, J.D. and McBean, D.M. 1970. Temperature and dipping treatment effects on drying rates and drying times of grapes, prunes and other waxy fruits. Food Technol. 24(12): 86.
Possingham, J.V. 1972. Surface wax structure in fresh and dried grapes. Ann. Bot. 36: 993.
Stafford, A.E., Fuller, G., Bolin, H.R., and Mackey, B. 1974. Analysis of fatty acid esters in processed raisins by gas chromatography. Ag. & Food Chem. 22: 478.
Studer, H.P. and Olmo, H.P. 1974. Raisin pick-up from a continuous paper tray. Transactions Am. Soc. Agr. Eng. 17: 20.
Ms received 1/30/75; revised 4/9/75; accepted 4/15/75.

The authors express thanks to Dr. Bruce Mackey for biometrical assistance.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Dept. of Agriculture to the exclusion of others than may be suitable.

TAKESHI MATSUURA, A. G. BAXTER and S. SOURIRAJAN
Div. of Chemistry, National Research Council of Canada, Ottawa, Canada K1A 0R9

REVERSE OSMOSIS RECOVERY OF FLAVOR COMPONENTS FROM APPLE JUICE WATERS

INTRODUCTION

THE PRACTICAL SUCCESS of reverse osmosis for fruit juice concentration depends on adequate recovery of the flavor components which escape through the membrane during the process. Since reverse osmosis separations of the flavor components could be significantly higher with dilute feed solutions essentially free from sugars (Matsuura et al., 1974c), it was suggested (Matsuura et al., 1973; 1974a) that the process be carried out in more than one stage. In the first stage, the primary objective could be the recovery of most (> 99%) of the sugars present in the fruit juice; a part of the flavor components is also recovered along with the sugars in this first stage operation. In the second and subsequent stages, the primary objective could be the recovery of most of the remaining flavor components by repeated reverse osmosis treatment of the membrane permeated fruit juice waters (obtained from the first stage operation) under appropriate experimental conditions; the small fraction of the sugars left in the fruit juice waters from the first stage operation may be expected to be recovered essentially completely during the second and subsequent stages along with the flavor components. The possibility of such recovery of flavor components has been experimentally demonstrated using apple juice waters and cellulose acetate membranes (Matsuura et al., 1974a).

Recent work (Matsuura et al., 1974d; Dickson et al., 1975) has indicated that polar organic solutes of the type present as flavor components in fruit juices are separated to a much higher extent with aromatic polyamide membranes than with cellulose acetate membranes with about the same average pore size on the membrane surface. Consequently, aromatic polyamide membranes appear to be better suited for reverse osmosis recovery of the flavor components present in fruit juice waters. This paper is concerned with an exploration of the effectiveness of aromatic polyamide membranes developed earlier (Matsuura et al., 1974d) for the separation of some typical apple flavor components present in dilute aqueous solutions.

This exploration utilizes and extends the fundamental physicochemical criteria approach to reverse osmosis separations based on the preferential sorption-capillary flow mechanism (Sourirajan and Matsuura, 1973). According to this mechanism reverse osmosis separation is governed both by the porous structure of the membrane surface and preferential sorption at the membrane-solution interface under the given experimental conditions. Preferential sorption at the membrane-solution interface is a function of the solute-solvent-polymer (membrane material) interactions. These interactions arise in general from polar-, steric-, nonpolar-, and/or ionic-character of each one of the above three components in the reverse osmosis system. For a given membrane (i.e., specified membrane material and surface porosity), in a reverse osmosis system involving preferential sorption of water (solvent) at the membrane-solution interface, when the solute is un-ionized and when its molecular structure does not contain more than three straight chain carbon atoms not associated with a polar functional group, solute separation in reverse osmosis is governed by polar and steric effects only. Many volatile flavor components fall in the above category of solutes. This discussion is concerned with such solutes.

The polar and steric effects in reverse osmosis have been discussed (Matsuura et al., 1974b; 1975a). It has been shown that the polar and steric effects governing reverse osmosis separations can be expressed quantitatively in terms of appropriate free energy and steric parameters respectively (Matsuura et al., 1975a). These parameters can then be built into transport equations enabling the prediction of solute transport parameter $D_{AM}/K\delta$ (treated as a single quantity) for a large number of un-ionized polar solutes from data on membrane specifications only, given in terms of pure water permeability constant A and solute transport parameter $D_{AM}/K\delta$ for sodium chloride (Matsuura et al., 1974a; 1975b; Sourirajan, 1970b).

The above prediction procedure was used in this work to calculate obtainable solute separations for some of the typical apple flavor components in dilute aqueous solutions using aromatic polyamide membranes. The data on solute transport parameter used for such prediction were also used in system analysis (Matsuura et al., 1974a) to calculate obtainable overall solute separations as functions of fraction product recovery and operating pressure. Finally, some reverse osmosis concentration experiments were carried out with actual apple juice waters (permeate from the first stage) using aromatic polyamide membranes to compare data on overall recovery of flavor components with those predicted for the individual flavor components studied.

EXPERIMENTAL

Reverse osmosis experiments

Two types of reverse osmosis cells, flow type and nonflow type, were used in this work. Details of cell design and experimental procedure are given in the literature (Sourirajan, 1970a). The nonflow type cell (effective film area = 9.6 cm^2) was used for the first stage concentration of apple juice to obtain the membrane permeated juice water, and the subsequent reverse osmosis treatment of the above apple juice water to concentrate the flavor components dissolved in it. Commercially available Allen's apple juice was used in the experiments. The flavor components present in this particular juice sample tested are however not known. The flow type cell (effective film area = 13.2 cm^2) was used for the reverse osmosis experiments involving the ester solutes listed in Table 1. Laboratory made aromatic polyamide membranes reported earlier (Matsuura et al., 1974d) were used in all the experiments.

The membranes used are specified (Sourirajan, 1970b) in Table 2 in terms of the pure water permeability constant A (in g moles H_2O/cm^2 sec atm) and the solute transport parameter $D_{AM}/K\delta$ (in cm/sec) for sodium chloride at the indicated operating pressure. Some data on membrane performance for the system $NaCl$-H_2O are also given in Table 2 for the specified feed concentration and mass transfer coefficient k (in cm/sec) on the high pressure side of the membranes. Unless otherwise stated, the reported product rates are those corrected to 25°C using the relative viscosity and density of pure water. In all experiments, the terms "product" and "product rate" refer to membrane permeated solutions.

Unless otherwise stated, all experiments were carried out at the laboratory temperature (23–25°C). Some experiments on juice water concentration were carried out at 7.5°C with the nonflow-type cell placed in a cold box; the temperature of the cell in the cold box could

Table 1—Physicochemical data on ester solutes studied[a]

Solute no.	Esters	Formula (R_1, R_2 in R_1-C(=O)-O-R_2)	Mol wt	ΣE_s	k X 10^4 cm/sec	ΔG_B/RT	ΔG_I/RT	$-\Delta\Delta G$/RT
1	Methyl acetate	CH_3, CH_3	74.08	0.0	18.0	8.37	5.97	2.40
2	Ethyl acetate	CH_3, C_2H_5	88.10	−0.07	16.3	8.66	6.55	2.11
3	Methyl n-butyrate	C_3H_7, CH_3	102.13	−0.36	15.2	8.95	7.12	1.83
4	Ethyl propionate	C_2H_5, C_2H_5	102.13	−0.14	15.1	8.95	7.12	1.83
5	Butyl acetate	CH_3, C_4H_9	116.16	−0.39	14.1	9.23	7.70	1.53

[a] T = 298°K; k_{NaCl} = 22 X 10^{-4} cm/sec; Data on ΣE_s from Taft (1956)

be held constant within ±1°C during the experiment. The operating pressure used was either 500 or 1000 psig as indicated.

The fraction solute separation f for the different feed solutions used was calculated as follows. For aqueous sodium chloride, and organic ester feed solution,

$$f = \frac{\text{solute ppm in feed} - \text{solute ppm in product}}{\text{solute ppm in feed}}$$

For the separation of volatile flavor components present in the membrane permeated fruit juice waters obtained from the first stage concentration process,

$$f = \frac{\text{equiv EtOH ppm in feed} - \text{equiv EtOH ppm in product}}{\text{equiv EtOH ppm}}$$

Analysis

The concentration of sodium chloride in aqueous solutions was determined using a conductivity bridge. A Tracor (model 160) gas chromatograph was used for the analysis of ester solutes and the flavor components present in solution in the apple juice waters.

For the analysis of the ester solutes, a 100 ft long, 0.020 in. i.d. support coated open tubular (S.C.O.T.) column containing Carbowax 1540 was employed. The column temperature was 50°C. Helium carrier gas flow rate was 12 cm^3/min. Sample size used was 2 μl. The entire effluent from the column passed into a hydrogen flame ionization detector. Methyl ethyl ketone or acetone was used as the internal standard.

The flavor components present in apple juice waters were analyzed in terms of equivalent ethyl alcohol using standard ethyl alcohol solutions for calibrations. The details of the column used and the experimental conditions employed were the same as those reported earlier (Matsuura et al., 1974a).

The overall accuracy of the analysis with respect to the individual ester components and the flavor components referred to above was estimated to be within 3%.

It is recognized that it is rather crude to treat the concentration of flavor components in terms of equivalent ethyl alcohol concentration; such treatment however does not affect the general conclusions reached in this paper.

RESULTS & DISCUSSION

Un-ionized polar aliphatic organic solutes in dilute aqueous solutions

General expression for solute transport parameter, $D_{AM}/K\delta$. For the case where reverse osmosis separations are governed by preferential sorption of water at the membrane-solution interface *and* the polar- and steric-character of the solute molecule, a general expression for solute transport parameter for un-ionized polar aliphatic and alicyclic organic solutes in dilute aqueous solutions can be given as

$$\ln (D_{AM}/K\delta) = \ln C^*_{NaCl} + \ln \Delta^* + \left(\frac{-\Delta\Delta G}{RT}\right) + \delta^* \Sigma E_s \qquad (1)$$

The basis of Eq. (1) has been discussed (Matsuura et al., 1975a). The meaning of the four parameters given on the right side of Eq. (1), and the method of obtaining numerical values

Table 2—Specifications of the aromatic polyamide membranes used and some performance data[a]

Film no.	1[b]		2[b]		3[b]		4[c]	
Operating pressure, psig	500	1000	500	1000	500	1000	500	1000
Pure water permeability constant A, $\frac{\text{g mole } H_2O}{cm^2.sec.atm} \times 10^6$	0.39	0.37	0.32	0.31	0.41	0.39	0.67	0.64
Solute transport parameter $(D_{AM}/K\delta)_{NaCl}$, (cm/sec) X 10^5	3.40	3.83	3.95	4.45	8.44	9.52	1.30	1.47
$\ln (D_{AM}/K\delta)_{NaCl}$	−10.29	−10.17	−10.14	−10.02	− 9.38	− 9.26	−11.25	−11.13
$\ln C^*_{NaCl}$	− 9.56	− 9.44	− 9.41	− 9.29	− 8.65	− 8.53	−10.52	−10.40
$\ln \Delta^*$	− 2.08	− 2.12	− 2.12	− 2.12	− 2.12	− 2.12	− 1.58	− 1.65
Solute separation, %	85.5	94.2	81.0	90.3	71.6	79.7	88.2	97.2
Product rate, g/hr	10.5	20.5	8.6	17.4	11.2	21.8	12.3	24.1

[a] Sodium chloride concentration in feed: 3500 ppm
[b] Film 1–3: Flow type cell, film area 13.2 cm^2, k = 22 X 10^{-4} cm/sec
[c] Film 4: Nonflow type cell, film area 9.6 cm^2, k = 20 X 10^{-4} cm/sec

for the above parameters are given briefly as follows with reference to the aromatic polyamide (membrane material)-water (solvent) system under consideration.

The quantity $\ln C^*_{NaCl}$ is a measure of the average pore size on the membrane surface; this quantity can be calculated from data on membrane specifications obtained from a dilute aqueous sodium chloride feed solution using the relation:

$$\ln (D_{AM}/K\delta)_{NaCl} = \ln C^*_{NaCl} + \left\{ \left(\frac{-\Delta\Delta G}{RT}\right)_{Na^+} + \left(\frac{-\Delta\Delta G}{RT}\right)_{Cl^-} \right\} \quad (2)$$

The values of $(-\Delta\Delta G/RT)_{Na^+}$ and $(-\Delta\Delta G/RT)_{Cl^-}$ at 25°C are −2.08 and +1.35 respectively from previous work (Dickson et al., 1975).

The quantity $\ln \Delta^*$ is an adjustment parameter to cancel the overlap in representation of the effect of pore size included in the combined term $(\ln C^*_{NaCl} + \delta^* \Sigma E_s)$ in Eq. (1). This adjustment parameter is expressed in Figure 1 in the form of $\ln \Delta^*$ versus $\ln C^*_{NaCl}$ correlation on the basis of previous work (Matsuura et al., 1975a). As already pointed out, this correlation is independent of the nature of the functional group in the solute molecule under consideration.

The quantity $(-\Delta\Delta G/RT)$ is the polar free energy parameter for the solute molecule. This parameter involves, besides the universal gas constant R and the absolute temperature T, the quantity $\Delta\Delta G$ which can be obtained from the relation:

$$\Delta\Delta G = \Delta G_I - \Delta G_B \quad (3)$$

where ΔG represents the free energy of solute-solvent interaction (i.e., the free energy of hydration in the present discussion) and the subscripts I and B refer to the membrane-solution interface and the bulk solution phase respectively. It has been shown (Butler, 1937; Diamond and Wright, 1969; Matsuura et al., 1975a) that the free energy of hydration in the bulk solution phase, and the corresponding quantity in the membrane-solution interface are additive functions of the structural groups in the solute molecule concerned. Consequently, each of the quantities ΔG_B and ΔG_I for the solute molecule can be considered to be the sum of appropriate incremental free energy of hydration for the structural groups involved in the molecule so that,

$$\Delta G_B = \Sigma \gamma_B \text{ (structural group)} + \gamma_{B,O} \quad (4)$$

and

$$\Delta G_I = \Sigma \gamma_I \text{ (structural group)} + \gamma_{I,O} \quad (5)$$

where γ_B (structural group) is the incremental free energy of hydration for the structural group concerned, and $\gamma_{B,O}$ is a characteristic constant applicable to all structural groups in the bulk solution phase, and γ_I (structural group) and $\gamma_{I,O}$ are the corresponding quantities applicable for the membrane-solution interface. The available data on the numerical values of γ_B (structural group), $\gamma_{B,O}$, γ_I (structural group), and $\gamma_{I,O}$ are listed in Table 3.

The quantity $\delta^* \Sigma E_s$ in Eq. (1) represents the contribution of steric effect to solute transport parameter. The quantity ΣE_s represents Taft's steric parameter for the substituent group in the solute molecule; numerical values of ΣE_s for many substituent groups are obtainable from Taft's table (Taft, 1956). The coefficient δ^* associated with ΣE_s is a function of the porous structure of the membrane surface, and the chemical nature of both the membrane material and solute molecule; this function has to be determined experimentally. Figure 2 represents the available data on δ^* pertinent to this work (Matsuura et al., 1975a).

Thus from data given in Tables 2 and 3, Taft's table (Taft, 1956), and Figures 1 and 2, the quantities on the right side of Eq. (1), and hence $D_{AM}/K\delta$ for the different polar organic solutes can be calculated for a given membrane.

Free energy parameters for ester-solutes. The major flavor components present in apple juice waters consist of alcohols, aldehydes and esters (Merson and Morgan, 1968; Chandrasekaran and King, 1971). While the data given in Table 3 are adequate for obtaining the free energy parameters for many alcohol- and aldehyde-solutes using Eq. (3), similar data for ester-solutes cannot be obtained from Table 3 since it does not contain the γ_I value for the structural group -C-O-. This latter value was obtained in this work by an interpolation technique based on a consideration of the specific free energy of hydration of the polar functional groups in the bulk solution phase and in the membrane-solution interface, represented by the symbols $\Delta G_{B,sp}$ (polar functional group) and $\Delta G_{I,sp}$ (polar functional group) respectively. These latter quantities were calculated as follows for different polar functional groups

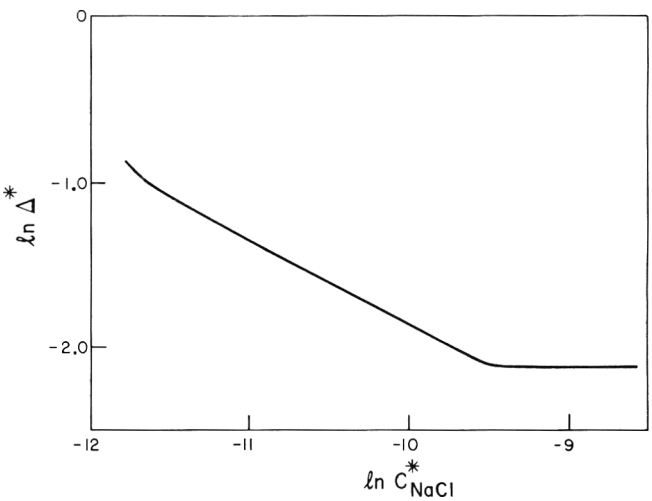


Fig. 1—Correlation of ln Δ vs ln C*NaCl for aromatic polyamide membranes.*

Table 3—Structural group contribution to ΔG_B and ΔG_I for aromatic polyamide membrane[a]

Structural groups	$\gamma_{B,O} = -12.04$ γ_B	$\gamma_{I,O} = 10.70$ γ_I
$-CH_3$	11.07	−1.16
$>CH_2$	0.17	0.34
$>CH_2'$[b]	—	−0.25
-C-H	−10.62	1.82
-C-	−21.50	3.33
cyclic	20.49	−4.17
-OH	3.99	−8.33
-COO-	− 5.14	—
>O	− 4.03	−3.59
-CN	5.02	—
>C=O	− 5.80	−5.55
-CHO	5.80	−6.34

[a] Data from Matsuura et al. (1975a)

[b] $>CH_2'$ refers to methylene groups in the molecular structure, which are separated from a functional group by three or more carbon atoms in a straight chain.

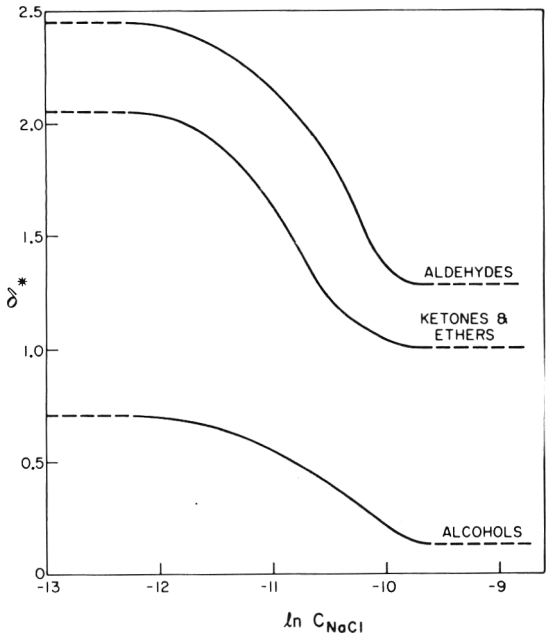


Fig. 2—Correlation of δ vs ln C*NaCl for aromatic polyamide membranes.*

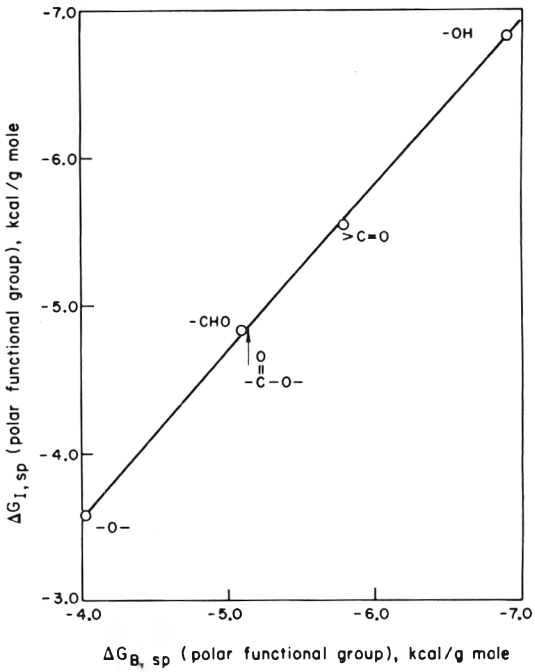


Fig. 3—Correlation of $\Delta G_{I,sp}$ (polar functional group) vs $\Delta G_{B,sp}$ (polar functional group) for aromatic polyamide membranes.

using the data given in Table 3 and the method indicated by Diamond and Wright (1969). For the bulk solution phase,

$\Delta G_{B,sp}$ (-OH) $= \Delta G_B (CH_3CH_2OH) - \Delta G_B (CH_3CH_3)$
$= 3.19 - 10.10 = -6.91$;

$\Delta G_{B,sp}$ (-CHO) $= \Delta G_B (CH_3CH_2CHO) - \Delta G_B (CH_3CH_3)$
$= 5.00 - 10.10 = -5.10$;

$\Delta G_{B,sp}$ (>C=O) $= \Delta G_B (CH_3COCH_3) - \Delta G_B (CH_3CH_3)$
$= 4.30 - 10.10 = -5.80$;

$\Delta G_{B,sp}$(-O-) $= \Delta G_B (C_2H_5OC_2H_5) - \Delta G_B (C_2H_5C_2H_5)$
$= 6.41 - 10.44 = -4.03$; and

$\Delta G_{B,sp}$ (-C(=O)-O-) $= \Delta G_B (CH_3COOCH_3) - \Delta G_B (CH_3CH_3)$
$= 4.96 - 10.10 = -5.14$.

Similarly for the membrane (aromatic polyamide)-solution interface,

$\Delta G_{I,sp}$ (-OH) $= \Delta G_I (CH_3CH_2OH) - \Delta G_I (CH_3CH_3)$
$= 1.55 - 8.38 = -6.83$;

$\Delta G_{I,sp}$ (-CHO) $= \Delta G_I (CH_3CH_2CHO) - \Delta G_I (CH_3CH_3)$
$= 3.54 - 8.38 = -4.84$;

$\Delta G_{I,sp}$ (>C=O) $= \Delta G_I (CH_3COCH_3) - \Delta G_I (CH_3CH_3)$
$= 2.83 - 8.38 = -5.55$;

$\Delta G_{I,sp}$ (-O-) $= \Delta G_I (C_2H_5OC_2H_5) - \Delta G_I (C_2H_5C_2H_5)$
$= 5.47 - 9.06 = -3.59$.

A plot of $\Delta G_{B,sp}$ versus $\Delta G_{I,sp}$ (Fig. 3) for the structural group -OH, -CO, -CHO, and -O- shows that the correlation is a straight line from which $\Delta G_{I,sp}$ for the structural group -C(=O)-O- can be obtained by interpolation as −4.84. Comparing Eq. (4) and (5) and the method used above for calculating $\Delta G_{B,sp}$ and $\Delta G_{I,sp}$ values, it is clear that, for the structural group -C(=O)-O-,

$$\Delta G_{B,sp}(\text{-C(=O)-O-}) \equiv \gamma_B(\text{-C(=O)-O-})$$

and

$$\Delta G_{I,sp}(\text{-C(=O)-O-}) \equiv \gamma_I(\text{-C(=O)-O-})$$

Therefore the γ_I value for the structural group -C(=O)-O- is −4.84 which can be used for obtaining the free energy parameter data for ester-solutes.

Values of δ* applicable for ester-solutes. The ln C^*_{NaCl} versus δ* correlations given in Figure 2 are those established earlier experimentally for the alcohol-, aldehyde-, ketone-, and ether-solutes (Matsuura et al., 1975a). Similar correlation for ester-solutes has not yet been firmly established. It may however be noted that the correlation is identical for both ketone- and ether-solutes. Since the specific free energy of hydration ($\Delta G_{B,sp}$) for the polar functional group -C(=O)-O- (= −5.14) lies between the corresponding data for the functional groups >C=O (= −5.80) and -O- (= −4.03), it seemed reasonable to consider that the ln C^*_{NaCl} versus δ* correlation given in Figure 2 for the ketone- and ether-solutes was also applicable for the ester-solutes. The following experimental results obtained in this work justified the above conclusion.

Experimental reverse osmosis data were obtained using the five ester-solutes listed in Table 1 and the polyamide films 1, 2 and 3 specified in Table 2. The operating pressure used was 500 psig and the solute concentrations in the feed solutions used were in the range 220–350 ppm (0.003g mole/liter).

The obtainable solute separations for the ester-solutes were also calculated on the basis of $D_{AM}/K\delta$ values predicted by Eq. (1). For the purpose of this prediction, the ln C^*_{NaCl} versus δ* correlation given in Figure 2 for the ketone and ether solutes was assumed valid for the ester-solutes also; the

applicable values of ln Δ^*, $(-\Delta\Delta G/RT)$, and ΣE_s were taken from Figure 1 and Table 1. Using the $D_{AM}/K\delta$ values predicted by Eq. (1), solute separation f was calculated from the following relation derived earlier (Matsuura and Sourirajan, 1973):

$$D_{AM}/K\delta = \frac{PR}{3600\,S\,d}\,\frac{1-f}{f}\left[\exp\left\{\frac{PR}{3600\,S\,k\,d}\right\}\right]^{-1} \qquad (6)$$

where PR = product rate in grams/hour per given area S of film surface (= 13.2 cm^2 in the present case), d = solution density which is essentially the same as that of pure water (in gram/cm^3) and k = mass transfer coefficient (in cm/sec) on the high pressure side of the membrane calculated by the method described earlier (Matsuura et al., 1974c). Because of the negligible osmotic pressure of the feed solutions involved, the quantity PR was assumed equal to the pure water permeation rate (PWP) for the given area of the membrane surface; the latter quantity was calculated from data on A given in Table 2. The values of k applicable for the experimental conditions used are listed in Table 1.

The calculated and experimental data on solute separations were found to be in reasonable agreement. This is illustrated in Figure 4. On the basis of this agreement, the ln C^*_{NaCl} versus δ^* correlation given in Figure 2 for the ketone and ether-solutes was assumed valid for the ester-solutes also in this work.

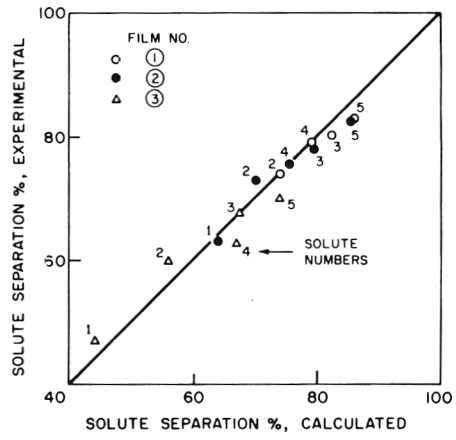


Fig. 4—Comparison of experimental and calculated separation of ester solutes by aromatic polyamide membranes. Solute numbers same as in Table 1.

Separation of some typical flavor components present in apple juice waters

Twelve flavor components which have been identified in apple juices (Merson and Morgan, 1968; Chandrasekaran and King, 1971; Bednas, 1974) are listed in Table 4. One can calculate reverse osmosis separations obtainable for each of these flavor components with a given polyamide film using Eqs. (1) and (6) and the method described below.

Considering, for illustration, the polyamide film 4 at the operating pressure of 1000 psig, the applicable value of ln C^*_{NaCl} is −10.4 from Table 2, that of ln Δ^* is −1.65 from Figure 1. The free energy parameter $(-\Delta\Delta G/RT)$ for each of the flavor components can be calculated using Eqs. (3), (4) and (5) and the data given in Table 3; the values of $(-\Delta\Delta G/RT)$ so calculated are given in Table 4. The applicable values of δ^* for the film are 0.36, 1.77 and 1.18 for the alcohol-, aldehyde-, and the ester-solutes respectively from Figure 2 and previous discussion. The ΣE_s values for each of the substituent groups involved, except $(s\text{-}C_4H_9)CH_2$, can be obtained from Taft's table (Taft, 1956); these values of ΣE_s are also included in Table 4. The value of ΣE_s for the substituent group $(s\text{-}C_4H_9)CH_2$ was obtained by an interpolation technique. A plot of Taft's data on ΣE_s for the substituent group R versus that for the substituent group R-CH_2 gives a straight line correlation as shown in Figure 5 from which ΣE_s for $(s\text{-}C_4H_9)CH_2$ can be read to be −1.31 corresponding to the ΣE_s value of −1.13 for s-C_4H_9 given in Taft's table. Thus all

Table 4—Physicochemical parameters and solute separations of typical flavor components in apple juice[a]

Flavor components	Formula	Mol wt	ΣE_s	$\frac{-\Delta\Delta G}{RT}$	k_{NaCl} = 20 X 10^{-4} cm/sec: k X 10^4 cm/sec	Solute sep %	k_{NaCl} = 50 X 10^{-4} cm/sec: k X 10^4 cm/sec	Solute sep %
Methyl alcohol	CH_3OH	32.0	0.0	3.06	22.4	84.3	56.0	87.7
Ethyl alcohol	C_2H_5OH	46.1	−0.07	2.76	18.6	87.1	46.4	90.5
i-Butyl alcohol	i-C_4H_9OH	74.1	−0.93	2.42	14.8	91.7	37.0	94.5
n-Hexyl alcohol	n-$C_6H_{13}OH$	102.2	−0.40	2.81	13.0	84.8	32.5	90.2
i-Butyraldehyde	i-C_3H_7OH	72.1	−0.70	2.40	15.4	96.6	38.5	97.8
n-Hexanal	n-$C_5H_{11}CHO$	100.2	−0.40	2.19	13.2	94.9	33.1	96.8
Ethyl acetate	$C_2H_5O\overset{O}{\overset{\|}{C}}CH_3$	88.1	−0.07	2.11	14.8	92.1	37.1	94.7
n-Butyl acetate	n-$C_4H_9O\overset{O}{\overset{\|}{C}}CH_3$	116.2	−0.39	1.53	12.9	96.5	32.2	97.8
Ethyl butyrate	$C_2H_5O\overset{O}{\overset{\|}{C}}C_3H_7$	116.2	−0.43	1.53	12.9	96.6	32.2	97.9
2-Methyl-1-butyl acetate	$C_2H_5CH(CH_3)CH_2O\overset{O}{\overset{\|}{C}}CH_3$	130.2	−1.31	1.46	12.2	98.8	30.5	99.3
Ethyl 2-Methyl-butyrate	$C_2H_5O\overset{O}{\overset{\|}{C}}CH(CH_3)C_2H_5$	130.2	−1.20	1.46	12.2	98.7	30.5	99.2
n-Hexyl acetate	n-$C_6H_{13}O\overset{O}{\overset{\|}{C}}CH_3$	144.2	−0.40	3.95	11.6	69.2	29.0	79.7

[a] T = 298° K, ΣE_s values from Taft (1956).

quantities on the right side of Eq. (1) are known so that ln $(D_{AM}/K\delta)$ for each of the flavor components listed can be calculated for film 4.

The data on solute transport parameter calculated above can be used in Eq. (6) to calculate solute separation f, for which purpose the applicable data on PR, S, d and k are needed. Since the flavor components are present in very low concentrations in apple juice waters, PR is essentially the same as PWP which can be obtained from the pure water permeability constant for the film given in Table 2. The density d of the apple juice water may be considered to be essentially the same as the density of pure water. The effective membrane area S was taken here as 9.6 cm^2 which is the area applicable for the nonflow type apparatus used in this work. The value of the mass transfer coefficient k for the flavor component on the high pressure side of the membrane may be obtained from the relation (Matsuura et al., 1974c):

$$k = k_{NaCl}\left[\frac{D_{AB}}{(D_{AB})_{NaCl}}\right]^{2/3} \quad (7)$$

where k_{NaCl} = mass transfer coefficient for NaCl on the high pressure side of the membrane with the reference feed solution NaCl-H_2O, and $(D_{AB})_{NaCl}$ and D_{AB} refer to diffusivity of NaCl and the flavor component respectively in water. The values of D_{AB} may be calculated as before (Matsuura and Sourirajan, 1973) from the empirical equation of Wilke and Chang (1955). The relative values of k so calculated are also included in Table 4.

Following the above method for calculating f, two sets of calculations were made corresponding to k_{NaCl} values of 20 × 10^{-4} cm/sec and 50 × 10^{-4} cm/sec. The former value of k was the one actually obtained in the apparatus used in this work, and the latter value of k is the one which is more desirable for industrial applications (Matsuura et al., 1974a). The values of f for each flavor component corresponding to the above values of k_{NaCl} are given in Table 4. It has already been shown that mixed solutes in dilute solution behave independently in reverse osmosis (Matsuura et al., 1974c). Therefore, for the polyamide membrane used, Table 4 gives the obtainable values of solute separations for the flavor components listed. The data show that these values are much higher than those reported (Matsuura et al., 1974b, c) for the same or similar solutes with comparable cellulose acetate membranes studied earlier. Hence the aromatic polyamide membranes are particularly suitable for the concentration of flavor components present in apple juice waters.

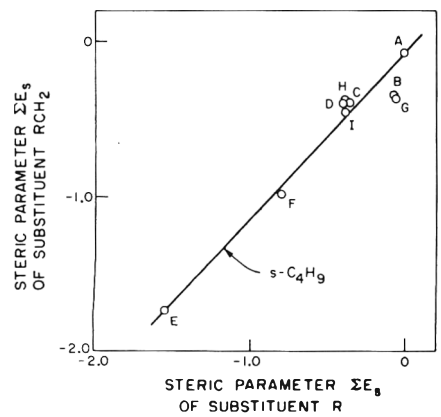


Fig. 5—Correlation of steric parameter ΣE_S of substituent RCH_2 vs steric parameter ΣE_S of substituent R. Substituent groups:

	R		
A	CH_3	F	cyclo-C_6H_{13}
B	C_2H_5	G	C_6H_5
C	C_3H_7	H	$C_6H_5CH_2$
D	C_4H_9	I	$C_6H_5(CH_2)_2$
E	t-C_4H_9		

Results of system analysis

The application of system analysis (Ohya and Sourirajan, 1971) for parametric studies on concentration of apple juice waters has been illustrated (Matsuura et al., 1974a). The results of similar analysis with respect to individual flavor components can be of practical interest. A set of such results was calculated for film 4 with respect to the flavor components 2-methyl-1-butyl acetate (whose reverse osmosis separation was the highest for the film considered), n-hexyl acetate (whose reverse osmosis separation was the lowest) and ethyl alcohol (whose reverse osmosis separation was in the intermediate range). It may be recalled that the parameters of system specification and system performance are defined as follows:

Parameters of system specification

$$\gamma = \frac{\text{osmotic pressure of initial feed solution}}{\text{operating pressure}}$$

$$\theta = (D_{AM}/K\delta)/(\text{pure water permeation velocity})$$

and $\lambda = k/(D_{AM}/K\delta)$.

Parameters of system performance

Δ = volumetric fraction water recovery

$$= 1 - \frac{V_1}{V_1^{\,o}} \text{ in batch operation}$$

$$\bar{C}_3 = \frac{\text{avg product conc for given } \Delta}{\text{initial conc of feed}}$$

and $\tau = Sv_w^* \, t/V_1^{\,o}$ in batch operation,

where S = membrane area, cm^2; t = time, sec; v_w^* = pure water permeation velocity, cm/sec; V_1 = volume of solution on the high pressure side of membrane at any time in a batch process, cm^3 and $V_1^{\,o}$ = value of V_1 at the start of batch operation, cm^3.

The system specifications for the reverse osmosis concentration of the three flavor components chosen for illustration are given in Table 5 for the operating pressures of 500 and 1000 psig. The values of $\lambda\theta$ given in Table 5 are based on the actual values of k_{NaCl} obtained in the apparatus used in this work. The data on system specifications show that both θ and $\lambda\theta$ decrease with increase in operating pressure. At a given level of Δ, a decrease in θ tends to decrease $\bar{C}_3$ (which means higher retention of the flavor component in the concentrate), while a decrease in $\lambda\theta$ tends to increase $\bar{C}_3$. Therefore it is not obvious which operating pressure is desirable in order to obtain a higher retention of the flavor compound in the concentrate i.e., a decrease in $\bar{C}_3$. The results of system analysis based on the above system specifications are given in Figure 6. These results show precisely that the values of $\bar{C}_3$ are lower at 1000 psig than at 500 psig for all the three flavor components studied. These results mean that the higher operating pressure is desirable in order to obtain a higher recovery of flavor components in the concentrate by the reverse osmosis treatment of apple juice waters.

Reverse osmosis treatment of apple juice waters

The foregoing analysis on the effect of operating pressure, and the analytical and experimental results reported earlier (Matsuura et al., 1974a) lead to the conclusion that a higher

Table 5–System specifications for some flavor components

Parameters of system specification	500 psig			1000 psig		
	Flavor component			Flavor component		
	2-Methyl-1-butyl acetate	Ethyl alcohol	n-Hexyl acetate	2-Methyl-1-butyl acetate	Ethyl alcohol	n-Hexyl acetate
v_w*, (cm/sec) X 10^4	4.09	4.09	4.09	7.84	7.84	7.84
$(D_{AM}/K\delta)$, (cm/sec) X 10^5	0.51	8.52	18.05	0.53	8.96	18.97
k, (cm/sec) X 10^4	12.2	18.6	11.6	12.2	18.6	11.6
γ	0	0	0	0	0	0
θ	0.012	0.208	0.441	0.007	0.114	0.242
$\lambda\theta$	2.975	4.531	2.840	1.554	2.366	1.481

Table 6–Experimental details and design parameters for recovery of apple flavor components[a]

Run no.	Experimental details			Design parameters at $\Delta = 0.6$		
	Operating pressure psig	Operating temp °C	Initial feed conc ppm eq. EtOH	$(c_A)_f/(c_A)_i$	$\bar{C}_3$	Processing capacity gal day^{-1} ft^{-2}
1	1250	7.5	160	2.22	0.16	9.1
2	1000	25	694	1.65	0.56	19.2
3	1000	7.5	735	2.19	0.19	8.6
4	1000	7.5	160	2.22	0.15	7.7
5	500	7.5	163	2.07	0.28	4.8
6	250	7.5	185	1.81	0.46	1.9

[a] Membrane: Aromatic polyamide film 4

operating pressure together with a lower operating temperature can result in a relatively higher recovery of flavor components in the concentrate during the reverse osmosis treatment of apple juice waters. This conclusion was verified experimentally by a set of six experiments using the polyamide film 4 and actual apple juice waters. In these experiments, the operating temperature used was either 7.5°C or 25°C. The operating pressure used was in the range 250–1250 psig. The volumetric fraction of membrane permeated juice water was in the range 60–70%. The concentration of flavor components in the feed expressed in terms of equivalent ethyl alcohol concentration was in the range 160–735 ppm. The experimental results obtained are illustrated in Figure 7 in the form of correlations of the concentration ratio $(c_A)_f/(c_A)_i$ [i.e., concentration of flavor components in the final concentrate, $(c_A)_f$/concentration of flavor components in the initial feed $(c_A)_i$] versus Δ, $\bar{C}_3$, and processing capacity of the membrane. The quantity "processing capacity" is defined as the volume of feed solution that one square foot of film surface can handle per day in a batch concentration process to obtain a given value of $(c_A)_f/(c_A)_i$. Table 6 gives the experimental details for the runs noted in Figure 7.

Table 6 also gives data on $(c_A)_f/(c_A)_i$, $\bar{C}_3$ and processing capacity of the film at $\Delta = 0.6$ arbitrarily chosen for comparison. From the point of view of higher recovery of flavor components in the concentrate, it is desirable to have a higher value for $(c_A)_f/(c_A)_i$ and a lower value of $\bar{C}_3$ at a given value of Δ; it is also desirable to have a higher value for processing

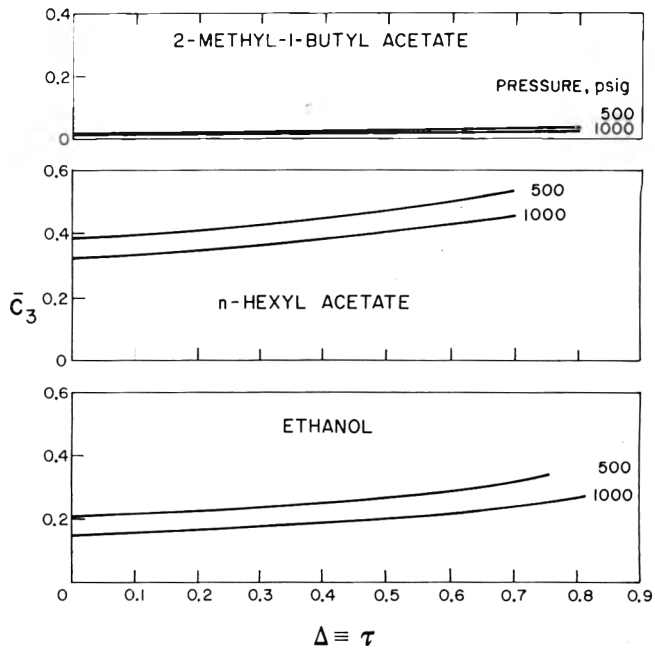


Fig. 6–Results of reverse osmosis system analysis for the recovery of some apple flavor components by aromatic polyamide membrane (film 4).

capacity so that the membrane area needed for practical use in recovery of flavor components is within economic limits.

The results given in Table 6 show that $(c_A)_f/(c_A)_i$ increases and $\bar{C}_3$ decreases significantly by (i) decrease in operating temperature (run numbers 2 and 3), (ii) increase in operating pressure in the range 250–1000 psig (run numbers 4, 5 and 6), and (iii) decrease in feed concentration (run numbers 3 and 4). The results also show that an increase in operating pressure from 1000 to 1250 psig (run numbers 4 and 1) had no significant effect on $(c_A)_f/(c_A)_i$ and $\bar{C}_3$. All the above results are consistent with the results of system analysis discussed above (Fig. 6) and in the previous paper (Matsuura et al., 1974a).

The cellulose acetate film J21 used earlier (Matsuura et al., 1974a) and the aromatic polyamide film 4 used in this work (Table 2) gave essentially the same level of solute separation for sodium chloride under identical experimental conditions. On this basis, the relative performance of the above two films is comparable. At $\Delta = 0.6$, the values of $(c_A)_f/(c_A)_i$ and $\bar{C}_3$ for the above cellulose acetate film were 1.41 and 0.74 respectively at 1000 psig and 7.5°C; the corresponding values for the polyamide film were 2.22 and 0.15 respectively (run no. 4 in Table 5). These data express precisely the advantage of the polyamide membrane for higher recovery of flavor components by reverse osmosis treatment of apple juice waters. Further, under the above experimental conditions, the processing capacities were 32 gal/day ft² and 7.7 gal/day ft² respectively for the cellulose acetate and the polyamide membranes studied. These data also express precisely the need for the development of more productive aromatic polyamide membranes for their more economic utilization in the practical recovery of flavor components by reverse osmosis treatment of apple juice waters. As Eq. (6) indicates, the use of more productive aromatic polyamide membranes (i.e., those giving higher PR values for the same $D_{AM}/K\delta$ values) can result in even greater separation, and hence higher recovery, of flavor components than those obtained in this work.

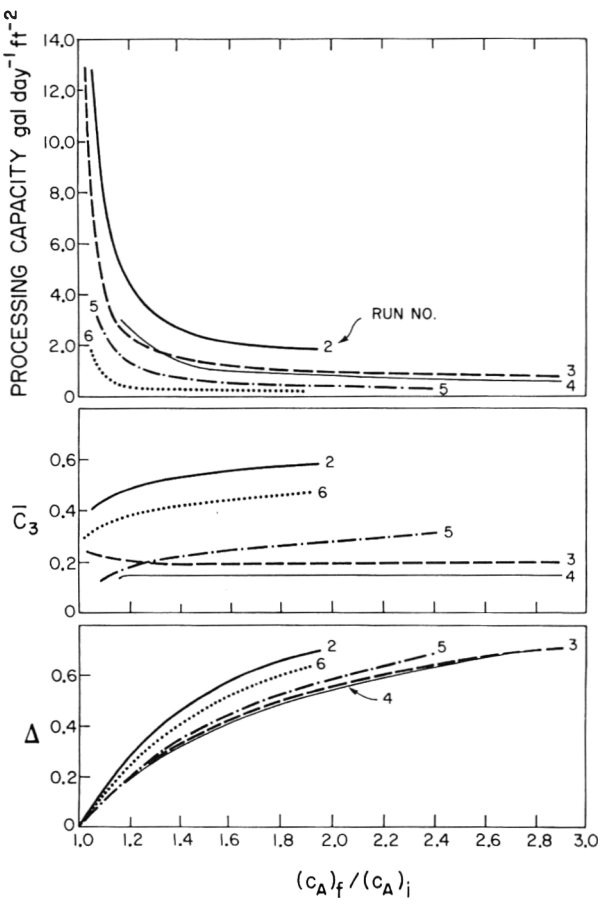


Fig. 7–Experimental results on reverse osmosis concentration of apple juice water by aromatic polyamide membrane (film 4). Experimental details, given in Table 6.

CONCLUSIONS

THIS PAPER illustrates the application of a fundamental physiocochemical criteria approach to the problem of recovery of flavor components in fruit juices by reverse osmosis. With appropriate additional research, this approach is capable of extension to a wide variety of solutes, solvents and membrane materials involved in reverse osmosis. With reference to the problem considered in this paper, if the object is to recover more specifically any particular group of flavor components, the above approach is capable of predicting what membrane specifications and operating conditions can accomplish the desired objective.

REFERENCES

Bednas, M.E. 1974. (Unpublished)

Butler, J.A.V. 1937. The energy and entropy of hydration of organic compounds. Trans. Faraday Soc. 33: 229.

Chandrasekaran, S.K. and King, C.J. 1971. Retention of volatile flavor components during drying of fruit juices. A.I.Ch.E. Chem. Eng. Progress Symp. Ser. 67(108): 122.

Diamond, J.M. and Wright, E.M. 1969. Biological membranes: the physical basis of ion and non-electrolyte selectivity. In "Annual Review of Physiology," Ed. Hall, V.E., p. 581. Annual Review Inc., Palo Alto, Calif.

Dickson, J.M., Matsuura, T., Blais, P. and Sourirajan, S. 1975. Reverse osmosis separations of some organic and inorganic solutes in aqueous solutions using aromatic polyamide membranes. J. Appl. Polym. Sci. 19: 801.

Matsuura, T., Baxter, A.G. and Sourirajan, S. 1973. Concentration of fruit juices by reverse osmosis using porous cellulose acetate membranes. Acta Alimentaria 2(2): 109.

Matsuura, T., Baxter, A.G. and Sourirajan, S. 1974a. Studies on reverse osmosis for concentration of fruit juices. J. Food Sci. 39: 704.

Matsuura, T., Bednas, M.E., Dickson, J.M. and Sourirajan, S. 1974b. Polar and steric effects in reverse osmosis. J. Appl. Polym. Sci. 18: 2829.

Matsuura, T., Bednas, M.E. and Sourirajan, S. 1974c. Reverse osmosis separation of single and mixed alcohols in aqueous solutions using porous cellulose acetate membranes. J. Appl. Polym. Sci. 18: 567.

Matsuura, T., Blais, P., Dickson, J.M. and Sourirajan, S. 1974d. Reverse osmosis separations for some alcohols and phenols in aqueous solutions using aromatic polyamide membranes. J. Appl. Polym. Sci. 18: 3671.

Matsuura, T., Dickson, J.M. and Sourirajan, S. 1975a. Free energy parameters for reverse osmosis separations of undissociated polar organic solutes in dilute aqueous solutions. Ind. Eng. Chem. Process Des. Develop (submitted).

Matsuura, T., Pageau, L. and Sourirajan, S. 1975b. Reverse osmosis separation of inorganic solutes in aqueous solutions using porous cellulose acetate membranes. J. Appl. Polym. Sci. 19: 179.

Matsuura, T. and Sourirajan, S. 1973. Physicochemical criteria for the reverse osmosis separation of monohydric and polyhydric alcohols and some related hydroxy compounds in aqueous solutions using porous cellulose acetate membranes. J. Appl. Polym. Sci. 17: 1043.

Merson, R.L. and Morgan, A.I. Jr. 1968. Juice concentration by reverse osmosis. Food Technol. 22: 631.

Ohya, H. and Sourirajan, S. 1971. Reverse osmosis system specification and performance data for water treatment applications. Thayer School of Engineering, Dartmouth College, Hanover, N.H.

Sourirajan, S. 1970. "Reverse Osmosis," (a) Chapter 1; (b) Chapter 3. Academic Press, New York.

Sourirajan, S. and Matsuura, T. 1973. Physicochemical criteria for reverse osmosis separation of inorganic and organic solutes in aqueous solutions using porous cellulose acetate membranes. Proc. Symp. on Membranes in Separation Processes, May 8–10, 1973, p. 24. Case Western Reserve Univ., Cleveland, Ohio.

Taft, R.W. Jr. 1956. Separation of polar, steric and resonance effects in reactivity. In "Steric Effects in Organic Chemistry," Ed. Newman, M.S., p. 598. Wiley, New York.

Wilke, C.R. and Chang, P. 1955. Correlation of diffusion coefficients in dilute solutions. A.I.Ch.E.J. 1: 264.

Ms received 2/25/75; revised 5/9/75; accepted 5/14/75.

Issued as NRC No. 14784.

N. PALAMIDIS and P. MARKAKIS
Dept. of Food Science & Human Nutrition, Michigan State University, East Lansing, MI 48824

STABILITY OF GRAPE ANTHOCYANIN IN A CARBONATED BEVERAGE

INTRODUCTION

THE CONTINUING REDUCTION in the number of coal tar dyes allowed for addition to foods has recently renewed interest in natural pigments as food colorants. Anthocyanins are widely distributed among plants and have been considered as tinctorial additives to foods. Red grapes are grown in large quantities (one-fourth of all fruit produced in the world is grapes), especially for wine and juice production. The grape anthocyanins are not quantitatively transferred to the wine or juice; considerable quantities of them are left in the pomace, which may eventually become an important source for the extraction of these pigments. Already in Italy a powder and a solution containing grape anthocyanins are marketed under the name Enocianina, or Enocyanin (Garoglio, 1965). These products are intended primarily for fortifying the red color of wines. Peterson and Jaffe (1969) patented a process resulting in extracts rich in color and flavor by treating grape pomace with water or alcohol containing 200–2,000 ppm SO_2. Recently Philip (1974) suggested anthocyanin recovery from grape wastes by extraction with methanol (or ethanol) containing 0.1–1.0% tartaric acid and subsequent precipitation of the excess tartaric acid as potassium hydrogen tartrate.

It is also known that anthocyanins are not very stable pigments (Markakis, 1974). Among the factors that affect their stability in foods are certain enzymes, pH, temperature, oxygen, light, etc. (Jurd, 1972; Van Buren et al., 1968).

The objective of this work was to extract by a simple method the anthocyanins of fermented grape skins and study the stability of these pigments in a nonalcoholic carbonated beverage to which they were added as colorants.

EXPERIMENTAL

Anthocyanin source

Fresh wine pomace of the grape variety Napa-Gamay was obtained from the Christian Brothers Winery in California. The pomace was shipped to this laboratory by air mail in plastic bags and frozen at −15°C upon arrival.

Extraction

Just before extraction the pomace was thawed and the skins were separated from the seeds and stems by hand. An effort to dry the pomace in an air draft to facilitate the separation of skins resulted in a low recovery of anthocyanin. The weight ratio of skins to seeds-plus-stems and the moisture of the skins were determined. The moist skins were divided into 5-g portions and each portion was ground in a Polytron mill (marketed by Brinkman Inc., N.Y.), in the presence of 25 ml of either hot water (100–95°C) or one of three SO_2 solutions: 500 ppm, 1,000 ppm and 2,000 ppm aqueous SO_2, at room temperature. The grinding lasted 3 min. The macerate along with the liquid used to wash the grinder was filtered under suction on Whatman No. 1 paper. The filtrate was saved and the sediment was ground a second time with fresh solvent. Filtration and re-grinding were repeated twice more. To evaluate the efficiency of extraction each filtrate was subjected to total anthocyanin determination. For the preparation of a quantity of pigment to be used as a beverage colorant, only two consecutive extractions were deemed advisable and the corresponding two filtrates were combined.

Concentration

The filtrates, single or combined, were concentrated to about one-fifth of the original volume in a flash evaporator at 50°C and the concentrates were made up to an exact volume with water. The suspended material was removed by centrifugation at 30,000 × G in a Sorvall RC2-B centrifuge. The supernatant was used for anthocyanin determination and for the preparation of a powder by freeze drying in a Vir-Tis 10-145 MR-BA lyophilizer.

Measurement

The anthocyanin content of the extracts, the concentrates and the beverages was measured by differential absorption spectrophotometry (Sondheimer and Kertesz, 1948). The following two buffers were used: (a) 0.13M HCl–0.05M KCl, pH 1.0; and (b) 0.05M HCl–0.5M CH_3COONa, pH 4.5. The mixtures of buffer and sample were equilibrated in darkness for 1 hr and their absorbance was measured at 520 nm in a Beckman DU spectrophotometer. The pigment concentration was expressed in Enocyanin equivalents (EE), obtained from a reference curve based on differential absorbance at 520 nm vs. commercial Enocyanin concentration (mg/ml).

Beverage preparation and storage

Two beverages were prepared differing only in the solvent used for anthocyanin extraction, namely hot water or 500 ppm SO_2. The composition of the beverages, in g per 100 ml, was: anthocyanin powder 0.7; citric acid 0.1; sucrose 13.0; grape flavor 0.2; and sodium benzoate 0.05. The beverage also contained CO_2, 1.7 volumes. The syrup was boiled for 5 min before mixing with the other ingredients, and the beverage was carbonated to saturation at 0°C (Jacobs, 1959). The pH of the beverages was 3.7. The color (absorbance at 520 nm) of the beverage prepared with hot water anthocyanin extract was that of a beverage containing 581 mg Enocyanin per 100 ml, while the beverage prepared with aqueous SO_2 (500 ppm) anthocyanin extract had a color corresponding to 640 mg of Enocyanin per 100 ml. Ten ml ampoules were filled with beverage at 0°C, the small head space was flushed with CO_2 and the ampoules were sealed in a flame. The filled ampoules were stored under the following conditions: (a) darkness at 3.5 ± 2°C; (b) darkness at 10 ± 2°C; (c) darkness at 20 ± 2°C; (d) darkness at 38 ± 1°C; (e) diffuse daylight (northern window) at 20 ± 2°C; and (f) continuous fluorescent light (80 foot candles) at 22 ± 2°C.

The anthocyanin content of the beverage was determined every 15 days. Two ampoules were opened from each group and duplicate determinations were made on each ampoule, using differential spectrophotometry. The results are given as the averages of the four values, which were found to vary not more than ± 5%.

RESULTS & DISCUSSION

Pigment extraction

The skins represented 55% of the weight of the pomace, the remaining 45% being seeds and stems. The moisture of the skins was 60%. The recovery of anthocyanin from the skins with hot water and 500 ppm SO_2 solution using four consecutive extraction steps is presented in Table 1.

There was no appreciable difference between these two solvents regarding the total anthocyanin extracted from the grape skins. With 1,000 ppm and 2,000 ppm SO_2 solutions the pigment yields were 33.8g and 34.8g of EE per 100g of moisture-free skins, respectively. In subsequent experiments, however, only the 500 ppm SO_2 solution was used. The two first extraction steps resulted in recovering approximately 94% of the total amount of anthocyanin which could be extracted with all four steps. For this reason only the two-step extraction was used for the preparation of the pigment which was later added to the carbonated beverage. Although after four consecutive extractions with either solvent the pomace looked

bleached, soaking was tried for the possible recovery of still greater quantities of anthocyanin. After the first maceration, the pomace was left at ambient temperature either with water or with 500 ppm SO_2 for periods up to 16 hr before the three final extraction steps were performed as in the no-soaking procedure. The yield in anthocyanin with water as an extractant did not increase upon soaking, while that with 500 ppm SO_2 increased by 7% after 4 hr and 10% after 8 or 16 hr of soaking. In a similar attempt to extract anthocyanin from cranberry pomace, Chiriboga and Francis (1970) found that a combination of maceration and repeated quick extractions with acidified methanol resulted in high pigment recovery and rendered lengthy extraction times unnecessary. In an industrial operation for anthocyanin recovery, the process of preference would probably be based on countercurrent extraction, which is more efficient than the simple multiple laboratory extractions. Hours rather than minutes of contact between solvent and the large masses of grape pomace would be involved and that would result in higher pigment yields, should SO_2 solution be used for extraction.

The anthocyanin-containing dry powder which was obtained by hot water extraction for use as beverage colorant was found to have 788 mg EE per g, while that obtained by SO_2 solution had 905 mg EE per g. Hot water extracted more nonanthocyanin plant material than the room temperature SO_2 solution, although the total amount of anthocyanin extracted by the two solvents was almost the same.

Pigment stability

Figures 1 and 2 illustrate the effect of two storage parameters, temperature and light, on the stability of grape anthocyanin added to a carbonated beverage. Increasing the storage temperature accelerated greatly the pigment destruction in the beverage. In darkness, at 38°C, after 135 days, only 23% of the original amount of hot-water-extracted pigment was left in the beverage, while at 3.5°C, under the same conditions of storage, 92% of the pigment was reatined. Similarly significant was the temperature effect on the pigment extracted with the SO_2 solution. On the average the loss of anthocyanin in the beverage stored at 20°C was almost double that at 10°C, indicating a Q_{10} of approximately 2.

Exposure to light also accelerated the degradation of the pigment; e.g., after 135 days, in the dark, at 20°C, approximately 30% of the hot-water-extracted pigment was lost, while for the same period and temperature, but with a northern window exposure of the beverage, the pigment loss was 50%. Under continuous fluorescent light, for the same period (135 days), and at a slightly higher temperature (22°C), the anthocyanin destruction was 70%.

A comparison of Figure 1 with Figure 2 reveals a higher stability for the anthocyanin extracted with 500 ppm SO_2 solution than with hot water. The stability differences due to extraction methods and storage conditions were easily quantified when the data were plotted semilogarithmically (log of % retention vs. time) and it was determined that the degradation of the pigment in the beverage followed first order reaction kinetics. The reaction rate constants were calculated by the formula of averages (King, 1963):

$$k = \frac{1}{(\text{anth. conc})_{avg}} \times \frac{\Delta \text{ anth. conc}}{\Delta t}_{avg}$$

and they are listed in Table 2. The half-lives, $T1/2 = 0.693/k$, for each treatment are also shown in Table 2. The pigment extracted with 500 ppm SO_2 appears to be from 30% (high storage temperatures) to 60% (low storage temperatures) more stable than the pigment extracted with hot water. The reason for the difference in stability between the two extraction methods is not clear. Paper chromatograms of the two pigment extracts, using Whatman 3 MM paper and a solvent containing

Table 1—Recovery of anthocyanin from grape skins

	With hot water		With 500 ppm aqueous SO_2	
	As g Enocyanin per 100g moisture-free skins	% of total recovery	As g Enocyanin per 100g moisture-free skins	% of total recovery
1st extraction	26.66	82.2	26.17	79.9
2nd extraction	3.93	12.2	4.39	13.4
3rd extraction	1.36	4.2	1.43	4.4
4th extraction	0.47	1.4	0.75	2.3
Total	32.42	100.0	32.74	100.0

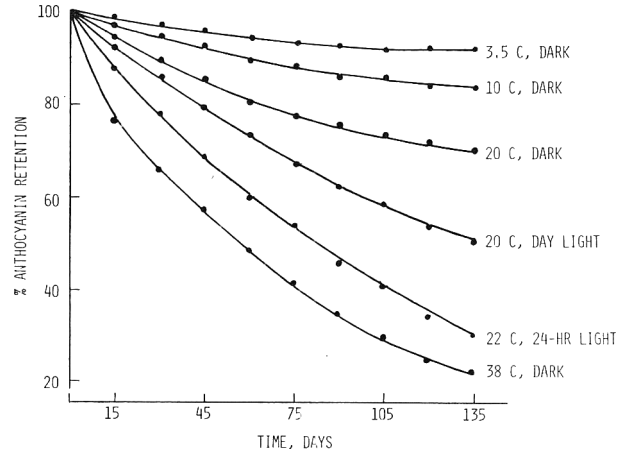


Fig. 1—Effect of storage temperature and light on anthocyanin retention in a carbonated beverage colored with anthocyanin extracted from grape skins by hot water.

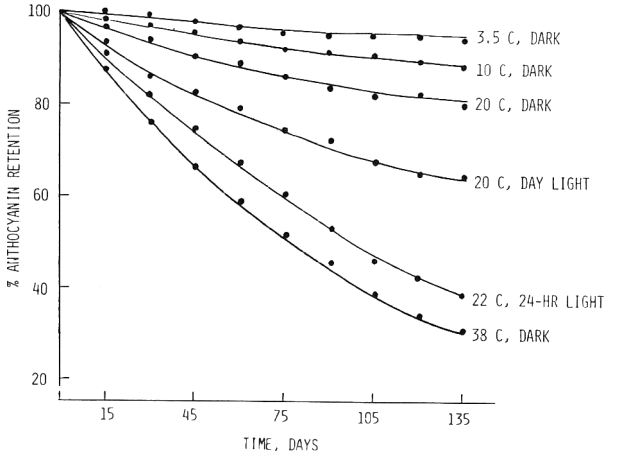


Fig. 2—Effect of storage temperature and light on anthocyanin retention in a carbonated beverage colored with anthocyanin extracted from grape skins by a 500 ppm SO_2 solution.

Table 2—Reaction rate constants (k) (days^{-1}) and half-life ($T_{½}$) (days) of anthocyanin in the stored beverage

Storage conditions	Anthocyanin extracted with			
	Boiling water		500 ppm Aqueous SO_2	
	k	$T_{½}$	k	$T_{½}$
3.5°C, dark	0.000736	941	0.000451	1536
10°C, dark	0.001320	525	0.000901	769
20°C, dark	0.002684	258	0.001665	416
20°C, day light	0.005068	136	0.003518	197
22°C, continuous light	0.008715	79	0.006836	101
38°C, dark	0.011123	62	0.008684	80

butanol:acetic acid:water (4:1:5 v/v/v) according to Mattick et al. (1969), did not reveal visually detectable differences in anthocyanin composition. The zinc nitroprusside test for residual SO_2 in the beverages containing pigment extracted with 500 ppm SO_2 solution (limit of detection 3.5 μg; Feigl, 1958) was negative. Possibly, the hot water extract of the skins contained substances which accelerated the degradation of anthocyanins in the beverage. As has been noted previously, hot water extracted more nonanthocyanin material from the skins than the SO_2 solution.

Using the anthocyanin retention data at 3.5, 10, 20 and 38°C, in dark storage, and the Arrhenius equation, the activation energy for the pigment degradation reaction was calculated. For the hot-water-extracted pigment the activation energy was 13.74 kcal/mole and the SO_2-extracted pigment 14.73 kcal/mole.

REFERENCES

Chiriboga, C. and Francis, F.J. 1970. An anthocyanin recovery system from cranberry pomace. J. Amer. Soc. Hort. Sci. 95: 233.
Feigl, F. 1958. "Spot Tests in Inorganic Analysis." Elsevier Publ., N.Y.
Garoglio, P.G. 1965. La Nuova Enologia. Instituto di Industrie Agrarie., Florence, Italy.
Jacobs, M. 1959. "Manufacture and Analysis of Carbonated Beverages." Chem. Publ., N.Y.
Jurd, L. 1972. Some advances in the chemistry of anthocyanin-type plant pigments. In "The Chemistry of Plant Pigments." Ed. Chichester, C.O. Academic Press, N.Y.
King, L.E. 1963. "How Chemical Reactions Occur." Benjamin Publ., N.Y.
Markakis, P. 1974. Anthocyanins and their stability in foods. CRC Critical Reviews in Food Technology. 4: 437.
Mattick, L.R., Weirs, L.D. and Robinson, W.B. 1969. Effect of pH on precipitation of anthocyanins of wine as their lead salts. Am. J. Enol. Citic 20: 206.
Peterson, R.G. and Jaffe, E.B. 1969. Berry and fruit treating process. U.S. Patent 3,484,254 (Dec. 16).
Phillip, T. 1974. Anthocyanin recovery system from grape wastes. J. Food Sci. 39: 859.
Sondheimer. E. and Kertesz, Z.I. 1948. Anthocyanin pigments. Colorimetric determination in strawberries and strawberry products. Anal. Chem. 20: 245.
VanBuren, P.J., Bentino, J.J. and Robinson, B.W. 1968. The stability of wine anthocyanins on exposure to heat and light. Am. J. Enology & Vitic. 19: 147.
Ms received 2/14/75; revised 4/23/75; accepted 4/28/75.

Michigan Agricultural Experiment Station Journal Article No. 7146. Deep appreciation is expressed to the Coca-Cola Export Corp. for partial financial support of this work and procurement of the grape skins and Enocyanin.

JUHANI OLKKU[1] and CHO KYUN RHA[2]
Food & Agricultural Engineering Dept., University of Massachusetts, Amherst, MA 01002

TEXTURAL PARAMETERS OF CANDY LICORICE

INTRODUCTION

THE OBJECT of this study was to investigate textural parameters of high-solid mixtures containing common food ingredients. Candy licorice was selected as a model because the product contains a high concentration of sugar, starch and protein, and the textural properties play the most important role in acceptability, and also product identity.

Candy licorice is a confection consisting mainly of wheat flour and sugar syrup. It is manufactured traditionally by gelatinizing starch within the flour-syrup-water system in an open kettle with constant mixing. The sugar-water ratio during cooking ranges from about 2:3 to 3:2 by weight. The resulting paste, having a moisture content of approximately 30%, is extruded and dried to moisture content of 15–25% of the total weight. The product is characteristically chewy, still not tough candy that suggests viscoelastic characteristics. Depending on the concentrations of sugar, flour and water and processing parameters, a wide range of textural characteristics can be built into the final product. In the candy licorice industry it is known that the texture of the product is one of the major criteria for consumer acceptability. Voisey (1973) recently studied the firmness of some licorice products and found that large differences in firmness of licorice can be easily detected. He also suggests that the ratio of firmness at two compression speeds may provide an easily obtained index related to the viscoelastic properties of licorice.

In this study, the textural parameters of candy licorice were characterized objectively applying a texture profile and penetration test. The textural parameters of five popular brands of candy licorice produced in Finland were studied in order to characterize their textural properties. The commercial samples were purchased to represent the typical textures that a consumer is likely to find.

The General Foods Texture Profile (G.F.T.P.) developed by Szczesniak (1963), Szczesniak et al. (1963) and Friedman et al. (1963), and later modified for a Universal Testing Machine by Bourne (1969) was applied. Samples from the same set were also subjected to a penetration test with a tapered plunger which was found to be suitable for this material in preliminary testing.

G.F.T.P. as applied to the Instron Universal Testing Machine has been used by Bourne (1969), Schmidt and Ahmed (1971), Ahmed and Dennison (1971) to measure the texture of fruits; by Breene et al. (1972) for cucumbers; and by Reidy and Heldman (1972) for freeze-dried meat. In general, there is only limited reference or elaboration for selecting a particular rate of deformaton. Shama and Sherman (1973a) recently studied the effect of rate of force application on the measurements of texture with a universal testing machine. The effect of stress relaxation during force-compression testing on foods with a universal testing machine was also studied by Shama and Sherman (1973b). From these studies it is evident that the selection of deformation speed is of importance in force-deformation testing like G.F.T.P. on food materials. The present study was carried out over a range of deformation speeds up to the limit of recording ability of the standard Instron Universal testing machine. This was determined to be deformation speed of 10 cm/min with the accompanying recorder speed of 100 cm/min for G.F.T.P. measurements.

EXPERIMENTAL

THE COMMERCIALLY purchased samples which were packaged in polyethylene bags were kept in aluminum foil to minimize moisture loss.

Samples were cut into 1.5-cm lengths with a wire cutter. The diameter of the steel wire was 0.02 cm. To determine the cross-sectional areas of the samples, the longest and shortest diameters of the samples were measured with a caliper. The cross-sectional area was then calculated from the ellipse formula with the coefficient 0.95 to compensate for irregularities in the cross-sectional form. This coefficient was determined by comparing areas calculated from the ellipse formula to the areas obtained by tracing the sample surface on the graph paper, pressing an image and counting the number of squares in the actual area of the sample cross section. The process was repeated five times for each brand. The cross-sectional areas of the samples ranged from approximately 0.8 cm^2 to 2.0 cm^2.

Instron Universal Testing Machine Model TM-M with 2 and 100 kg cells was used for G.F.T.P. and tapered plunger testing. Deformation speeds 0.05, 0.10, 0.50, 1.0, 5.0 and 10.0 cm/min and recorder speeds of 0.5, 1.0, 5.0, 10.0, 50.0 and 100.0 cm/min were used to give a 1:10 ratio of deformation speed to recorder speed. The temperature at which measurements was taken was 20°C.

G.F. texture profile

A flat plate (5 cm in diam) was used as the compression body in G.F.T.P. measurements. The maximum preselected deformation in both strokes was 1.0 cm which was equivalent to 2/3 of the sample height. The force-deformation curves were analyzed by taking force readings from the charts at intervals each corresponding to 0.05 cm sample deformation. A Fortran IV program was developed to compute the parameters fracturability, hardness, cohesiveness, springiness, gumminess, chewiness and adhesiveness as defined in a typical force-deformation curve (Fig. 1). In practice, a term such as fracturability would not be properly descriptive for candy licorice. Nevertheless, this term is used here to represent the first peak of the force-deformation curve. The ratio of the area under the upstroke to total area was determined in order to find out the significance in the calculation of the parameter, cohesiveness. Energies required for the first downstroke were recorded separately. Since there were large differences in the sample cross-sectional areas, all G.F.T.P. results were standardized to a cross-sectional area of 1.0 cm^2.

Penetration testing

A flat bottomed circular plunger was used (Fig. 2). The diameter of the bottom, 0.2 cm, was small enough so that all the sample diameters were significantly larger and thus the effect of sample area was eliminated from the values. The cross-sectional area of the plunger was reduced above the bottom surface so that the licorice did not contact the sides of the plunger shaft during penetration. Force-deformation curves were analyzed in a manner similar to G.F.T.P. curves for the parameters shown in Figure 3. Maximum predetermined penetration was 1.0 cm from the sample surface. Force-deformation curves were analyzed as above, and a Fortran IV program was developed to compute the parameters.

All measurements were made with four samples at each deformation speed. Moisture contents of the samples were measured by drying dupli-

[1] Present address: Queen Elizabeth College, University of London, Campden Road, London, W8 7AH, England
[2] Present address: Dept. of Nutrition & Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139

cate 5-g samples at 105°C for 24 hr, and checking for constant weight. Nitrogen contents of the samples were determined by the Kjeldahl method (AOAC, 1955).

RESULTS & DISCUSSION

CHANGES in the parameters slope or from logarithmic to linear function of textural parameter and deformation speeds were apparent in several cases. Thus the mean values of the textural parameters were fit with regression lines for the following: (1) textural parameter vs. deformation speed; (2) textural parameter vs. log of deformation speed; and (3) combination of the above, with a change of slope. A best fit curve was then selected on the basis of correlation to the experimental data, and statistical significance.

The G.F.T.P. parameters, hardness, cohesiveness, springiness, gumminess and chewiness, and the penetration test parameter, force at maximum penetration, are given for the five samples in Figures 4 through 11. In all but three cases the parameters increased with the increasing deformation speed. The three exceptions, all in sample 2, were decreases in cohesiveness and distance to surface failure in penetration testing, and the springiness which was independent of the deformation speed. In general, the nature of the change in textural parameters vs. deformation speed was not similar in the different samples.

The parameter vs. deformation curves for different samples intersected frequently. Approximately one-third of these curves had a change in slope, most of which occurred in the experimental region of 0.5–1.0 cm/min deformation speed. This confirms that in instrumental measurements of a material like candy licorice, in order to fully characterize the textural parameters, the deformation speed cannot be selected at random; rather, a series of measurements is necessary.

Texture profile measurements

Fracturability and hardness increased with an increase in deformation rate and between them, there was a high correlation coefficient. Samples 4 and 5 behaved in a similar manner and samples 1, 2 and 3 showed similar behavior. Fracturability of sample 3 was, however, higher while it showed lower hardness at lower deformation than those of samples 1 and 2. At 1.0 cm/min, deformation speed, samples 4 and 5 change their functional form and sample 3 has a change in slope. Cohesiveness of sample 2 decreased with an increase in deformation speed and the cohesiveness curves for different samples intersected. Sample 3, which could be broken with the fingers with least effort and had a granular appearance at the broken surface, had the lowest cohesiveness values. The upstroke areas as percentage of respective downstroke areas ranged from 4.5–9.6% for the first stroke and from 25–41% for the second stroke in the G.F.T.P. measurements. This represents a significant contribution of upstroke in determination of cohesiveness

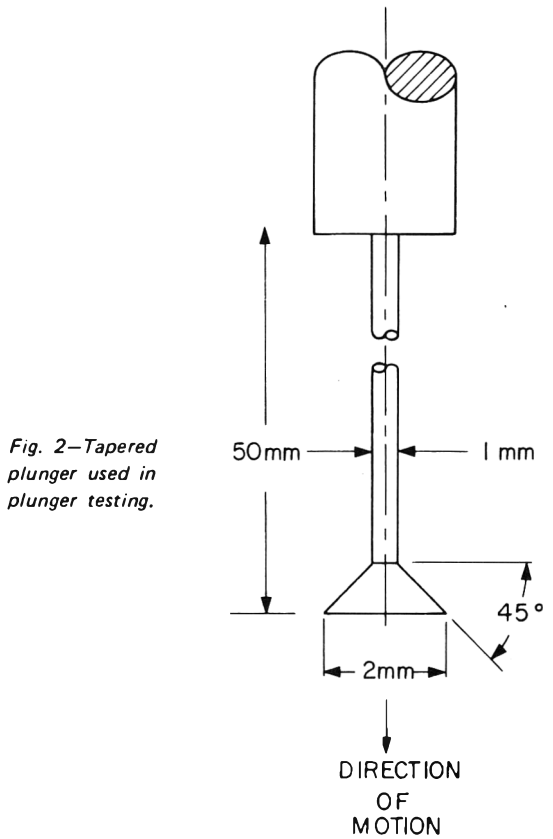


Fig. 2–Tapered plunger used in plunger testing.

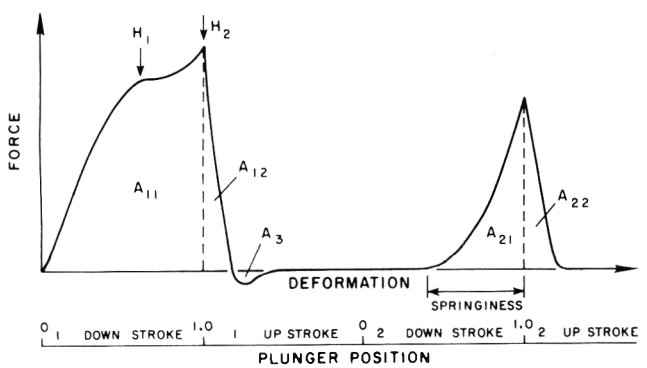


Fig. 1–Typical instron G.F. texture profile force deformation curve for candy licorice. [H_1 = Brittleness; H_2 = Hardness; A_{11} = Area of first downstroke; A_{12} = Area of first upstroke; A_{21} = Area of second downstroke; A_{22} = Area of second upstroke; and A_3 = Adhesiveness.] [Cohesiveness = $(A_{21} + A_{22})/(A_{11} + A_{12})$; Gumminess = Cohesiveness × Hardness; and Chewiness = Cohesiveness × Hardness × Springiness.]

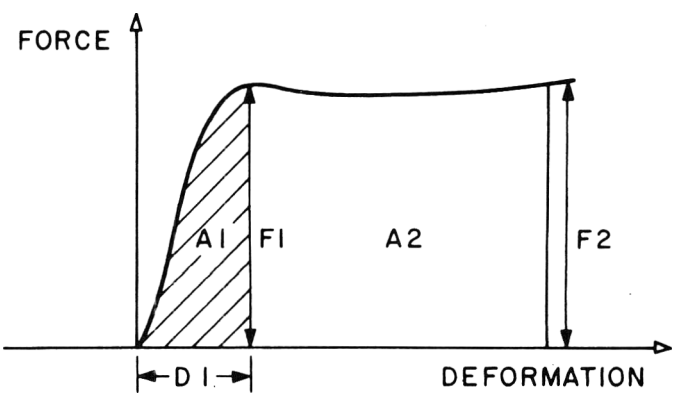


Fig. 3–Typical plunger testing force-distance curve for candy licorice. [F1 = Force at surface penetration; F2 = Force at max penetration deformation (deformation predetermined); D1 = Distance from sample height to surface penetration; A1 = Energy required for surface penetration; and A1 + A2 = Energy required for predetermined penetration deformation.]

in highly viscoelastic material such as candy licorice. Springiness for samples 1, 3, 4 and 5 increases as a linear function of the deformation speed, with samples 3 and 5 curves intersecting and sample 2 independent of the deformation speed and the value well below those of other samples.

Gumminess, which is a product of hardness and cohesiveness, has a high correlation to hardness, and an even higher correlation to cohesiveness in all the samples except sample 2. The value of and changes in hardness are a few orders of magnitude greater than cohesiveness, and thus numerically affect the value of gumminess to a much greater extent. However, in the absence of accompanying organoleptic tests it is difficult to attribute the effects of hardness and cohesiveness in the gumminess.

Chewiness is a product of hardness, cohesiveness and stringiness. It has high correlation to hardness for all the samples, and a relatively high correlation to cohesiveness and springiness for all samples except sample 2. In this case, at high deformation speeds samples 4 and 5 again had very close values, but at low deformation speeds all the samples had close values. Adhesiveness of the samples were different depending upon the surface material of the measurement cell. In most cases, the value was close to 0, and it is excluded from the results.

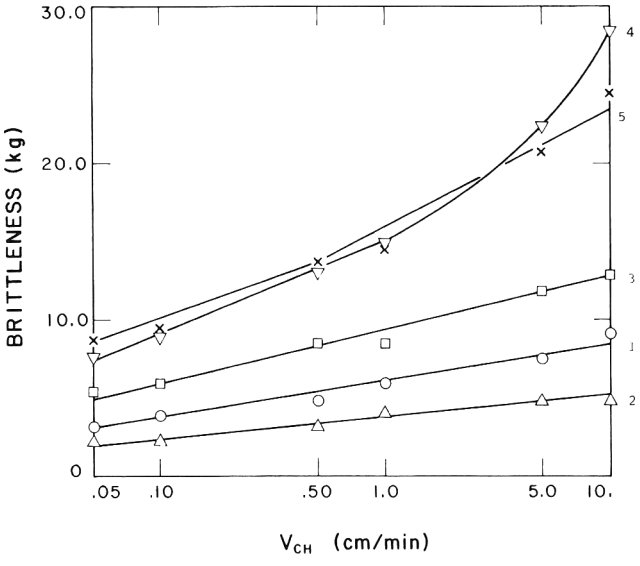


Fig. 4–G.F.T.P. fracturability vs. log of crosshead speed for candy licorice.

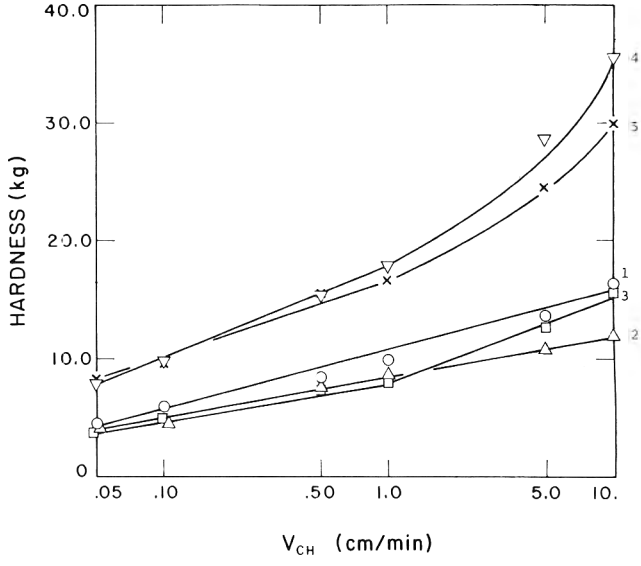


Fig. 5–G.F.T.P. hardness vs. log of crosshead speed for candy licorice.

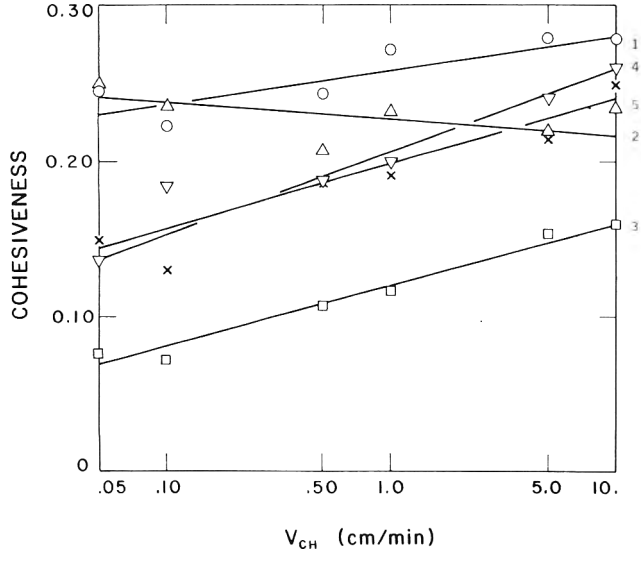


Fig. 6–G.F.T.P. cohesiveness vs. log of crosshead speed for candy licorice.

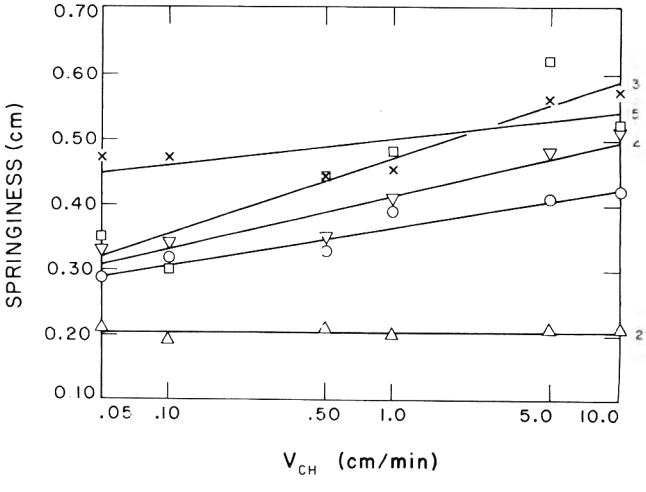


Fig. 7–G.F.T.P. springiness vs. log of crosshead speed for candy licorice.

Penetration test

In general, the force required for surface penetration was well correlated with fracturability. The force for maximum penetration to hardness and distance to surface failure to springiness also had statistically good correlations. These good correlations and the similar behavior between the force at maximum penetration and hardness, in terms of relative ranking of samples, indicates that the correction for the sample areas is justified. For instance, samples 4 and 5 had the largest and smallest cross-section area respectively, and accordingly the values of raw hardness data were widest apart. Incidentally, very limited organoleptic tests also support the result of the penetration test.

Visual observation indicated that samples 4 and 5 had the most homogenous structure, while sample 3, which could be easily broken, revealed a grainy appearance. Samples 1 and 2 were in between these extremes.

The most common functional form in the results is the parameter vs. log of deformation speed. This is in agreement with the observation of Shama and Sherman (1973b) about the influence of stress relaxation during compression testing.

In restressing, the importance of selecting an appropriate deformation speed is evident from the results of this study. It seems that in order to characterize the texture parameters the approach of Voisey and Crete (1973) should be considered. The range of the force application rates used in subjective texture evaluation should be evaluated first, and the measure-

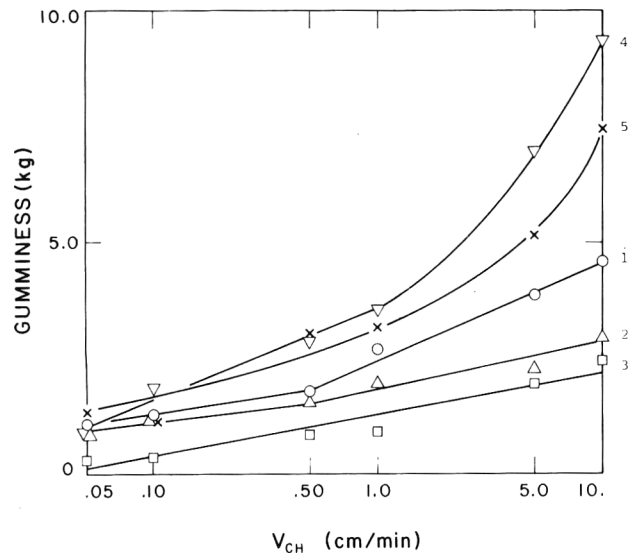


Fig. 8–G.F.T.P. gumminess vs. log of crosshead speed for candy licorice.

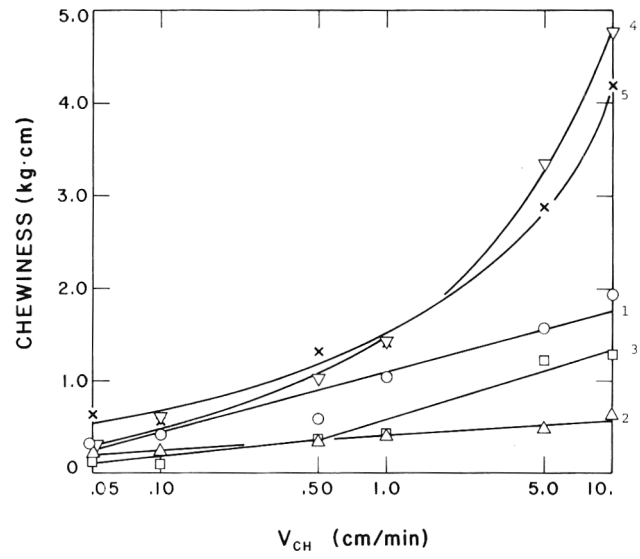


Fig. 9–G.F.T.P. chewiness vs. log of crosshead speed for candy licorice.

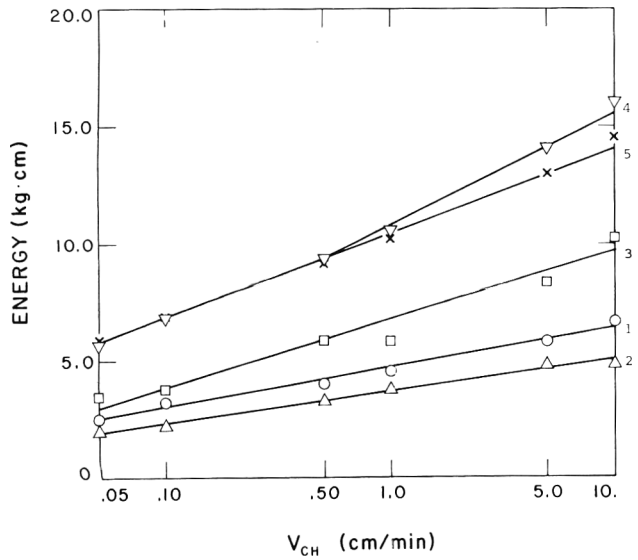


Fig. 10–G.F.T.P. area under first downstroke vs. log of crosshead speed for candy licorice.

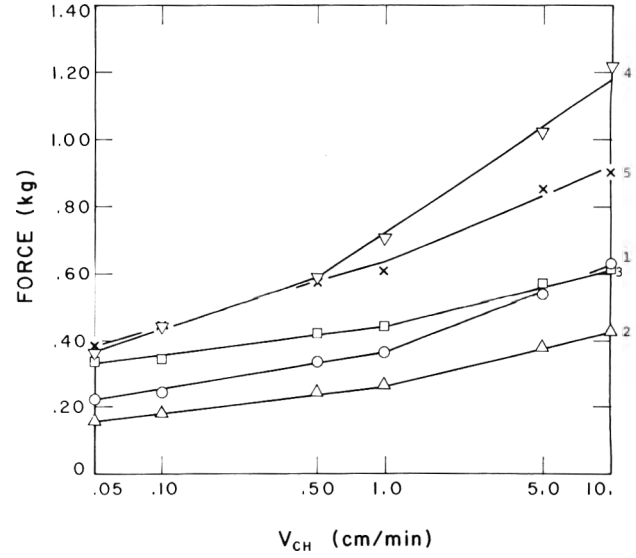


Fig. 11–Penetration testing force of maximum penetration vs. log of crosshead speed for candy licorice.

ments then made at near the region of mean value. In order to find the parameter behavior in the organoleptic testing range, the measurements should be made either at the extreme values of the range or at a distance of one or two standard deviations from the mean.

CONCLUSIONS

THE FOLLOWING were concluded from this study:

1. Several G.F.T.P. parameters have a high correlation with each other in candy licorice. Some of these parameters are also highly correlated with punctural testing parameters.
2. The upstroke areas in the G.F.T.P. measurements should be included in the area calculations for materials like candy licorice.
3. The effect of changes in the deformation speed on each textural parameter is not consistent. Therefore, the selection of rate of deformation should depend on the textural characteristics of the material based on a series of measurements and considering the rate of deformation of the product.
4. The textural behavior of commercial candy licorice cannot be correlated with moisture or nitrogen content in this experimental range.

REFERENCES

AOAC. 1955. "Official Methods of Analysis," p. 805. Assoc. Official Ag. Chemists, Washington, D.C.

Ahmed, E.M. and Dennison, R.A. 1971. Texture profile of irradiated mangoes and peaches. J. Texture Studies 2: 489.

Bourne, M.C. 1968. Texture profile of ripening pears. J. Food Sci. 33: 223.

Breene, W.M., Davis, D.W. and Chou, H.E. 1972. Texture profile analysis of cucumbers. J. Food Sci. 37: 113.

Friedman, H.H., Whitney, J.E. and Szczesniak, A.S. 1963. The Texturometer—A new instrument for objective texture measurement. J. Food Sci. 28: 380.

Reidy, C.A. and Heldman, D.R. 1972. Measurement of texture parameters of freeze-dried beef. J. Texture Studies 3: 218.

Schmidt, T.F. and Ahmed, E.M. 1971. Textural and elastic properties of Irish potatoes. J. Texture Studies 2: 460.

Shama, F. and Sherman, P. 1973a. Evaluation of some textural properties of foods with the Instron Universal Testing Machine. J. Texture Studies 4: 344.

Shama, F. and Sherman, P. 1973b. Stress relaxation during force-compression studies of foods with the Instron Universal Testing Machine and its implications. J. Texture Studies 4: 353.

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

Szczesniak, A.S., Brandt, M.A. and Friedman, H.H. 1963. Development of standard rating scales for mechanical parameters of texture and correlation between the objective and sensory methods of texture evaluation. J. Food Sci. 28: 397.

Voisey, P.W. 1973. Firmness of candy licorice. Report No. 7319, Eng. Res. Service, Agr. Can., Ottawa.

Voisey, P.W. and Créte, R. 1973. A technique for establishing instrumental conditions for measuring food firmness to simulate consumer evaluation. J. Texture Studies 4: 371.

Ms received 4/27/75; revised 5/1/75; accepted 5/6/75.

This study was partially supported by a W.K. Kellogg Fellowship and by Massachusetts Experiment Station Hatch 334 Project.

Part of the measurements reported were done in the Technical Research Centre of Finland, Food Research Laboratory, SF-02150 Otaniemi, Finland.

The authors thank Mr. Peter W. Voisey for his contribution to this study.

DAVID F. BROWN,[1] CARL M. CATER, KARL F. MATTIL and JAMES G. DARROCH
Soil & Crop Sciences and Statistics Departments, Texas A&M University, College Station, TX 77843

EFFECT OF VARIETY, GROWING LOCATION AND THEIR INTERACTION ON THE FATTY ACID COMPOSITION OF PEANUTS

INTRODUCTION

THE FATTY ACID composition of the endogenous fats and oils in agricultural products plays an important role in determining shelf life, nutrition and flavor of the ensuing food products (Lea, 1962; Alfin-Slater and Deuel, 1960). Direct incorporation of peanut oil into food products or incorporation of oil-containing peanut meals and peanut protein concentrates could be expected to alter shelf life, nutrition and flavor. Evidence that the long-chain saturated fatty acids, behenic and arachidic, in peanut oil may be implicated in heart disease has been published (Nutrition Foundation, Inc., 1973). The high linoleate content of some peanut varieties has been shown to decrease the shelf life of roasted peanut products (Young et al., 1972).

Environment, genotype and growing location are reported to influence the fatty acid composition of most oilseeds (Canvin, 1965; Hitchcock and Nichols, 1971). The influence of genotype on the fatty acid composition of peanuts has been well demonstrated by Worthington and Hammons (1971). Variations in lipid composition from one season to another (within the same year) also have been reported (Su and Chen, 1973). Small differences in the oil composition of peanuts have been reported for widely separated growing locations during the same year (Su and Chen, 1973; Young et al., 1974); but whether or not the differences were due to environmental factors or cultural practices was uncertain. The purpose of the present investigation is to more clearly establish the effect of growing location, variety and their interaction on the fatty acid composition of peanuts.

MATERIALS & METHODS

Materials

Peanut (*Arachis hypogaea* L.) samples used in these experiments were drawn from pooled replicates from the 1972 National Regional Peanut Variety Trials (NRT) and were grown with supplemental irrigation on the respective state experiment stations and at the latitudes indicated: Yoakum, Texas (29° 18′N), Bryan, Texas (30° 41′N), Marianna, Fla. (30° 45′N), Tifton, Ga. (31° 27′N), Stephenville, Texas (32° 12′N), Ft. Cobb, Okla. (35° 08′N) and Holland, Va. (36° 43′N). Varieties tested were: (a) Spanish botanical type; Comet, Starr, Spanhoma, TP 716-2-1, TP 931, (b) Virginia botanical type; Va. 72R, (c) Virginia market type; Florigiant, (d) runner market type; F 439-16-6, Florunner, and (e) Spanish market type; Wilco I. Traditionally the runner market type and Virginia market type have been arbitrary divisions of the Virginia botanical group for purposes of commerce, the runner market type having smaller pods and higher seed counts. Florigiant, Florunner and F 439 were derived from infraspecific crosses between Spanish and Virginia botanical types and are marketed as Virginia and runner market types on the basis of their physical similarity to the traditional market types (Hammons, 1973). Wilco I is of uncertain ancestry. It has some characteristics of both botanical groups and is sold as a Spanish variety.

All planting seed for each variety was from the same seed lot. Harvesting and curing were done according to the recommended procedures for each state. Samples were received "in shell" shortly after curing. They were shelled within 30 days of receipt and passed over standard screens (15/64 × 3/4 in. for Spanish and 16/64 × 3/4 in. for Wilco I, runner and Virginia type varieties). Peanuts riding the respective screens were then hand-sorted to remove visually immature and off-color nuts, and the resulting sound mature kernels were stored at 4°C in sealed glass jars.

Florigiant, F 439-16-6 and Va. 72R varieties were not grown at Ft. Cobb, Okla., due to the normally short growing season, and all of the later maturing varieties in the tests at Holland, Va., were lost due to heavy rains during the harvest period.

Analytical methods

Oil samples were obtained by cold-pressing in polyethylene bags as described previously (Brown et al., 1974). Oven keeping times (60°C) were determined according to the procedure outlined by Young (1970). Fatty acids in the oils were determined by gas chromatography after transesterification to their methyl esters by the procedure of Metcalfe et al., (1966). Esters were separated using a Beckman GC-5 chromatograph equipped with a FID using a 72 × 1/4 in. stainless steel column packed with 10% EGSS-X on Gas Chrom P and an oven temperature of 180°C: after on-column injection the averages from three or more disc integrator tracings were used to compute peak areas, and the peak areas were normalized to total 100%.

Statistical methods

Principal component analyses were obtained using the computer program designed by Barr and Goodnight (1972). A total of 62 'variety by location' vectors for the seven fatty acids were available for statistical analysis due to the missing varieties at Ft. Cobb, Okla. (3) and Holland, Va. (5). These data vectors were used in principal components procedures. The correlation coefficients between fatty acids were by-products of these analyses.

RESULTS

Fatty acid analyses

All of the fatty acids commonly found in peanut oil except lignoceric and linolenic acid were quantitated. Although linolenate is partially resolved from arachidate and eicosenate on EGSS-X columns, its presence was not detected in the samples. Operating parameters used in the GLC analyses were not very satisfactory for quantitative determination of lignocerate, which was present in the range of about 1–3%. The normalized results (lignocerate excluded) of the complete fatty acid analyses of the oils from all of the varieties grown at Yoakum (South Texas), Tifton (Southwestern Georgia) and Ft. Cobb (South Central Oklahoma) are shown in Table 1. In addition to the fatty acids reported in Table 1, traces of palmitoleic were found in all of the samples.

Both variety and location affected the composition of peanut oil. In general the three major fatty acids, palmitic, oleic and linoleic, were affected more than the minor fatty acids. Corresponding trends were recorded at the other locations tested in 1972. Comparative data for palmitate, oleate and linoleate in all the varieties from all seven of the test locations, listed in the order of increasing north latitude, are reported in Table 2. With increasingly more northern growning locations, the oleate and palmitate contents of all varieties tended to

[1] Present address: USDA Southern Regional Research Center, ARS, New Orleans, LA 70179

decrease, while the linoleate content tended to increase. The oil composition of the Virginia varieties was affected more strongly than the composition of the Spanish type and F 439-16-6 varieties.

The overall changes in fatty acid composition of the large-seeded varieties, Va. 72R, Florigiant and Florunner, were relatively large, and increases in linoleate and decreases in palmitate and oleate with increasing latitude, Yoakum, Bryan, Marianna, Tifton, Stephenville, Ft. Cobb and Holland, were observed. The one exception was at Marianna where the composition of the Virginia botanical type peanut oils was more like the Yoakum samples than the Bryan samples.

Correlation coefficients for the fatty acids from the oils of the 10 varieties at the seven locations were calculated for the variety and the location effects. The correlation coefficients for the relationships between fatty acids in the 10 varieties within the seven locations (varietal effects) were similar to those reported by Worthington and Hammons (1971) for 110 varieties grown at Tifton, Ga., in 1968 and are not shown. The only exception was the relationship between eicosenoic and arachidic acids. These two acids were positively correlated at the 0.05 rejection level, while Worthington and Hammons reported a negative correlation which was significant at the 0.001 level. The discrepancy probably reflects the analytical difficulties associated with determining the low concentrations of eicosenoate in the samples.

Correlation coefficients listed in Table 3 are statistical estimates of the effect of location on the relationships between fatty acids in the individual varieties. Location affected the relationships between several pairs of fatty acids differently than the usual variety effects. Within a location stearic and oleic acids normally decrease with increasing palmitate content, but the stearate and oleate content of the varieties was positively correlated with palmitate over the seven locations. The high positive correlations between palmitic and linoleic and the correlation between palmitic and behenic acid became negative when the effects of location were tested. The negative correlation between eicosenoic and arachidic acids among varieties became positive over the locations.

Principal component analyses

The data on fatty acid composition plus total protein content data for these varieties and locations in the 1972 NRT reported earlier by Rhee et al. (1973) were subjected to principal component analysis. Using this technique, the unique and independent information characterizing the varieties and locations contained in the data was extracted and recombined into a smaller number of latent variates, i.e., principal components (Blackith and Reyment, 1971). Through this technique, which is often utilized in taxonomic studies, the degree of relatedness between the varieties and locations could be compared. The first and second components representing 86 and 9% of the total variability between the varieties are plotted in Figure 1. The five true Spanish botanical varieties were closely clustered and are closely related in terms of the variables which were measured. By this analysis the F 439-16-6 variety appeared to have Spanish characteristics and Florunner, Florigiant and Va. 72R possess characteristics which are relatively similar to each other. Plotting the first and second components representing

Table 1—Fatty acid composition of peanuts from three locations in the National Regional Peanut Variety Trials in 1972 (Normalized to 100%)

Variety	Palmitic	Stearic	Oleic	Linoleic	Arachidic	Eicosenoic	Behenic
			Yoakum, Texas				
Spanhoma	11.8	2.9	45.5	34.7	1.5	0.9	2.8
Comet	11.9	2.4	44.7	36.0	1.4	1.0	2.5
Starr	12.2	2.4	45.6	35.1	1.4	1.0	2.3
TP 716-2-1	12.0	3.2	45.6	34.0	1.5	0.8	2.9
TP 931	11.8	2.0	47.7	34.7	0.9	0.7	2.1
Wilco I	11.5	1.7	49.1	33.6	1.3	1.1	1.8
Florunner	9.6	1.5	59.1	25.8	1.0	1.1	1.8
F 439-16-6	11.2	1.9	45.1	38.2	0.8	1.1	2.4
Florigiant	9.1	2.2	60.3	24.2	1.1	1.0	1.8
Va. 72R	9.0	2.1	61.7	23.4	1.4	1.0	1.5
			Tifton, Georgia				
Spanhoma	10.7	2.8	46.6	34.9	1.3	0.8	2.6
Comet	11.3	2.3	46.5	35.0	1.4	0.9	2.5
Starr	11.4	2.0	46.4	36.0	1.8	1.6	2.8
TP 716-2-1	11.3	2.6	46.6	35.0	1.4	0.9	2.3
TP 931	11.4	2.6	47.9	33.3	1.4	0.7	2.6
Wilco I	11.1	1.4	46.7	36.6	0.4	1.4	2.2
Florunner	9.6	1.3	55.5	28.8	1.2	1.3	2.2
F 439-16-6	10.8	1.7	41.3	41.9	0.7	1.1	2.4
Florigiant	9.0	2.4	56.4	26.8	1.8	1.1	2.4
Va. 72R	8.8	1.6	60.0	26.2	0.8	0.9	1.6
			Ft. Cobb, Oklahoma[a]				
Spanhoma	11.0	2.2	42.7	38.7	0.9	0.8	2.9
Comet	11.5	2.6	42.5	37.5	1.8	1.1	3.0
Starr	11.3	2.3	45.0	36 7	1.0	1.1	3.0
TP 716-2-1	10.8	2.4	43.3	38.2	1.2	1.2	3.0
TP 931	10.8	2.6	43.0	37.1	2.0	1.3	3.1
Wilco I	10.5	1.6	41.1	40.1	1.7	2.2	2.6
Florunner	9.0	1.4	48.0	36.5	1.1	1.8	2.3

[a] Florigiant, Va. 72R and F 439-16-6 not tested at Ft. Cobb

60 and 27% of the same biochemical characteristics over locations resulted in a clustering of Bryan, Yoakum and Marianna and to a lesser degree Holland, Ft. Cobb, Tifton and Stephenville (Fig. 2).

Oleate/linoleate ratios

Oleate/linoleate (O/L) ratios in the samples were calculated for all 10 varieties grown at the seven locations (Table 4). The highest O/L ratios for the standard Spanish botanical type varieties were obtained at Marianna, averaging 1.47, while the other three southern locations, Yoakum, Bryan and Tifton averaged, respectively, 1.31, 1.32 and 1.35. The highest O/L ratios for the other varieties, all of which are normally harvested later than the true Spanish varieties, were recorded at Yoakum. The values from Marianna, Bryan and Tifton were quite similar and ranked in the order given for the locations.

Table 2—Linoleate, oleate and palmitate contents of 10 peanut varieties grown in the National Regional Peanut Variety Trials in 1972 (Normalized to 100%)

Variety	Yoakum Texas	Bryan Texas	Marianna Florida	Tifton Georgia	Stephenville Texas	Ft. Cobb Oklahoma[a]	Holland Virginia[a]
				Linoleate content			
Spanhoma	34.7	35.7	33.2	34.9	38.5	39.7	38.4
Comet	36.0	34.9	33.5	35.0	37.4	37.5	39.5
Starr	35.1	34.3	32.1	36.0	38.1	36.7	37.0
TP 716-2-1	34.0	35.0	32.2	35.0	37.3	38.2	37.3
TP 931	34.7	33.9	33.3	33.3	37.8	37.1	38.1
Wilco I	33.6	34.0	34.5	36.6	40.7	40.1	–
Florunner	25.8	28.3	27.9	28.8	35.6	36.5	–
F 439-16-6	38.2	40.0	38.6	41.9	46.0	–	–
Florigiant	24.3	25.5	24.9	26.8	33.4	–	–
Va. 72R	23.4	25.8	25.1	26.2	33.5	–	–
				Oleate content			
Spanhoma	45.5	45.2	47.8	46 6	42.5	42.7	43.9
Comet	44.7	45.1	47.7	46 5	43.7	42.5	42.6
Starr	45.6	44.9	48.5	44.4	43.4	45.0	43.3
TP 716-2-1	45.6	46.1	49.6	46.5	43.4	43.3	43.6
TP 931	47.7	47.3	47.9	47.9	43.5	43.0	43.8
Wilco I	49.1	47.1	47.7	46.7	41.5	41.1	–
Florunner	59.1	54.3	55.2	55.5	49.9	47.9	–
F 439-16-6	45.1	41.4	44.1	41.3	38.5	–	–
Florigiant	60.3	57.5	58.1	56.4	51.7	–	–
Va. 72R	61.7	57.9	58.2	60.0	52.4	–	–
				Palmitate content			
Spanhoma	11.8	11.9	11.7	10.7	10.9	11.0	11.4
Comet	12.0	12.3	12.4	11.3	11.3	11.5	11.6
Starr	12.2	12.3	12.0	11.4	11.4	11.3	11.6
TP 716-2-1	12.0	12.4	11.9	11.3	11.2	10.7	11.0
TP 931	11.8	12.2	12.2	11.4	11.7	10.8	11.3
Wilco I	11.5	10.7	11.3	11.1	10.8	10.5	–
Florunner	9.6	9.6	9.5	9.6	9.5	9.0	–
F 439-16-6	11.2	10.9	11.2	11.4	9.9	–	–
Florigiant	9.1	9.4	10.5	9.0	8.8	–	–
Va. 72R	9.0	9.1	9.4	9.0	9.0	–	–

[a] –Not available from Ft. Cobb, Okla.; lost in heavy rains at Holland, Va.

Table 3—Correlation coefficients (r) of fatty acids within 10 varieties over seven locations determined for the National Regional Peanut Variety Trials samples from 1972[a]

Fatty acid	Stearic	Oleic	Linoleic	Arachidic	Eicosenoic	Behenic
Palmitic	0.305*	0.503**	–0.561**	–0.052	–0.431**	–0.511**
Stearic		0.367**	–0.553**	0.463**	–0.233	–0.069
Oleic			–0.948**	0.015	–0.470**	–0.521**
Linoleic				–0.252	0.325*	0.372**
Arachidic					0.527**	0.312*
Eicosenoic						0.616**

[a] d.f. = 55
* $P \leq 0.05$
** $P \leq 0.01$

The lowest O/L ratios were obtained for most of the varieties at Ft. Cobb, but the ratios at Stephenville and Holland, the other northern locations, differed very little from Ft. Cobb. The O/L ratios from Florunner responded differently. A considerably lower ratio was obtained at Ft. Cobb than Stephenville.

DISCUSSION

THE DATA in Tables 1 and 2 and the principal component analysis in Figure 1 indicate that the fatty acid compositions of the five Spanish botanical type varieties and F 439-16-6 were quite similar within a given location, and that the compositions of the three large-seeded Virginia varieties, Florunner, Va. 72R and Florigiant, also were quite similar to each other within a given location. The composition of Wilco I differed from the other Spanish varieties and expressed some of the characteristics of the large-seeded Virginia botanical type. Su and Chen (1971), Worthington and Hammons (1971) and Young et al. (1974) reported similar differences between several varieties grown commerically in the U.S.

The fatty acid composition of comparable varieties grown on Taiwan (Su and Chen, 1971) were quite similar to our values from Yoakum and Tifton. Data for Starr and Spanhoma variety peanuts grown at Tifton and Ft. Cobb, in 1968 and 1969 were reported by Young et al. (1974). Agreement between their data and our data for Ft. Cobb was very close. On the other hand we found larger differences between Georgia grown and Oklahoma grown Starr and Spanhoma Spanish peanuts than they reported. They reported more palmitate (1.3%), more linoleate (0.5%) and less oleate (2.2%) than in the 1972 NRT samples from Tifton. Higher values for stearate (0.8%) and lower values for arachidate, eicosenoate and behenate also were reported by Young et al. (1974). Both groups of researchers reported relatively large differences in composition from year to year and Su and Chen (1971) also reported sizeable differences in composition from fall and spring plantings in Taiwan of the same varieties within the same year.

The data in Tables 1–3 show that latitude related effects play more significant roles in peanut lipid composition than east-west effects. Differences between the fatty acid composition of the peanuts grown at Bryan and Yoakum, Texas and Tifton, Ga. and Marianna, Fla. are relatively small despite the fact that the former locations are about 850 miles, or more than 11°, west of the Georgia and Florida locations. On the other hand, the data in Table 2, which is arranged in the order of increasing north latitude, reveal definite latitude effects. The oleate contents of the Virginia peanuts became progressively lower with increasing latitude and a similar decreasing trend was noted with palmitate. Linoleate content of the Virginia peanuts became progressively greater with increasing north latitude. For example in Florunner peanuts grown at Ft. Cobb (35° 08′N), the linoleate content was 11% greater or 1-1/2 times the linoleate content of the Florunner oil from Yoakum (29° 18′N). The oleate content of the Ft. Cobb grown Florunner variety is 3/4 of the value at Yoakum.

Changes in the fatty acid composition of Spanish botanical type peanuts were not as large as those in the Virginia and runner types. The oleate and palmitate contents of the Spanish varieties grown at the three northernmost locations were lower than at the four southern locations, but the order within the northern and southern groups was scrambled with respect to latitude. Linoleate content at the three northern growing locations was higher than at Yoakum, Bryan, Marianna and Tifton, but the order within the two groups again did not parallel increasing latitude. The extreme values in composition were from Holland and Marianna. The oleate content of Spanish varieties grown at Holland (36° 43′N) was 5% lower than at Marianna (30° 45′N), while the linoleate content at Holland was about 5% greater than in the Spanish peanuts grown at Marianna.

The facts that the compositions of the Spanish and Virginia peanut oils and the O/L ratios of the Marianna oils were more like those from Yoakum than would have been anticipated from its latitude, and that the deviation was greater among the Spanish peanuts than the later maturing Virginia and runner peanuts were noted earlier. The deviations may simply reflect an extreme yearly effect on composition. However, the agronomic history and the composition of the peanuts grown at Marianna suggest that the high oleate and low linoleate content are due to moisture stress.

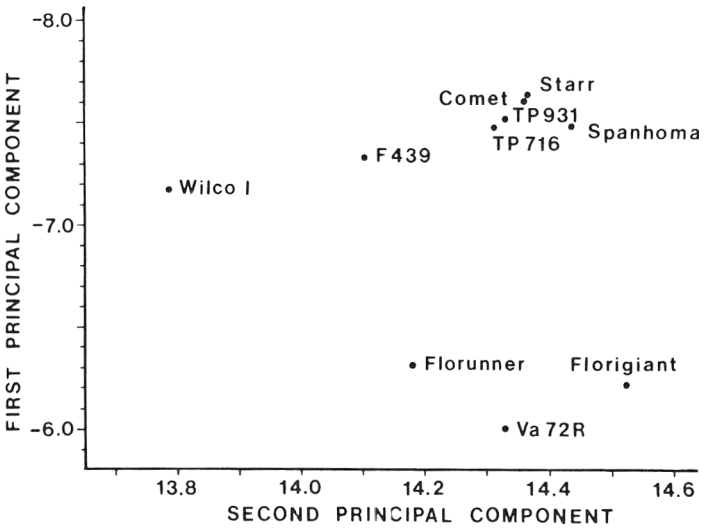


Fig. 1–Clustering of 10 peanut varieties in the 1972 National Regional Peanut Variety Trials on the basis of the first and second principal components derived from the location data vectors within the varieties.

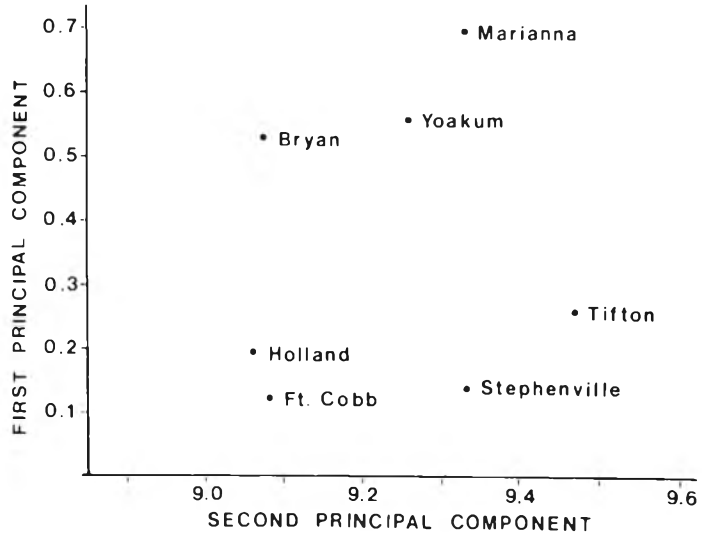


Fig. 2–Clustering of seven growing locations in the 1972 National Regional Peanut Variety Trials on the basis of the first and second components derived from the variety data vectors within the locations.

Similarly lowered linoleate and increased oleate contents were reported for dry land grown peanuts in Oklahoma and Georgia for 1968 and 1969 by Young et al. (1974). The Marianna Spanish peanuts received supplemental irrigation in June only, whereas the Virginia and runner varieties also received supplemental irrigation in September when the pods on these varieties were in their later stages of maturation. The additional irrigation after the Spanish botanical variety harvest would be expected to partially relieve the effects of moisture stress on the Virginia peanuts and result in a more 'normal' composition. The fatty acid composition of the Marianna grown Virginia type peanuts were much closer to the expected composition and, thus, lends support to the stress hypothesis.

The atherogenicity (proliferation of granular, collagen-containing lesions, particularly in the aorta) of peanut oil has been reviewed recently (Nutrition Foundation, Inc., 1973). The relatively high content of long-chain saturated fatty acids, principally arachidic and behenic, along with positional isomerism has been implicated with the unusually high atherogenicity of peanut oil in comparison to other vegetable oils. The potentially adverse effect of these two fatty acids should be considered in evaluating various peanut varieties.

The results in Table 1 and the respective correlation coefficients with linoleate in Table 3 indicate that variety and growing location affect the percentage of behenate in peanut oil, while the percentage of arachidate is relatively insensitive to location and variety. Data (not shown) for arachidate and behenate at the other locations also confirms the variety and location effects. The relative proportion of behenic acid increased with more northerly growing locations and was lower in the Virginia type varieties. Generally, varieties possessing high palmitate and linoleate percentages were higher in behenate as well. Since the arachidate percentage remains relatively constant over varieties and locations, total saturated long-chain fatty acids (behenate plus arachidate) also are higher in Spanish varieties and higher at increasing more northerly growing locations. It is noteworthy that the F 439 variety was much lower in arachidate and relatively low in behenate as well. Whether or not the 20–30% higher behenate content at the three northern-most growing locations, the average 10–20% greater behenate content of Spanish peanuts or the unusual composition of the F 439-16-6 variety are nutritionally significant is not known. However, these observations suggest that the composition of peanut oil can be improved somewhat from the standpoint of potential health effects through genetic selection.

Considerable importance has been ascribed to the role of the oleate/linoleate ratio in governing product shelf life (Young et al., 1974; Worthington et al., 1972). Higher O/L ratios usually lead to increased oil-stability and are thought to be indicative of potentially longer shelf life in roasted peanut products. In fact, some manufacturers blend Spanish and Virginia type peanuts to obtain products, particularly peanut butter, with better texture, flavor and stability (Worthington and Hammons, 1971).

The O/L ratios for the 1972 NRT peanuts represent the combined effect of year, location, environment and agronomic factors on composition. Conclusions drawn from these data are obviously tentative and need to be validated over a period of several years. However, application of the O/L theory to the results shown in Table 4 leads to the conclusion that products prepared from the northern grown peanuts from the NRT in 1972 would be less stable than products from the corresponding varieties grown at the southern locations. Furthermore, peanut products made from Virginia peanuts grown at Yoakum would be expected to have the longest shelf lives, while products made from Marianna grown Spanish peanuts should be more stable than Spanish peanut products from the other locations. The least stable products would be anticipated from the F 439-16-6 variety, and the most stable products from Va. 72R and Florigiant peanuts. The O/L ratios reported by Young et al. (1974) for nine Spanish peanut varieties grown at Tifton in 1968 and 1969 averaged about 0.15 units higher than at Ft. Cobb for the same year and support the validity of our O/L ratio-location data. Whether or not differences in O/L ratios will be reflected in product stability is unknown, and much more research on this subject is needed.

Young (1970) and Worthington et al. (1972) demonstrated that oil stability (oven-keeping times at 60°C) can be correlated with O/L ratios. However, the correlations are highly variable from year to year, and Worthington et al. (1972) were able to account for only 10–73% of the variation on the basis of O/L ratios. The reasons for the large variation in oil stability values from one year to another are unknown, but Worthington and Hammons (1971) have suggested that variations in climatic conditions, in soil moisture during maturation and in temperature during curing are possible causes.

In a preliminary study we have found a correlation of $r = 0.63$ for the relationship between O/L ratios and the oven-keeping times of the oils from the 10 varieties grown at the seven locations sampled in the 1972 NRT. This value accounts for only about 39% of the variation recorded. Thus, it would seem unlikely that the small differences in composition between peanuts from within the southern portions or within the northern portions of the U.S. production area will be noticeable in product stability, except when Virginia and Spanish market types or when northern and southern grown Virginia and runner peanuts are compared. These hypotheses regarding

Table 4—Oleate/linoleate ratios of peanuts grown in the National Regional Peanut Variety Trials in 1972

Variety	Yoakum Texas	Bryan Texas	Marianna Florida	Tifton Georgia	Stephenville Texas	Ft. Cobb Oklahoma[a]	Holland Virginia[a]
Spanhoma	1.31	1.27	1.44	1.34	1.10	1.10	1.15
Comet	1.24	1.29	1.43	1.33	1.17	1.13	1.08
Starr	1.30	1.31	1.51	1.29	1.14	1.23	1.17
TP 716-2-1	1.34	1.32	1.54	1.33	1.16	1.13	1.17
TP 931	1.38	1.39	1.44	1.44	1.15	1.16	1.15
Wilco I	1.46	1.38	1.38	1.28	1.02	1.02	–
Florunner	2.29	1.92	1.98	1.93	1.40	1.31	–
F 439-16-6	1.18	1.03	1.14	0.99	0.84	–	–
Florigiant	2.49	2.25	2.33	2.10	1.55	–	–
Va. 72R	2.63	2.24	2.32	2.29	1.57	–	–

[a] –Not available from Ft. Cobb, Okla.; lost in heavy rains at Holland, Va.

the NRT peanuts currently are being tested in our laboratory in shelf-life tests of peanut butter prepared from the NRT samples.

The changes in peanut oil composition with increasingly more northern growing locations are probably temperature induced effects. The fact that the composition of oils obtained from some plants, i.e., sunflower, rape and flax, varies widely according to the temperature at which the plants are grown has been recognized for a number of years (Kinman and Earle, 1964; Canvin, 1965). Generally monounsaturates increase and polyunsaturates decrease with increasing temperature. The ratio of monounsaturates is governed by the availability of oxygen and the rate of oxygen diffusion into the actively metabolizing cell system. Under field conditions increased temperature leads to lower polyunsaturation and higher monounsaturation (oleate, erucate, eicosenoate, etc.) due to the higher metabolic rate recorded at elevated temperatures (Bonner and Galston, 1951) and decreased availability of oxygen for reoxidizing the desaturase enzyme system required to synthesize linoleate and linolenate, etc.

Peanuts present a rather unique biological environment for the occurrence of the biochemical processes associated with maturation. The peanut seed is initiated above ground but matures in a subterranean environment 1 to 3 inches below the soil surface. The carbon utilized in lipid biosynthesis is translocated from the leaves to the seed through the peg in the form of carbohydrate and then is converted into lipid. Thus, soil temperature rather than air temperature is probably the governing factor in peanut seed lipid biosynthesis.

Southern grown peanuts are normally set and mature in July and August and are harvested in August or September. Soil temperatures approach the air temperature in fields with full plant canopies. In South Texas (Yoakum) the surface temperatures can easily rise to 40°C, and the soil temperatures an inch and a half below the soil surface under the canopy can stay above 30°C for days (Boswell, 1974). Peanuts grown in Stephenville, Ft. Cobb and Holland normally set fruit in September and are harvested in late October. Thus, they are exposed to maximum surface temperatures in the range of 20–35°C and subsurface temperatures in the range of 20–25°C. Conditions at Bryan and harvest dates are intermediate between the northern and southern locations.

The data leads one to postulate that F 439-16-6, Wilco I, Florunner, Va. 72R and Florigiant are probably more sensitive to temperature than the Spanish botanical type varieties and that the three varieties with the largest seeds are the most susceptible to location effects. The observed greater sensitivity of the large-seeded Virginia type peanuts to location effects could be due directly to the size of the seeds. Oxygen requirements for cellular metabolism in large seeds probably are similar to those in smaller seeds, while the rate of oxygen diffusion into the large seeds is probably slower because of the relatively lower surface to volume ratios in large seeds. Therefore, one might expect the desaturases responsible for converting oleate to linoleate to be depressed to a greater extent in large seeds and that the relative amount of linoleate should be decreased more than in Spanish peanuts produced under the same temperature regime. The observed closer similarity between O/L ratios in Virginia and Spanish peanuts grown at northern locations could also be expected on the basis of the temperature-oxygen diffusion explanation, since metabolic oxygen requirements should be lower at lower growing temperatures.

The possibility that differences in photoperiod, maturity and seed sources may have been responsible for the differences in lipid composition seems unlikely, although photoperiod and maturity cannot be completely excluded. The fact that all of the seeds for each variety were obtained from the same seed lot virtually eliminates the seed source and genetic differences as possible sources of variation. The fact that the lipids are synthesized in a subterranean environment argues strongly against the photoperiod explanation but does not completely eliminate the possibility that decreased or increased carbohydrate supplies might significantly alter the energy balance within the seed. The fact that the peanuts were sorted for maturity on the basis of seed size, pericarp color and for absence of wrinkling casts doubt on the possibility that the maturity of the nuts from any of the locations was significantly different. The foregoing hypotheses should be pursued in future investigations in order to more clearly identify the factors responsible for differences in lipid composition in peanuts.

REFERENCES

Alfin-Slater, R.B. and Deuel, H.F. 1960. In "Modern Nutrition in Health and Disease," Ed. Wohl, M.G. and Goodhart, R.S., p. 772. Lea and Febiger, Philadelphia, Pa.

Barr, J.A. and Goodnight, J.H. 1972. A user's guide to the statistical analysis system. Students' Supply Stores. North Carolina State University. Raleigh, N.C.

Boswell, T.E. 1974. Private communication. Texas A&M University Plant Disease Res. Sta. at Yoakum, Yoakum, Tx.

Blackith, R.E. and Reyment, R.A. 1971. "Multivariate Analysis," p. 146. Academic Press, New York, N.Y.

Brown, D.F., Cater, C.M. and Mattil, K.F. 1974. Effects of extraction, cold-pressing and period of storage on oven stability of peanut oils. J. Am. Oil Chem. Soc. 51: 502.

Bonner, J. and Galston, A.W. 1951. "Principles of Plant Physiology," p. 462. W.H. Freeman and Co., San Francisco, Cal.

Canvin, D.T. 1965. The effect of temperature on the oil content and fatty acid composition of the oils from several oil seed crops. Can. J. Bot. 43: 63.

Hammons, R.O. 1973. Private communication. USDA Ga. Coastal Plains Expt. Sta., ARS, Tifton, Ga.

Hitchcock, C. and Nichols, B.W. 1971. "Plant Lipid Biochemistry." Academic Press, New York, N.Y.

Kinman, M.L. and Earle, F.R. 1964. Agronomic performance and chemical composition of the seed of sunflower hybrids and introduced varieties. Crop. Sci. 4: 417.

Lea, C.H. 1962. In "Symposium on Foods: Lipids and Their Oxidation." Ed. Schultz, H.W., Day, E.A. and Sinnhuber, R.O., p. 3. Avi Publishing Co., Westport, Conn.

Metcalfe, L.D., Schmitz, A.A. and Pelka, J.R. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38: 514.

Nutrition Foundation, Inc. 1973. Atherogenicity of peanut oil in the rabbit. Nutr. Rev. 30: 70.

Rhee, K.C., Brown, D.F., Cater, C.M. and Mattil, K.F. 1973. Effects of variety and growing location on electrophoretic patterns and amino acid profiles of peanut proteins. Presented at the 58th Ann. Meeting Am. Assoc. Cereal Chem., November 4–8, at St. Louis, Mo.

Su, K.-C. and Chen, C.-S. 1973. Effects of varieties, locations and seasons on free fatty acid components of peanuts. J. Agric. Assn. China N.S. 74: 19.

Worthington, R.E. and Hammons, R.O. 1971. Genotypic variation in fatty acid composition and stability of Arachis hypogaea L. Oil. Oleagin. 26: 695.

Worthington, R.E., Hammons, R.O. and Allison, J.R. 1972. Varietal differences and seasonal effects on fatty acid composition and stability of oil from 82 genotypes. J. Ag. Food Chem. 20: 727.

Young, C.T. 1970. Biochemical studies of peanut (Arachis hypogaea L.) quality. Ph.D. dissertation, Oklahoma State University, Stillwater, Okla.

Young, C.T., Worthington, R.E., Hammons, R.O., Matlock, R.S., Waller, G.R. and Morrison, R.D. 1974. Fatty acid composition of Spanish peanut oils as influenced by planting location, soil moisture conditions, variety and season. J. Am. Oil Chem. Soc. 51: 312.

Young, C.T., Mason, M.E., Matlock, R.S. and Waller, G.R. 1972. Effect of maturity on the fatty acid composition of eight varieties of peanuts grown at Perkins, Oklahoma in 1968. J. Am. Oil Chem. Soc. 49: 314.

Ms received 6/5/74; revised 4/11/75; accepted 4/15/75.

Presented at the 34th Annual Meeting of the Institute of Food Technologists, New Orleans, La., May 12–15, 1974.

The authors acknowledge Wallace K. Bailey, Ray O. Hammons, ARS, USDA, and Olin Smith, Texas Agricultural Experiment Station, for consultation and peanut samples. Gifts of peanuts from the following individuals and their respective experiment stations are gratefully acknowledged: Dan Gorbet (Florida), James S. Kirby (Oklahoma) and Charles Simpson and Thurman Boswell (Texas).

D. D. BROWN and R. T. TOLEDO
University of Georgia, Food Science Dept., Athens, GA 30602

RELATIONSHIP BETWEEN CHOPPING TEMPERATURES AND FAT AND WATER BINDING IN COMMINUTED MEAT BATTERS

INTRODUCTION

FAT AND WATER BINDING by comminuted sausage meat batters is important as they relate to optimization of production practices and to the texture and juiciness of the cooked product. Reference to sausage meat batters as "emulsions" are widespread in the literature (Hansen, 1960; Swift et al., 1961, Helmer and Saffle, 1963; Baron, 1965; Rongey, 1965; Borchert et al., 1967; Saffle, 1968; Acton and Saffle, 1969). The prevalent concept in these reports is that sausage meat batters have properties very similar to oil in water emulsions, and that chopping actually results in simultaneous size reduction, salt soluble protein extraction, and emulsification. The salt soluble proteins are generally considered the emulsifying agent. Baron (1965) theorized that meat "emulsions" separate or become unstable when the fat particle size becomes so small that their total surface area becomes too large for the salt soluble proteins to cover.

The mechanism involved in the stabilization of sausage batters is not very well understood. Meyer et al. (1964) and Aboul-Saad (1970) reported that commercial food emulsifiers when added to sausage batters actually decreased rather than increased the fat binding. Furthermore, optimum fat and water binding in the chopped batters appear to be very strongly dependent upon the finish temperature in chopping. This temperature which is in the range of 15–22° (Hansen, 1960; Helmer and Saffle, 1963) is lower than the melting temperatures of the predominant fat present in these batters (Townsend et al., 1968). It is possible that some mechanically and thermally stable ordered layering of the protein fat and water molecules occur in the batter when a certain temperature range is reached during chopping. Mechanical entrapment of fat particles in a stable matrix of protein fat and water is probably responsible for the stability of comminuted meat batters.

In this report, the changes in stability of meat batters during comminution was studied to establish whether the temperatures attained during chopping have a direct relationship to stability or whether the observed temperature endpoint for optimum stability was merely a consequence of the extent of mechanical action in the chopper. The changes in the bound water level was also measured using Nuclear Magnetic Resonance techniques, to determine if some restriction of water mobility occurs at times during the chopping process where stability is optimum.

MATERIALS & METHODS

Formulation

Table 1 shows the proximate composition of the meats used in the experiments.

Two different formulations shown in Table 2 were chopped. The first two batches used for following change in fat and water binding and bound water content with comminution was formula 1. The ratio of boneless beef protein to fat in this formulation was 0.137–1. The third batch used for studying the role of fat in both fat and water binding and the effect of prolonged chopping at a constant temperature on stability had the composition of formula 2. The ratio of boneless beef protein to fat in this formulation was 0.374–1.

Comminution

Ingredients for a 13.6 kg batch were placed in the bowl of a Hely Jolly silent cutter (Hely Jolly Co., Tassin, France) and comminuted.

Table 1—Composition of meats used in comminuted meat formulations

Type of meat	Proximate composition		
	Fat (%)	Moisture (%)	Protein (%)
Boneless beef	20	56	21.8
Beef plates and flanks	42	42.3	18.3
Pork trimmings	45.7	38.1	13.6
Pork back fat	89.5	8.2	2.3

Table 2—Formulation of comminuted meat mixtures

Formula no.	Weight						Composition		
	Boneless beef (g)	Beef plates and flanks (g)	Pork trimmings (g)	Pork back fat trimmings (g)	Water (as ice) (g)	NaCl (g)	Protein (%)	Fat (%)	Water (%)
1	4080	3060	3060	–	3094	306	13.7	25.7	57.6
2	1140	–	–	3060	3094	306	11.9	30.6	53.7

Batter temperature was constantly monitored and recorded using thermocouples and a potentiometer recorder. Approximately 200-g samples were removed at various intervals and the chopping time and batter temperature were recorded at the time each of the samples were taken. The samples were stored inside screwcapped jars at 38°F (3°C) until used for analysis.

Chopping was continued until the batter temperature reached 77°F (25°C), at which time dry ice was added. Samples were taken just prior to addition of dry ice, and after approximately five revolutions of the chopper bowl past dry ice addition. Chopping was continued as before with continuous temperature monitoring and periodic removal of samples. In the first experiment, dry ice was added only once. To test for the effect of a second cycle of dry ice addition on the ability of the batter to regain the fat and water binding, two dry ice additions were done in the second experiment.

In a third experiment, pork trimmings were not added until the beef mixture was chopped for 6 min, and the first sample was not taken until 2 min of chopping had elapsed after fat addition. The batter temperature was allowed to rise to 55°F (12.7°C) at which time dry ice was added to drop the temperature to 35°F (1.7°C). Chopping was continued with controlled addition of dry ice to maintain the batter temperature at 55°F (12.7°C). After 40 min of chopping, more dry ice was added to drop the batter temperature and chopping was resumed until the batter temperature reached 77°F (25°C). More dry ice was added the second time to drop the temperature and chopping was allowed to proceed for an additional 16 min allowing the temperature to increase.

Determination of fat and water binding

Binding capacities of the batters were determined by taking cylindrical plugs of the batters weighing approximately 50g by inserting a cylinder into the batter and leveling the ends of the cylinder with a spatula. The plugs were then pushed out of the cylinder into tared 500 ml Erlenmeyer flasks, using a plunger. The flasks were covered with aluminum foil, placed in racks, and immersed into a pre-heated (80°C) water bath for 1 hr. At the end of the heating period, the liquid that was released from the sample in each flask was poured into a graduated cylinder and the ml fat and water lost by the sample was measured. This volume was corrected for the weight of the sample and expressed as ml fat or water released per 50g of sample.

Bound water determination

The bound water content in the batters was determined using a PA-7 Wide Line NMR Process Analyzer (Varian Associates, Palo Alto, Calif.) following the procedure reported by Shanbhag et al. (1970). Since the compositions of the samples from the same batch were the same, and since only the relative amount of bound water, rather than the absolute quantity present was of interest in the study, the results are presented in terms of NMR units per gram of sample, rather than the actual amount of bound water present. Subsequent analysis of the water content of the samples by the vacuum oven method reveal differences not exceeding 0.5% between samples.

RESULTS & DISCUSSION

FIGURES 1 AND 2 show the extent of fat and water binding in sausage batters during comminution. It can be seen that the increases in temperature with time of chopping are consistent for the two batters. The binding capacities for fat and water also increased with increasing temperature to 50–70°F (10–21°C) after which it decreased. After about 6 min of chopping where a temperature of 50°F (10°C) was attained, the batters had satisfactory fat binding capacities. The binding capacities remained satisfactory up to 14 min of comminution where the batter temperature at this point was 74°F (23–24°C). After that point, the profiles showed a steep increase in the amount of fat and moisture released. Townsend et al. (1968) showed that a 23–24°C beef fat should be in the liquid phase. The extent of fat and water release by the batters at this temperature (Figs. 1 and 2) show that the liquefication of fat decreased rather than increased the binding capacity.

The batters appear to lose their binding capacity for water earlier than for fat. The changes in water binding during comminution were more gradual compared to fat the first time the batters became unstable. This suggests that during the process of comminution and the formation of stable meat batters, water binding and fat binding are interrelated. It appears that as water is released, the ordered structure of protein water and

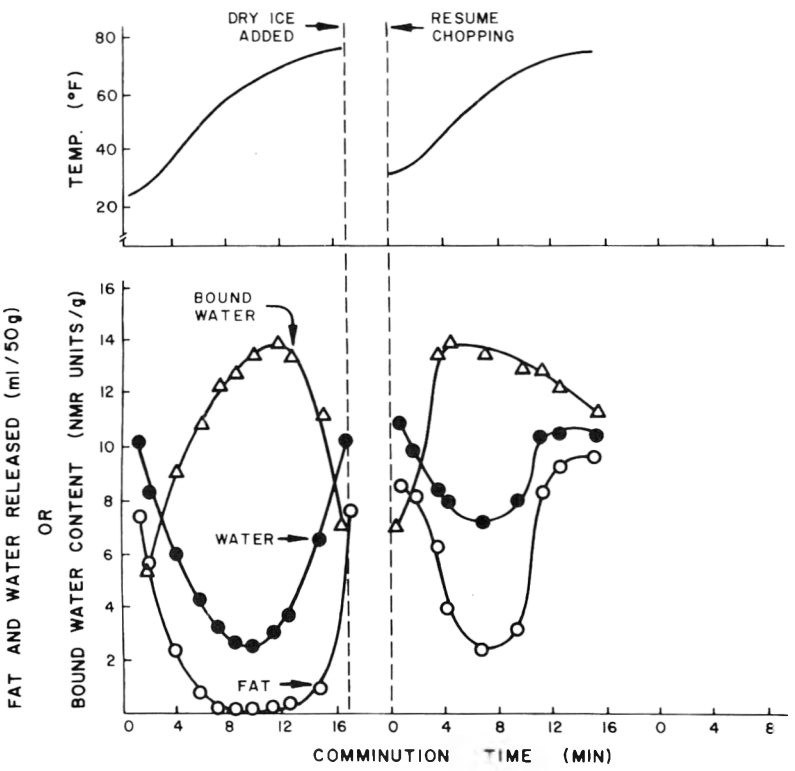


Fig. 1–Effect of chopping and recooling on fat and water binding by comminuted meat batters. Temperature curves represent batter temperature during comminution.

fat which mechanically traps the rest of the fat particles disintegrates and fat is released.

When the temperatures were dropped by the addition of dry ice to the batters, there was no immediate change in the water and fat binding. However, as the batters were comminuted, they gradually regained their fat and water binding capacities. The maximum binding capacity, however, was not at the same level as that prior to the addition of the dry ice. Apparently some changes have occurred on the protein such that the matrix formed was weaker than the original one. It took between 5 and 6 min of comminution after the addition of dry ice before the batters reached their maximum binding capacities for the second time. The temperature at the point was between 54 and 56°F (12.2–13.3°C). The batters maintained their maximum binding capacities for only 4 min of comminution after dry ice was added as opposed to 8 min before the addition of dry ice. Thus, the condition of the matrix not only affects the amount of fat and water bound, but also the resistance of the batters to stress.

In Figure 2, the temperature of the batter was dropped by adding dry ice for the second time. After the second cooling treatment for the batters the fat and water binding properties improved very slightly with additional comminution. After the second cooling treatment the batter practically had no resistance to stress and the time for maximum binding persisted for less than 4 min. These data indicate that the ability of the protein to form an ordered structure with fat and water was damaged each time batter temperatures exceeded a certain range during comminution. This impairment of protein quality was not completely reversible with a lowering of the temperature. This is in disagreement with Helmer and Saffle (1963) who concluded that there are no changes in the protein that could be observed at the point where the batters lose their fat and water binding characteristics during comminution.

Figures 1 and 2 also show the bound water content in the batters at various stages of comminution. This bound water content was determined using the rf saturation technique described by Shanbhag et al. (1970) on a wide-line NMR. Using this technique, free water does not register an NMR absorption peak whereas bound water absorbs giving a peak area directly proportional to the quantity of bound water present.

Corn oil and water emulsified using "Tween 60" as an emulsifier did not show any changes in the NMR absorption as the emulsion was formed. Thus, water is not "bound," as defined by this rf saturation NMR technique for bound water determination, during a regular emulsification process. The curves showing the changes in bound water content with time of comminution in both Figures 1 and 2 reveal that in the case of sausage batters an actual change in the relative mobility of water molecules can be observed. The bound water content increases as the batter approaches maximum stability, stays relatively constant at the region of maximum fat and water stability and again decreases as the batter begins to release increasing amounts of fat and water. Although quantitatively, the bound water content does not appear to be in direct proportion to the extent of fat and water retained by the batters, the trend of increasing bound water levels with decreasing fat and water released is very apparent.

The relationships between batter temperature and fat and water binding appears to be rather indefinite. Although the temperatures attained where fat and water binding are maximum are very consistent at the very first maxima in the fat and water binding curves, subsequent temperature cycling did not show a direct correlation between fat and water binding

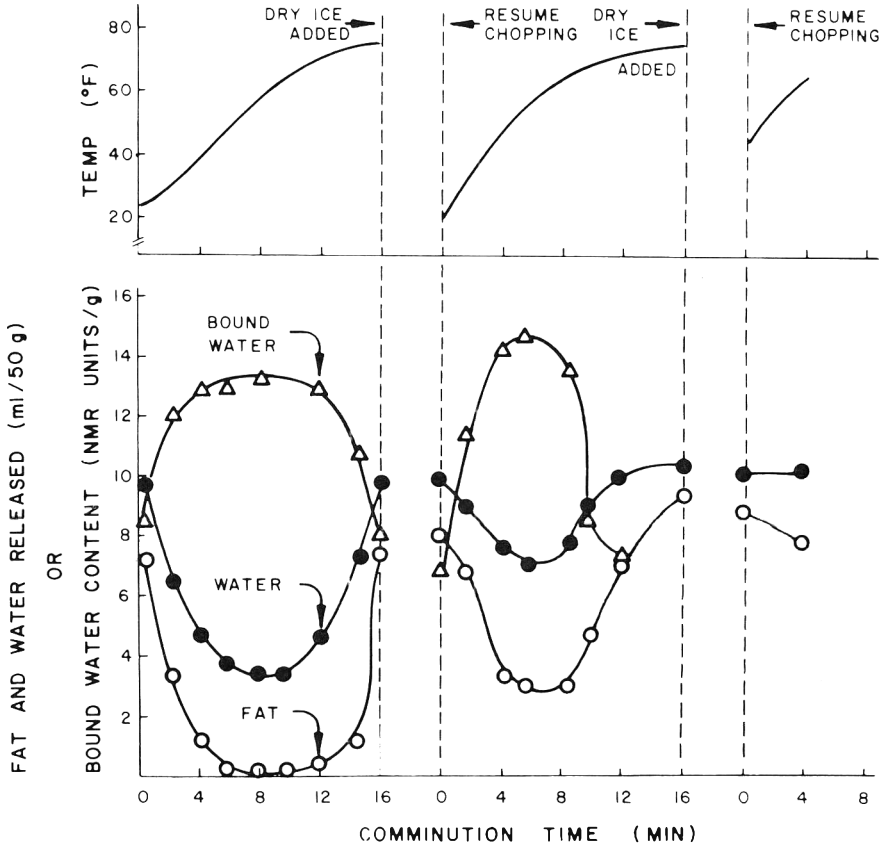


Fig. 2–Effect of chopping and two re-cooling cycles on fat and water binding by comminuted meat batters. Temperature curves represent batter temperature during comminution.

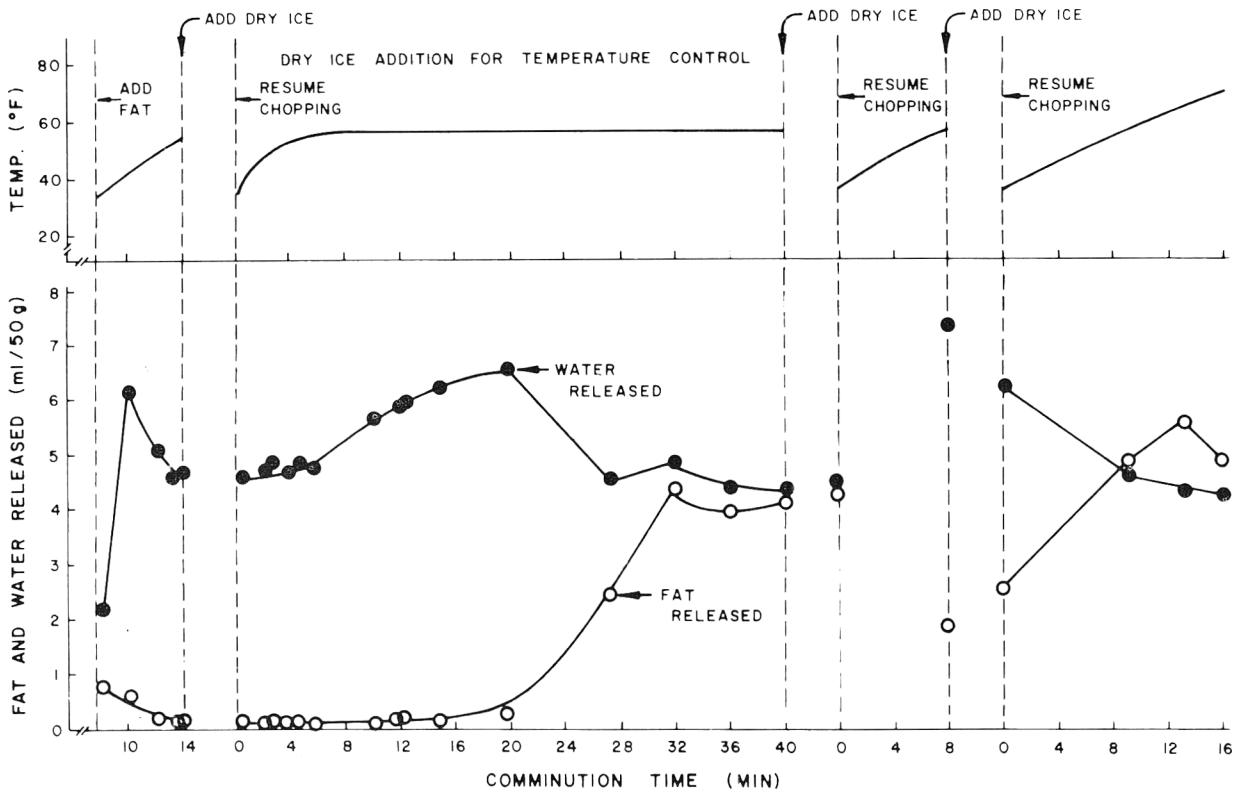


Fig. 3—Interrelationship between fat and water binding by a comminuted meat batter containing a high ratio of binder meat proteins to fat. Temperature curves represent batter temperature during comminution.

and temperature. In Figure 2, the batter never regained its original water binding property after the second cooling cycle. In Figure 3 where the temperature was held constant during prolonged chopping, changes in fat and water binding still continued to occur.

Figure 3 also shows the interrelationship of fat and water binding in comminuted meat batters. When fat addition was delayed in a meat batter having the composition shown in formula 2, the minima in the curve for water release as a function of time of comminution did not reach the levels attained in batters having the composition of formula 1. In this particular system it appears that there is an inverse relationship between fat and water binding. As fat binding increases, water binding decreases, and vice versa. Thus fat and water binding by comminuted meat batters do not always parallel each other and in this particular formulation and chopping procedure, they appear to be in competition with each other for binding in the system. No NMR data for bound water was obtained in this experiment.

CONCLUSIONS

STABILIZATION of fat and water binding by comminuted meat batters appear to be more involved than a simple emulsification process. Changes in the bound water content as measured by wide-line NMR techniques can be observed indicating a restriction in the mobility of the water present in the batters at the point in the chopping process where maximum binding is observed. The temperature range attained by the batter during chopping, where binding is maximum, occurs at 15–22°C but this is only true the first time a stable batter is produced from the original unchopped meat. Prolonged chopping results in changes in the fat and water binding regardless of temperature control.

REFERENCES

Acton, J.C. and Saffle, R.L. 1969. Preblended and prerigor meat in sausage emulsions. Food Technol. 23(3): 93.
Aboul-Saad, H.G. 1970. Studies on food emulsions. Ph.D. thesis, University of Georgia, Athens, Ga.
Baron, A. 1965. Stability of sausage emulsions. The National Provisioner 152(15): 14.
Borchert, L.L., Greaser, M.L., Bard, J.C., Cassens, R.G. and Briskey, E.J. 1967. Electron microscopy of a meat emulsion. J. Food Sci. 32: 419.
Brown, D.D. 1972. A study of factors affecting stability and quality characteristics in sausage systems. Ph.D. thesis, University of Georgia, Athens, Ga.
Hansen, C.J. 1960. Emulsion formation in finely comminuted sausage. Food Technol. 14: 565.
Helmer, R.C. and Saffle, R.L. 1963. Effect of chopping temperature on the stability of sausage emulsions. Food Technol. 17: 115.
Meyer, J.A., Brown, W.L., Giltner, M.E. and Guinn, J.R. 1964. Effect of emulsifiers on the stability of sausage emulsions. Food Technol. 18: 1976.
Rongey, E.H. 1965. A simple objective test for sausage emulsion quality. Proceedings of the Meat Industry Research Conference. American Meat Institute Foundation, 59 East Van Buren St., Chicago, Ill.
Saffle, R.L. 1968. "Meat Emulsions." In Adv. Food Res. 16: 105.
Shanbhag, S., Steinberg, M.P. and Nelson, A.I. 1970. Bound water defined and determined at constant temperature by wide line NMR. J. Food Sci. 35(5): 612.
Swift, C.E., Lockett, C. and Fryar, A.J. 1961. Comminuted meat emulsions—The capacity of meats to emulsify fats. Food Technol. 15: 468.
Townsend, W.E., Witnauer, L.P., Riloff, J.A. and Swift, C.E. 1968. Comminuted meat emulsions—differential thermal analysis of fat transitions. Food Technol. 22(319): 71.
Ms received 2/13/75; revised 5/3/75; accepted 5/6/75.

The authors express thanks to Dr. Marvin Steinberg at the University of Illinois for the use of the wide-line NMR process analyzer.

S. R. DRAKE, L. C. HINNERGARDT, R. A. KLUTER and P. A. PRELL
U.S. Army Natick Laboratories, Natick, MA 01760

BEEF PATTIES: THE EFFECT OF TEXTURED SOY PROTEIN AND FAT LEVELS ON QUALITY AND ACCEPTABILITY

INTRODUCTION

THE USE OF textured soy protein (TSP) products to extend, and thus reduce the cost of ground beef patties has become widespread, but the acceptance of these products has been variable. The Food and Nutrition Service (USDA) has approved the use of TSP in the school lunch program. Many manufacturers of meat patties use these extenders in school lunch patties but not in the patties sold directly to the consumer. Robinson (1972) reported that 71% of all persons questioned were prejudiced against meat analogs, but this figure was reduced to 58% after exposure to good meat analogs.

Judge et al. (1974) suggested that cook shrinkage (decrease in patty diameter) is one of the criteria of ground beef quality, and reported that soya additives substantially reduced shrinkage in samples containing 20 and 30% fat. Anderson and Lind (1974) found that retention of water and retention of fat was directly proportional to the amount of TSP in ground beef. Nollman and Pratt (1972) found that the addition of 2% TSP to meat loaves had no effect in total cooking loss or individual influence on moisture or fat retention. Wolf (1970) indicated that TSP added to meat loaves increased moisture retention.

Law et al. (1971) reported that consumers preferred ground beef with the relatively low fat content of 15–20% and that moisture content varied inversely with the fat content. As the fat content of ground beef was increased the amount of fat lost in cooking increased; however, high total weight losses consisted mainly of moisture. Kaiser et al. (1970) found that fat content had no influence on consumer acceptance of cooked ground beef. In contrast to the findings of Law et al. (1971), Cole et al. (1960) reported that laboratory and family taste panels rated ground beef with 15% fat less acceptable than ground beef with 25, 35 and 45% fat.

This study was initiated to determine if a synergistic effect existed between fat and TSP levels that would: (1) optimize the quality and acceptability of ground beef containing TSP and (2) affect the fat analysis as determined by either heat or solvent extraction.

EXPERIMENTAL

USDA CHOICE TOP ROUNDS, 7–10 days postmortem, were obtained from a commercial source along with cod and kidney fat. A 2^4 factorial design was used that consisted of four fat levels (15, 20, 25 and 30% by raw weight of patties) plus four levels (0, 15, 20 and 25% by raw weight of patties) of Archer Daniels Midland Co. Minced 180 textured soy protein (TSP). Levels of TSP added were calculated on a wet weight basis; moisture content of the hydrated form was 65% in all instances (USDA, FNS Notice 219).

The rounds were trimmed of cover fat and cut into strips about 5 cm thick. A 1.27 cm plate was used to grind the meat and fat separately. Prior to fat and TSP addition to the lean ground beef, the fat content of the lean ground beef was determined using a Hobart Fat Percent Indicator described by Bellis et al. (1967). Ground beef, textured vegetable protein and fat in the proper amounts were combined on a wt/wt basis into the 16 treatments. Each treatment (4.54 kg) was mixed for 3 min in a Hobart Mixer (Model No. H-600-T) to insure thorough blending of the ingredients. After mixing, each treatment was ground through a 0.3 cm plate. A Hollymatic Food Portioning machine (Model 54) was used to produce patties 7.6 cm in diameter, 1.27 cm thick and approximately 71g ± 0.3g in weight. The patties were wrapped in freezer-paper, frozen at −23.3°C and stored at −17.8°C until analysis.

Total cooking loss of the patties was determined by weight difference. Moisture content of raw and cooked patties was determined by placing the samples in a 70°C oven with 507 mm of vacuum for 24 hr. Fat content of the raw patties was determined by a Hobart Fat Percent Indicator, and of the raw and cooked patties by the Soxhlet extraction method.

Sensory quality evaluations were performed with a screened and trained panel of 12–14 members. Eight sessions were required, and four samples of one-third of a patty each were served at each session. In four of the sessions, one of the four fat levels was held constant and TSP varied; in the other four sessions the procedure was reversed. All test samples were evaluated on a nine-point descriptive quality rating scale ranging from 9 = Excellent, 5 = Fair and 1 = Extremely Poor. Five general attributes of quality were rated in this order: color, odor, flavor, texture and appearance. Samples were served in balanced random order. The frozen patties were grilled 5 min per side in an electric frypan calibrated to an average surface temperature of 176°C. They were held warm (about 63°C) until served. Water and unsalted crackers were used between samples to reduce flavor carryover.

For sensory acceptance evaluation, 32 consumer judges were selected at random from a roster of 500 Natick Labs staff volunteers. The nine-category Hedonic Scale was used to rate flavor and texture separately. Samples were presented one at a time to judges seated at a partitioned counter. Judges were provided with a standard instructional set describing the Hedonic method and were advised upon entering the laboratory that they were to "rate flavor and texture of grilled beef patties." Optional salt in portion-control packets was offered with each sample (one-half patty).

RESULTS & DISCUSSION

TABLE 1 indicates that as the amount of fat was reduced, total cooking losses were also reduced; as the amount of TSP was increased total cooking losses decreased. A significant increase in cooking loss due to fat content was found, and the largest reduction (2.5g) occurred between 25 and 20% fat. Another significant reduction (1.7g) occurred between 20 and 15% fat.

Table 1—Total cooking loss in grams for ground beef patties (71g ea) with added increments of textured vegetable protein and fat[a]

Amount of fat (%)	Amount of TSP (%)				$\bar{X}$ for effect of fat
	0	15	20	25	
30	20.95	19.32	18.43	13.36	18.01a
25	21.16	16.40	16.97	14.97	17.28a
20	17.00	14.66	14.50	12.97	14.78b
15	16.57	11.66	11.80	12.12	13.04c
$\bar{X}$ for effect of TSP	18.92a	15.51b	15.42b	13.26c	

[a] Values followed by the same letter in columns or rows did not differ significantly from each other at $P > 0.95$.

As the amount of TSP was increased, total cooking loss was significantly reduced. Patties containing no TSP had a total mean cooking loss of 18.92g and this figure was significantly reduced by the addition of 15% TSP. Increasing the level of TSP to 25% resulted in further significant reduction in total cooking losses.

As shown in Table 2, the amount of TSP incorporated into ground beef had no effect on fat losses. As the amount of TSP increased from 0 to 25% no binding effect of TSP on fat content was observed. The amount of fat lost during cooking depended upon the level of fat originally incorporated into the raw ground beef patties before cooking.

As the amount of fat in ground beef was increased from 15 to 30%, an increase in fat losses occurred during cooking, but the only significant change in fat loss was between the 15% and 30% fat levels.

Moisture losses during cooking of ground beef containing selected levels of fat and TSP are presented in Table 3. The initial amount of fat in raw ground beef patties had no significant influence on the final moisture content of the cooked product. The presence of TSP, however, had a significant influence on the amount of moisture retained in the cooked product. Cooked ground beef patties containing 15, 20 and 25% TSP lost equivalent amounts of moisture during cooking, and significantly less than patties containing no added TSP.

Raw ground beef patties containing TSP had significantly higher amounts of moisture than patties containing no TSP. Some of the increased moisture in the cooked patties containing TSP, was possibly due to water used to rehydrate the TSP. However, the moisture loss was equivalent at all levels of TSP studied and significantly less than patties with no TSP; thus it can be concluded that TSP did bind moisture during cooking.

Ground beef is purchased by the military on the basis of fat content, and the Hobart Fat Percent Indicator is used by the Military to determine the amount of fat present. In a study using 20% fat in the ground beef, Bellis et al. (1967) determined that fat content could be determined by either the Hobart Method or the Soxhlet Method with equal accuracy. However, the question arose whether or not these two methods would compare favorably when the ground beef contains increments of TSP with certain levels of fat.

There was no evident statistical interaction between fat and TSP content. Both methods of fat determination produced results that were comparable regardless of TSP content. At all calculated fat levels both the Hobart and Soxhlet methods produced comparable results that were within ± 1%. The amount of fat and TSP present in the raw patties had no effect on either Hobart or Soxhlet method of fat extraction. The Hobart Method for fat determination indicated a variation of 1.75% between ground beef with 0% and 25% TSP. The Soxhlet method of fat determination indicated a variation of 2.5% between samples with 0 and 25% TSP. At the four levels of TSP the differences between the two methods were 0.07, 1.02, 0.78 and 0.78, respectively. All differences in analyzed fat content were small and either method would produce accurate and comparable results regardless of TSP or fat content found in ground beef within the range of fat and TSP evaluated.

Results of quality and acceptance evaluations of cooked patties are exhibited in Tables 4, 5 and 6. Table 4 indicates, that of the five general attributes evaluated by the trained panel, the effects on color and texture were not significant for fat and TSP levels investigated and there were no significant interactions. The effect of TSP on odor scores was significant. However, the Newman-Keuls Test, computed when significant F ratios are found, revealed this was primarily due to scores at the 15% fat level wherein the 0% TSP patty was rated significantly higher than those containing any level of TSP. Although F ratios suggested that fat and TSP levels had a significant effect on appearance scores, the direction of the differences followed no consistent pattern with respect to level of either ingredient, and average ratings were within a narrow range of quality (less than 0.5 scalepoint).

Table 2—Grams fat lost during cooking of beef patties (71g ea) with selected amounts of textured vegetable protein and fat[a]

Amount of fat (%)	Amount of TSP (%) 0	15	20	25	$\bar{X}$ for effect of fat
30	6.33	8.33	6.78	4.91	6.58a
25	4.30	4.27	4.52	3.25	4.08ab
20	1.70	1.44	1.02	3.00	2.54ab
15	0.36	0.61	1.47	1.03	0.86b
$\bar{X}$ for effect of TSP	3.17a	3.66a	3.39a	3.04a	

[a] Values followed by the same letter in columns or rows did not differ significantly from each other at $P > 0.95$.

Table 3—Grams moisture lost during cooking of beef patties (71g ea) with selected amounts of textured vegetable protein and fat[a]

Amount of fat (%)	Amount of TSP (%) 0	15	20	25	$\bar{X}$ for effect of fat
30	14.19	10.81	11.46	7.78	11.06a
25	16.89	11.73	10.86	11.66	12.78a
20	14.30	12.97	13.17	9.99	12.60a
15	16.01	10.78	9.49	10.87	11.78a
$\bar{X}$ for effect of TSP	15.34a	11.57b	11.24b	10.07b	

[a] Values followed by the same letter in columns or rows did not differ significantly from each other at $P > 0.99$.

Table 4—F-ratios sensory quality evaluation of beef patties containing four levels of fat and TSP (trained panel)

Source of variation	Color	Odor	Flavor	Texture	Appearance
TSP	0.46^{NS}	5.44^{**}	79.51^{**}	1.83^{NS}	4.17^{**}
FAT	1.66^{NS}	1.63^{NS}	0.76^{NS}	0.86^{NS}	2.75^{*}
TSP & FAT	0.10^{NS}	0.40^{NS}	1.19^{NS}	0.40^{NS}	0.71^{NS}

** $P > 0.99$
* $P > 0.95$

Table 5—Mean sensory ratings of cooked patties for odor, flavor and appearance as affected by textural soy protein content (trained panel)[a]

Characteristic	Percent TSP 0	15	20	25
Odor	6.83a	6.59b	6.50b	6.41b
Appearance	6.99a	6.68b	6.79b	6.92a
Flavor	6.83a	5.27b	5.08b	4.72c

[a] Values followed by the same letter in rows did not differ significantly from each other at $P > 0.99$.

Table 6—Acceptance ratings for flavor and texture (consumer panel)[a]

Number of judgments	Percent		Attribute	
	Fat	TSP	Flavor	Texture
Acceptance Test 1				
32	30	0	7.2a	7.1a
32	15	0	6.9a	6.5a
32	30	25	5.8b	6.6a
32	15	25	5.5b	6.7a
Acceptance Test 2				
32	20 & 25	0	7.2a	6.9a
32	20 & 25	15	6.5b	7.0a
32	20 & 25	20	5.9b	6.7a
32	20 & 25	25	6.0b	6.7a

[a] Values followed by the same letter in columns did not differ significantly from each other at $P > 0.99$.

Table 4 shows that TSP level had a highly significant effect on flavor and fat level had no effect. Table 5 displays the ratings for the TSP levels averaged across the four fat levels. Patties at 0% TSP 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.

Since objective data suggested that the extremes of fat and TSP levels could produce the largest differences in fat and moisture retention (Table 5), the four out of 16 combinations representing these levels were selected for an initial preference evaluation. They were: 30% fat, 0% TSP; 30, 25; 15, 0; and 15, 25. As this experiment demonstrated that TSP, but not fat level had a significant effect on preference, a follow-up experiment was run wherein the two intermediate fat levels were combined and all four levels of TSP were presented.

In acceptance test 1, Table 6, patties with 0% TSP were significantly preferred for flavor at both initial raw-basis fat levels over those containing the highest level studied. Results from acceptance test 2 agreed with test 1: the panel significantly preferred the 0% TSP patty over all those containing TSP, and no preferences occurred between levels. In both tests, voluntary panel comments were few and did not provide clear reasons for acceptance expressed via the Hedonic scale. At 20 and 25% TSP levels, 25% or more of the panelists expressed some degree of dislike which could also be inferred from the relatively high standard deviations among their ratings. Texture ratings in both preference tests were, for practical purposes, identical, and there was little evidence from panel comments or authors' observations of any notable differences.

CONCLUSIONS

TOTAL COOKING LOSS can be reduced with the addition of TSP to the ground beef patties. TSP did not have a binding effect on the fat present in the product, but the addition of TSP to ground beef patties resulted in a product higher in moisture, both before and after cooking. The amount of fat lost during cooking was directly related to the original level of fat in patties and not to the level of TSP.

In the determination of the amount of fat incorporated into the beef patties, both the Hobart Fat Percent Indicator and the Soxhlet methods produced equally reliable results regardless of fat and TSP contents over the ranges investigated. Either method of fat determination was considered to be acceptable.

Sensory data indicated that: (1) TSP level had no effect on the quality attributes of appearance and color, and a limited effect on odor; (2) within TSP levels studied, neither trained nor consumer judges perceived differences in texture; (3) both trained and consumer judges found TSP addition to have a highly significant effect on flavor; (4) the various levels of fat had no effect on color, odor, flavor, texture or acceptance; (5) there was no interacting effect found between the TSP and fat levels and any addition of TSP resulted in lower quality and acceptance ratings regardless of fat content.

REFERENCES

Anderson, R.H. and Lind, K.D. 1974. Retention of water and fat in cooked patties of beef and beef extended with textured vegetable protein. Program of the 34th Annual Meeting of the Institute of Food Technologists, Paper No. 64.

Bellis, D.R., Secrist, J.L. and Lenskey, M.J. 1967. A comparison of solvent and thermal techniques for determining the fat content of ground beef. J. Food Sci. 32(5):

Cole, J.W., Ramsey, C.B. and Odom, L.O. 1960. What effect does fat content have on palatability of broiled ground beef? Tenn. Farm & Home Sci. 36: 5.

Judge, M.D., Hough, C.G., Zachariah, G.L., Parmelee, C.E. and Pyle, R.L. 1974. Soya additives in beef patties. J. Food Sci. 39(1): 137.

Kaiser, N.L., Rogers, R.W. and Haggan, F. 1970. Ground meat performance study. Miss. Farm Res. 33(2): 2.

Law, H.M., Young, S.P. and Mullins, A.M. 1971. Ground beef quality at the retail level. A study in Baton Rouge, La. J. Amer. Diet. Assoc. 58(3):

Lockmiller, N.R. 1972. What are textured protein products? Food Technol. 26(5): 56.

Nollman, D.S. and Pratt, D.E. 1972. Protein concentrates and cellulose as additives in meat loaves. J. Amer. Diet. Assoc. 61(6):

Pilgrim, F.J. and Peryam, D.R. 1958. Sensory testing methods, a manual. QMFCI Report No. 25-58.

Robinson, R.F. 1972. What is the future of textured protein products? Food Technol. 26(5): 59.

USDA, FNS Notice 219. 1971. Textured vegetable protein products (B-1) to be used in combination with meat for use in lunches and suppers served under Child Feeding Programs.

Wolf, W.J. 1970. Soybean proteins; their functions chemical and physical properties. J. Agric. Food Chem. 18: 969.

Ms received 1/15/75; revised 4/10/75; accepted 4/15/75.

The use of trade name does not represent an endorsement of the product by the Department of Defense.

MELISSA A. M. SHAFER and MARY E. ZABIK
Dept. of Food Science & Human Nutrition, Michigan State University, East Lansing, MI 48824

DIELDRIN, FAT AND MOISTURE LOSS DURING THE COOKING OF BEEF LOAVES CONTAINING TEXTURIZED SOY PROTEIN

INTRODUCTION

SEVERAL STUDIES have shown that pesticide levels in foods can be reduced by cooking (Ritchey et al., 1969; Reinert et al., 1972; Liska et al., 1967; Smith et al., 1973). Yadrick et al. (1971) reported that dieldrin residues in bacon could be reduced by pan frying and baking. Most of the losses were found in the drip and were attributed to fat rendering; although, heat destruction and codistillation were also cited as being responsible for some decrease in dieldrin levels.

Morgan et al. (1972) reported that simmering and pressure cooking chicken significantly reduced levels of several pesticides, including lindane, dieldrin, DDT and DDE. These pesticides were reported to be rendered into the broth with the fat. However, only 60–80% of the pesticides were recovered. Therefore, these authors indicated that volatilization and/or codistillation were responsible for some reduction in pesticide levels.

Yadrick et al. (1972) roasted pork muscles which had been trimmed of all surface fat thereby minimizing drip losses. They reported that dieldrin reduction accompanied volatile losses under these conditions. Ritchey et al. (1969) suggested that although the greatest reductions in DDT levels during cooking occurred through the leeching of fat, long heating times could also be responsible for some reduction in total residue levels. A further study by Ritchey et al. (1972) covering a wide range of pesticides indicated that while lindane and heptachlor epoxide could be reduced by heating alone, endrin, dieldrin and aldrin could not.

Rakosky (1974) reported that soy proteins are capable of absorbing water and binding fat in meat-soy combinations thereby reducing cooking losses, particularly drip losses. Although the use of 30% texturized soy in a meat-loaf system has been found to reduce overall cooking losses (Williams and Zabik, 1975; Nielsen and Carlin, 1974), the moisture content of the loaves containing 30% soy was found to be lower than that of the control loaves (Williams and Zabik, 1975). Both researchers found the fat content of the cooked loaves with 30% soy to be similar to that of the Control meat loaves even though substitution of ground meat with rehydrated soy containing 1% fat did reduce the fat content of the raw meat-loaf system.

Since the addition of soy has been found to reduce drip losses (Williams and Zabik, 1975), this may affect the reduction of pesticides during cooking. This study was conducted to determine if a substantial decrease in cooking losses would occur with the substitution of various levels of texturized soy protein (TSP) into a meat-loaf system, and to determine how changes in volatile and drip losses would affect the reduction of pesticides during cooking.

EXPERIMENTAL

Sample

Lean chuck sections of Hereford beef, which had been removed from the market since it contained greater than 0.3 ppm dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene–HEOD) from a tail head biopsy, was taken from freezer storage (−23°C) and made into meat loaves containing 0, 10, 20, 30 and 50% rehydrated TSP based on the weight of the meat. The TSP was Ultrasoy (a product of Far-Mar-Co., Inc.).

The formula used for the meat loaf mixture is given in Table 1. A common lot of meat loaf was made for each level of TSP substitution. Enough beef for five replications of each variable was ground through a Hobart Food Cutter, Model 84181D, using a plate with 4.7 mm extrusion holes. The ground beef and TSP which had been rehydrated with distilled water (1:2 by weight) were stirred together for approximately 30 sec. The meat-TSP mixture was then reground to assure homogeneity. The additional ingredients were combined with the meat using a Hobart mixer-Model A-200, at speed one for 3 min. One-half the meat loaf mixture was mixed at one time. A 1500-g batch of this mixture was weighed into a ham loaf press lined with Saran wrap and pressed for 3 min. The pressed loaves were wrapped in aluminum foil and stored at −18°C. Before baking, the loaves were defrosted for approximately 24 hr at 5°C. One-third of each loaf was removed for raw analysis and refrozen at −18°C.

Baking

The remaining portion of each loaf was weighed and placed unwrapped on a rack in a 9 × 12 × 2 in. aluminum pan. The loaves were baked in an Etco electric thermal convection oven which was regulated at 175°C ± 4° by a Honeywell controller. Each loaf was cooked to an internal temperature of 75°C, as indicated by an iron constantan thermocouple with a 4 in. immersion length which was positioned at the center of the loaf. Each loaf was allowed to cool at room temperature for 15 min. prior to weighing. The loaves were wrapped in aluminum foil and refrozen at −18°C along with the raw samples for later chemical analyses.

Cooking losses, expressed as total, volatile and drip were determined as outlined by Funk et al. (1966a). The drip was collected by rinsing the roasting pan and rack with acetone, and then evaporating the acetone from the drip. These samples were stored at −18°C for later chemical analyses. Samples were defrosted for approximately 24 hr at 5°C before being chemically analyzed.

Moisture analysis

The moisture content of the raw and cooked samples was determined in duplicate using the procedure outlined by Funk et al. (1966b). The percentage change in moisture between raw and cooked meat loaves was calculated by the formula [(% raw − % cooked)/(% raw)] × 100.

Table 1—Ingredients used in meat loaves[a]

Ingredient	Quantity %
Beef and rehydrated soy[b]	100
Bread crumbs	12
Catsup	11
Barbecue sauce	4
Salt (NaCl)	2
Onion (dry flakes)	2

[a] Quantities are based on the weight of the meat in the control.
[b] Soy was rehydrated by 2 parts distilled water to 1 part texturized soy (w/w).

Pesticide extraction

For pesticide analysis, 8g duplicate samples of the defrosted raw and cooked meat loaves were weighed to the nearest 0.0001g after three 1/2 in. slices had been homogenized in a 275 ml blender jar, using a Waring Blendor at the low speed for 30 sec. Two of the slices were taken from a distance of 1 in. from each end, and the third slice was taken from the center. The entire drip from each meat loaf was also analyzed.

Extraction with hexane-acetone (2:1), acetonitrile partitioning, and Florasil-Celite column cleanup were performed as described by Yadrick et al. (1971).

Fat analysis

The fat content of each sample was estimated by determining the percentage fat in a dried aliquot of hexane used to remove the fat (Yadrick et al., 1971). The percentage fat was converted to a dry weight basis.

Gas chromatographic analyses

Gas chromatographic analyses were carried out using a Beckman gas chromatograph, Model 72-5 interfaced to Digital Equipment Corporation PDP-8/E interfaced to a PDP-11/40 RSTS system. The gas chromatograph was equipped with a discharge electron capture detector and fitted with a 6 ft (1.83m) × 1/8 in. (2.0 mm) Pyrex column packed with 3% SE30 on 80/100 Gas Chrom Q with column, injector, and detector temperatures of 175°, 280°, and 260°C, respectively. The Helium flow rate was 95 ml/min for the discharge side of the detector and 40 ml/min for the column.

External standards were prepared from 99+% pure recrystallized HEOD (Shell Chemical Co.) in nanograde hexane. Quantitations were based on peak height. Spiked meat-loaf systems gave recoveries of approximately 91% using this method of analysis.

Dieldrin levels were expressed as total micrograms, ppm of solids and ppm of fat. Total micrograms in the cooked meat plus drip as compared with the total micrograms in the raw meat were used to calculate percentage recovery. Losses through codistillation were estimated using the formula One–(% recovery).

Mass spectrometry analyses

The presence of dieldrin was confirmed by mass spectrometric analysis run on a pool of all of the extracted samples. A GC-MS-CPU system was used which consisted of a Beckman GC-65 gas chromatograph interfaced to a DuPont 21-490 Mass Spectrometer which was in turn interfaced to a Digital PDP-12 computer. The mass spectra were obtained at an ionizing voltage of 70 eV with a source temperature of 150°C.

Data analyses

Data were analyzed for variances attributable to degree of soy substitution and interrelations due to state, i.e., cooked vs raw. Duncan's Multiple Range Test (1957) was used to pinpoint sources of significant differences. When appropriate, correlation coefficients were calculated (Dixon and Massey, 1957).

RESULTS & DISCUSSION

Fat content

The addition of TSP should have had a diluting effect on the fat content of the raw meat loaves proportional to the percentage of soy protein added, since the fat content of TSP was cited by the manufacturer to be 1.0%. From Table 2, it can be seen that the fat content of the raw meat loaves tended to decrease as the level of TSP substitution increased; however, the decrease which occurred was far smaller than had been expected for some of the levels of TSP substitutions. The fat content of the meat loaves containing 50% TSP was approximately one-half that of the Control; however, the fat content of the meat loaves containing 10% TSP was higher than that of the Control and the fat content of loaves containing 20% TSP was not significantly different from that of the Control. Although the beef came from the lean chuck portion of the same animal, obvious differences in the original fat content occurred.

As shown by previous studies, the use of TSP in the meat loaves has a binding effect on the fat (Williams and Zabik, 1975; Nielsen and Carlin, 1974). With increased levels of TSP substitution, smaller differences in the fat content between the cooked and raw samples were evident. The meat loaves substituted with 0% and 10% TSP contained significantly less fat after cooking ($p < 0.01$); however, the meat loaves containing 20, 30 and 50% TSP showed no significant fat reduction upon cooking.

The cooked loaves also showed smaller differences in fat content among loaves of varying levels of TSP substitution than was found in the raw loaves. Before cooking, the control contained significantly higher levels of fat than the loaves sub-

Table 2–Moisture, fat and cooking loss data for meat loaves substituted with texturized soy proteins[a,b]

Type of analysis	Level of substitution 0%	10%	20%	30%	50%	Statistical significance[c] p< 0.05
% Cooking losses						
Total	17.6 ± 0.5	16.1 ± 1.0	14.6 ± 0.6	13.7 ± 1.0	16.5 ± 0.6	0>50 10>20 30
Drip	2.0 ± 0.3	1.7 ± 0.3	0.9 ± 0.2	0.2 ± 0.1	0.0 ± 0.0	0>10>20>30 50
Volatile	15.5 ± 0.4	14.3 ± 1.0	13.8 ± 0.5	13.6 ± 1.0	16.5 ± 0.6	50>0>10 20 30
% Moisture						
Raw	57.88 ± 0.39	55.51 ± 0.31	56.47 ± 0.11	57.29 ± 0.18	56.05 ± 0.39	R>C
Cooked	51.84 ± 1.22	50.04 ± 0.70	52.05 ± 0.44	51.52 ± 1.15	47.50 ± 0.49	R_0 R_{30} R_{20} R_{50} R_{10}
% Difference	10.44	9.85	7.83	10.07	15.25	C_{20} C_0 C_{30}>C_{10}>C_{50}
% Fat						
Raw	20.93 ± 1.87	24.13 ± 1.80	19.61 ± 0.84	17.32 ± 1.53	10.89 ± 0.34	R_0>C_0 R_{10}>C_{10}
Cooked	17.20 ± 1.27	19.07 ± 0.81	18.19 ± 0.47	16.31 ± 1.08	11.31 ± 0.98	R_{10}>R_0 R_{20}>R_{30}>R_{50}
						C_{10} C_{20} C_0 C_{30}>C_{50}
Cooking time (min.)	71.4 ± 3.1	70.0 ± 4.8	66.8 ± 3.6	71.2 ± 3.3	80.4 ± 4.0	50>0 30 10 20

[a] Rehydrated soy protein was substituted for an equal weight of the meat in the Control
[b] Means and standard deviations were based on five replications.
[c] Values underscored with the same line are not significantly different (Duncan, 1957).

stituted with 30% TSP. In addition, the loaves with 10% TSP contained significantly higher levels of fat than that of loaves with 20% TSP. However, after cooking the fat content of the loaves with 0 and 30% TSP or with 10 and 20% TSP were not significantly different.

The loaves with 50% TSP showed no drip loss. However, the initial level of fat in the raw meat loaves was so much lower than the fat content of the other loaves, that after cooking, the fat content of the 50% TSP substituted loaves was still significantly lower.

Cooking time

Funk and Boyle (1972) found that cooking time was related to the fat content of the raw ground beef when cylinders of meat were roasted at several oven temperatures. At 121°C and 149°C, the fat content and cooking time were found to be inversely related; however, at 177°C no relationship was noted.

In this study (oven temperature 175°C) a very highly significant negative correlation was found between the fat content of the meat loaves and the cooking time (Table 2). The fat content of the cooked loaves correlated better ($r = -0.72$) with cooking time than did the fat content of the raw loaves ($r = -0.66$).

This relationship was most pronounced for meat loaves containing 50% TSP which had a significantly lower fat content before and after cooking as well as a significantly longer cooking time ($P < 0.001$).

Moisture and cooking losses

According to Rakosky (1974) soy proteins have an affinity for meat juices and, therefore, produce a juicier product. In contrast, Williams and Zabik (1975) found the moisture content of cooked meat loaves substituted with 30% soy to be lower than that of the Control meat loaves.

It can be seen (Table 2) that all of the raw meat loaves contained significantly more moisture than the cooked loaves. No consistent relationship was shown between degree of TSP substitutions and the moisture content in raw and cooked samples.

Volatile losses, previously found to be directly related to cooking time (Funk and Boyle, 1972), are in agreement with the findings of this study. A very highly significant correlation of $r = 0.76$ was calculated for cooking time and change in moisture content during roasting.

The greatest change in moisture content occurred in the meat loaves with 50% soy due to the very long cooking time. The moisture content of the other cooked meat loaves depended on the initial moisture content as well as cooking time.

The addition of soy to meat products has also been reported to decrease total cooking losses, particularly drip losses (Williams and Zabik, 1975; Nielsen and Carlin, 1974). Total losses (Table 2) tended to decrease with increased levels of TSP substitution. The meat loaves substituted with 50% TSP did not follow this trend. However, these loaves showed cooking losses which were not significantly different from the loaves substituted with 10% TSP. This was due to the extremely high volatile losses which occurred in meat loaves containing 50% soy. As expected, losses which occurred through drip showed a consistent decrease with increased substitution.

Dieldrin levels

The mass spectral analyses verified the presence of dieldrin in the meat loaves. Since chlorinated hydrocarbon pesticides are fat-soluble, a reduction in the level of fat by soy addition should have decreased dieldrin levels in the raw meat loaves. However, since the initial fat content of the meat varied, this did not occur (Table 3). The loaves with 30% TSP had significantly higher levels of dieldrin, but no other significant differences were noted among any of the other four levels of TSP substitutions. Differences in samples taken from the same portion of an animal have been related to differences in the metabolism of the pesticide (Yadrick et al., 1971).

On the fat basis, the raw meat loaves with 30 and 50% TSP showed significantly higher dieldrin levels than those with 0,

Table 3—The dieldrin content[a] of raw and cooked meat loaves substituted with various levels of soy protein[b]

Dieldrin content	Level of substitution					Statistical significance $p < 0.05$	
	0%	10%	20%	30%	50%	Level of substitution[d]	State
Total μg							
Raw	7.08 ± 0.55	8.24 ± 2.00	8.11[c] ± 1.16	9.98 ± 1.70	7.58 ± 0.72	30R>10R 20R 50R 0R	R & C>D
Cooked	6.13 ± 0.58	6.20 ± 1.02	7.59 ± 1.39	8.00 ± 1.16	4.93 ± 1.00	30C 20C>10C 0C 50C	10R>10C
Drip	0.66 ± 0.23	0.59 ± 0.20	0.31 ± 0.06	0.12 ± 0.09	0.00 ± 0.00	0D 10D>20D>30D 50D	30R>30C 50R>50C
ppm fat							
Raw	0.080 ± 0.014	0.076 ± 0.020	0.095[c] ± 0.014	0.133 ± 0.029	0.156 ± 0.011	50R 30R>20R 0R 10R	R & C>D
Cooked	0.091 ± 0.013	0.077 ± 0.013	0.103 ± 0.019	0.115 ± 0.016	0.101 ± 0.026	30C 20C 50C 0C 10C	50R>50C
Drip	0.040 ± 0.012	0.043 ± 0.009	0.045 ± 0.009	0.074 ± 0.049	0.000 ± 0.000	30D>20D 10D 0D>50D	
ppm dry							
Raw	0.017 ± 0.002	0.018 ± 0.004	0.019[c] ± 0.003	0.023 ± 0.004	0.017 ± 0.002	30R 20R 10R 50R 0R	D>R & C
Cooked	0.016 ± 0.002	0.015 ± 0.003	0.019 ± 0.003	0.019 ± 0.003	0.011 ± 0.002	30C 20C 0C 10C 50C	
Drip	0.033 ± 0.011	0.033 ± 0.007	0.038 ± 0.008	0.062 ± 0.043	0.000 ± 0.000	30D>20D 10D 0D>50D	
% Recovery	96.2 ± 10.6	85.3 ± 21.5	98.6 ± 20.1	82.5 ± 13.8	64.9 ± 9.4	20 0 10 30 50	

[a] Means and standard deviation were based on five replications.
[b] Substitution is based on the weight of the meat in the Control.
[c] Means and standard deviations were based on four replications.
[d] Values underscored by the same line are not significantly different (Duncan, 1957).

10 or 20% TSP. Smith et al. (1973) also found that the pesticide level was not related to fat content in fish. The dieldrin content of the cooked meat loaves was lower than that of the raw meat loaves for all levels of TSP substitutions. This observation supports previous findings that pesticide content can be reduced by cooking (Liska et al., 1967; Ritchey et al., 1969; Reinert et al., 1972).

Fat rendering has been suggested as the major mode for pesticide removal (Ritchey et al., 1969, 1972; Morgan et al., 1972). Although fat rendering has been found by all investigators to reduce pesticide levels, other data suggest that additional mechanisms also may be important (Yadrick et al., 1971; Funk et al., 1971). Funk et al. (1971) reported dieldrin losses during the baking of sausage patties to be 42 and 46%. However, the drip accounted for only 9 and 4% of these losses, respectively.

Yadrick et al. (1971) found that when bacon was baked, the dieldrin contents were reduced by 51, 82 and 62%, while the drip contained only 15, 8 and 19% of the dieldrin, respectively. Yadrick et al. (1972) reported that with minimal drip losses from roasting trimmed pork round muscles, most of the residue loss accompanied volatile losses. Ritchey et al. (1969) suggested that losses in total pesticide levels can be reduced by increased heating time. In a more recent study, Ritchey et al. (1972) found that lindane and heptachlor epoxide were reduced by heat alone, and that the reduction of lindane increased as the heating time increased. They found, however, that endrin, aldrin and dieldrin were not significantly decreased by heat.

In the current study, the losses which occurred through cooking were significant only for the meat loaves with 10, 30 and 50% TSP substitutions. In no case did the dieldrin content in the drip account for a significant loss in the pesticide content. Therefore, some other mode of removal such as codistillation or some other unidentified factor was responsible for these significant decreases.

In fact, the amount of dieldrin lost tended to be associated with volatile losses even though drip losses decreased as the level of TSP substitution increased. As the percentage moisture change increased, the amount of dieldrin recovered decreased ($r = -0.58$).

Dieldrin levels were lower in the drip than in the raw samples and tended to be higher in the cooked than the raw loaves. Zabik (1974) and Ritchey et al. (1969) indicated that chlorinated hydrocarbons are leeched into the drip with the fat. On the basis of percentage fat, Zabik (1974) found that the residual levels in the drip were similar to levels in the raw and cooked samples. Evidently, in the current study the dieldrin was bound to some extent in the meat-loaf system. Starch has been found to be capable of binding pesticides (Funk et al., 1972). Therefore, components such as bread crumbs could be responsible for this phenomenon.

Although significant losses did not occur through the drip, some residual levels were found in the drip. The quantity of dieldrin in the drip showed the same relationship as the drip losses since the quantity of dieldrin in the drip decreased as the levels of TSP substitutions increased.

On a dry weight basis, no significant differences were found between raw and cooked loaves for any level of TSP substitution or among any levels of TSP substitution for the raw or cooked systems. The drip contained significantly higher levels of dieldrin than either the raw or cooked loaves for all levels of substitution indicating that more dieldrin/g of dry material was present in the drip.

From these data it appears that with the use of increased levels of TSP substitution less dieldrin is lost in the drip upon cooking. However, other mechanisms such as codistillation appear to play an important part in the reduction of dieldrin levels in this meat-loaf system. The degree of codistillation seems to be related to change in moisture content and, therefore, cooking time and fat content of the meat loaves.

REFERENCES

Dixon, W.J. and Massey, F.J. Jr. 1957. "Introduction to Statistical Analysis," 2nd ed, Chap. 11. McGraw-Hill Book Co., Inc. New York.
Duncan, D.B. 1957. Multiple range test for correlated and heteroscedastic means. Biometrics 13: 164.
Funk, K., Aldrich, P.J. and Irmiter, T.F. 1966a. Delayed service cookery of loin cuts of beef. J. Am. Dietet. Assoc. 48: 210.
Funk, K., Aldrich, P.J. and Irmiter, T.F. 1966b. Force convection roasting of loin cuts of beef. J. Am. Dietet. Assoc. 48: 404.
Funk, K. and Boyle, M.A. 1972. Beef cooking rates and losses. J. Am. Dietet. Assoc. 61: 404.
Funk, K., Zabik, M.E. and Smith, S.L. 1971. Dieldrin residues in sausage patties cooked by three methods. J. Food Sci. 36: 616.
Funk, K., Zabik, M.E. and Smith, W.E. 1972. Pesticide residue levels in cooked rice and noodles. Home Ec. Res. J. 1: 44.
Liska, B.J., Stemp, A.R. and Stadelman, W.J. 1967. Effect of method of cooking on chlorinated insecticide residue content in edible chicken tissues. Food Technol. 21: 435.
Morgan, K.J., Zabik, M.E. and Funk, K. 1972. Lindane, dieldrin and DDT residues in raw and cooked chicken and chicken broth. Poultry Sci. 51: 470.
Nielsen, L.M. and Carlin, A.F. 1974. Frozen, pre-cooked beef and beef-soy loaves. J. Am. Dietet. Assoc. 65: 35.
Rakosky, J. 1974. Soy, grits, flour, concentrates, and isolates in meat products. J. Am. Oil Chem. Soc. 51: 123A.
Reinert, R.E., Stewart, O. and Seagram, H.L. 1972. Effects of dressing and cooking on DDT concentrations in certain fish from Lake Michigan. J. Fisheries Research Board of Canada 29(5): 525.
Ritchey, S.J., Young, R.W. and Essary, E.O. 1969. Cooking methods and heating effects on DDT in chicken tissues. J. Food Sci. 34: 569.
Ritchey, S.J., Young, R.W. and Essary, E.O. 1972. Effects of heating and cooking method on chlorinated hydrocarbon residues in chicken tissues. J. Agr. Food Chem. 20(2): 291.
Smith, W.E., Funk, K. and Zabik, M.E. 1973. Effects of cooking on concentrations of PCB and DDT compounds in chinook and coho salmon from Lake Michigan. J. Fisheries Research Board of Canada 30(5): 702.
Williams, C.W. and Zabik, M.E. 1975. Quality characteristics of soy-substituted ground beef, pork and turkey meat loaves. J. Food Sci. 40: 502.
Yadrick, M.K., Funk, K. and Zabik, M.E. 1971. Dieldrin residues in bacon cooked by two methods. J. Agr. Food Chem. 19: 491.
Yadrick, M.K., Zabik, M.E. and Funk, K. 1972. Dieldrin levels in relation to total, neutral, and phospholipid composition in selected pork muscles. Bull. Environ. Contam. & Toxicol. 8: 289.
Zabik, M.E. 1974. Polychlorinated biphenyl levels in raw and cooked chicken and chicken broth. Poultry Sci. 53: 1785.
Ms received 4/11/75; revised 5/31/75; accepted 6/2/75.

Michigan Agricultural Experiment Station Journal Article No. 7215
The authors express their appreciation to Robert M. Cook of the Dept. of Dairy Science for providing the beef and to Matthew Zabik of the Pesticide Research Center for mass spectrophotometric analyses and the use of gas chromatograph-computer equipment.

PAVEL JELEN
Dept. of Food Science, University of Alberta, Edmonton, Alberta T6G 2N2

USE OF COAGULATED LACTALBUMIN FROM CHEESE WHEY IN GROUND MEATS

INTRODUCTION

USE OF UNDENATURED soluble cheese whey proteins in human foods has become feasible as a result of several new technologies (Morr et al., 1973). However, these technologies may not be economical for smaller cheese manufacturers. The simple process of heat-acid coagulation offers an opportunity to recover the major whey protein fractions as a heat-coagulated lactalbumin curd (LC) (e.g., Webb and Hufnagel, 1946; Scott and McDonald, 1952; Pien, 1971, etc.). So far this product has found limited acceptance in human foods due to its insolubility and gritty character (Wingerd, 1971; Mann, 1971).

Various nonmeat proteins were investigated for use as emulsion stabilizers or binders (Inklaar and Fortuin, 1969; Smith et al., 1973) and as extenders or meat replacers in ground meats and sausages (Thomas et al., 1973; Anon., 1973; Yeo et al., 1974). Current interest has been focused on the soybean products added to meats as powders (Judge et al., 1974), curds (Yeo et al., 1974), or in the texturized form (TP). The TP-ground beef mixtures have been successfully marketed for both institutional and retail use.

Milk proteins in various forms—NFDM, Na-caseinate, co-precipitates-have also been utilized in meat sausages (Anon., 1973; Thomas et al., 1973). The simplicity of the heat-acid coagulation process and the availability, nutritive excellence and compatibility with particulate foods suggest LC as a potential meat additive. The purpose of this study was to evaluate the performance of the LC used as an extender in ground beef meat balls.

EXPERIMENTAL

Preparation of the lactalbumin curd

Fresh cottage cheese whey obtained from a local processor was adjusted to pH 6.5 with NaOH, heated in a cheese vat to 95°C, acidified to pH 4.5 with acetic acid, held for about 2 hr to cool gradually to ambient temperature, and the settled curd separated by decantation as described previously (Jelen et al., 1973). The curd was placed into a perforated stainless steel box lined with cheese cloth, and left to drain overnight at 5°C. The moisture content of the drained curd was 84.4%. Most of the curd was freeze dried in a pilot-plant drier (Virtis Co., Gardiner, N.Y.) in order to obtain constant and uniform supply of experimental material. The dry LC was reconstituted as needed to 30% total solids (TS) for subsequent mixing with the ground meats. The dehydration of LC irreversibly reduced the high water-binding capacity of the curd as discussed by Webb (1970). To obtain the crumbly structure typical of fresh LC, the freeze-dried material was used reconstituted to 30% TS. Reconstitution to 15% TS resulted in a rather fluid paste.

Preparation of meat balls

Commercial ground beef was thoroughly mixed with fresh (15.6% TS) or reconstituted (30% TS) LC in a 5 liter Hobart mixer (Model K5-A, Hobart Mfg. Co., Troy, Ohio), at the #2 speed using a flat beater. Most of the experiments in this work were carried out with mixtures containing up to 40% LC. Preliminary tests showed that with higher LC content the meat balls became organoleptically unacceptable. Higher levels of LC were used only in the moisture and fat retention study.

Meat balls of 25 ± 0.1g each were formed by hand and deep fried in an all-vegetable shortening at 175°C for predetermined times. The cooked meat balls were drained of excess oil on an absorbent paper towel before testing. For comparison, ground beef-TP mixture was also prepared using the same meat sample and rehydrated (30% TS) Promate III (Griffith Labs, Ltd., Scarborough, Ont.). Four replicate meat balls for each treatment were prepared at a time and fried simultaneously. Three or four different groups of meat ball replicates were fried in a single batch.

Experimental procedures

Proximate analysis. Moisture, protein and fat of the raw experimental materials were determined by vacuum oven (80°C for 20 hr), micro-Kjeldahl and Soxhlet standard procedures, respectively (Table 1). The same procedures were used for analyses of composite samples of the fried replicate meat balls.

Cooking loss and texture. All meat balls were weighed before and after frying and the weight loss in % of initial weight was calculated. The Lee-Kramer shear press model SP-12 (Lee Inc., Washington, D.C.) with the standard shear compression cell C-1S and the 3000 lb max electronic proving ring R-1E (experimental setting 0–300 lb), was used for texture measurements. A fried meat ball was placed into the cell and maximum force during the disintegration was recorded. Three replicates were used for the texture measurements at each replacement level.

Organoleptic evaluations. The basic method was the hedonic scale scoring using a nine-point numerical-verbal scale (extremely well liked–9, to extremely disliked–1) as described (Larmond, 1970). Taste panels were selected from a group of 15 members of the Dept. of Food Science staff with past experience in sensory evaluation. The panelists were not specifically instructed as to the particulars of the products tested. The taste panel data were evaluated by the Duncan's multiple range test.

RESULTS

Texture

The effect of lactalbumin curd on texture of the deep-fried meat balls is shown in Figure 1. A general trend of decreasing shear force with increasing levels of LC was observed at all frying times; with regard to doneness, 6–8 min frying was considered optimal. The final internal temperature was 80–85°C. With longer frying times the outer layer of the balls became too crusty and the shear force readings fell outside the

Table 1—Proximate analyses of the experimental materials

Sample	Moisture (%)	Protein (%)	Fat (%)
Fresh lactalbumin curd	84.6	n.d.[a]	n.d.[a,b]
Freeze-dried lactalbumin curd	2.2	51.3	n.d.[a,b]
Ground beef – batch 1	58.7	17.6	23.2
Ground beef – batch 2	60.2	18.0	21.6

[a] Not determined
[b] The use of cottage cheese whey implies absence of fat.

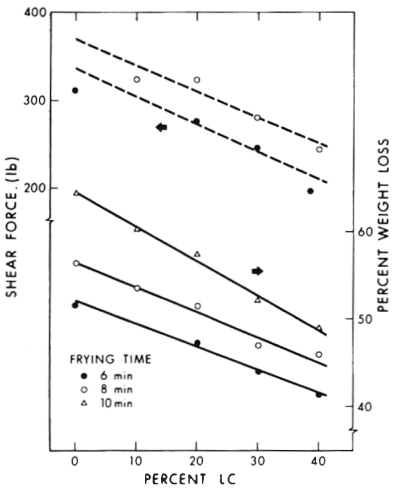


Fig. 1—Effect of percent lactalbumin curd (LC) in ground beef/LC mixtures on weight loss and texture of the deep-fried meat balls.

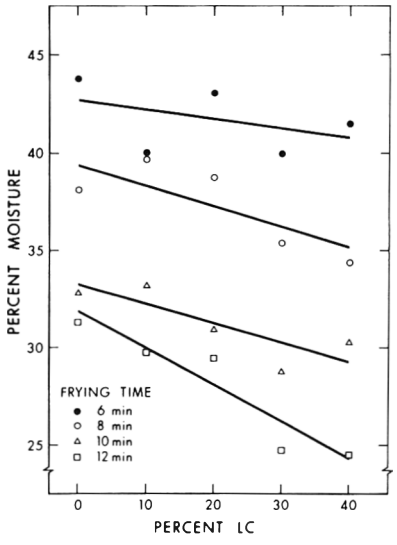


Fig. 2—Moisture of the deep-fried meat balls as affected by frying time and percent lactalbumin curd in LC/ground beef mixtures.

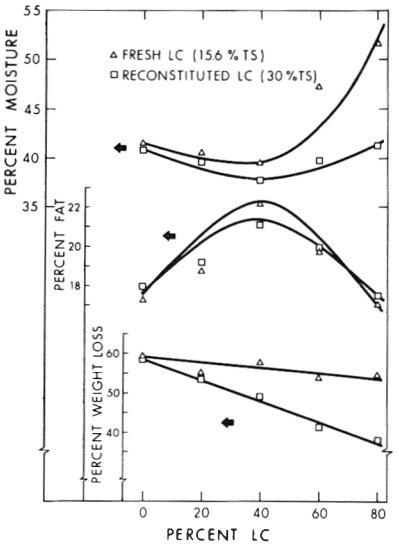


Fig. 3—Moisture content, fat content and weight loss after deep frying of LC/ground beef meat balls (8 min frying at 175°C).

range of the machine. Increasing levels of LC caused the balls to become noticeably gritty and more particulate than the all-beef controls. This probably accounted for the decrease in the shear force. Increasing grittiness or mealiness was also noted several times in the taste panel evaluations. Similar results have been reported for broiled beef-soy curd patties (Yeo et al., 1974).

Cooking loss

Weight losses upon cooking also decreased with increasing levels of LC at all frying times (Fig. 1). However, the consistent pattern of decreasing moisture with increasing levels of LC in the fried balls was observed with meat balls containing 0–40% LC (Fig. 2). Even though the individual data points are somewhat scattered, the trend toward lower moisture content with increasing LC at all four cooking times is clearly noticeable. Computer evaluation of the data revealed that the best-fit model (linear with respect to % LC) in the form

$$M = 53.160 - 1.692t - 0.0132t^2 + [0.163 - 0.043t + 0.0012t^2]\,L$$

(where M = % moisture content; t = cooking time; and L = % LC in the mixture) fits all the experimental data in Figure 2 with an average error of 2.8% (max error was 7.6%). Replicate experiments confirmed the negative correlation between moisture content after frying and the % LC in the meat ball mixture in the 0–40% LC content range, even though slightly different numerical results were obtained. This was probably due to the difficult temperature control in the frying procedure.

The seemingly contradictory observations of diminishing weight losses with diminishing moisture content indicated that increased water retention of the LC was not the reason for the higher cooking yields. Further experiments were carried out to establish whether the effect is due to higher fat absorption or higher solids retention or both.

Moisture and fat

Moisture, fat and cooking loss were determined with meat balls fried for 8 min. The substitution range was extended to 80% (i.e., 20 parts meat and 80 parts LC). Both fresh and reconstituted LC were used in order to gain more information about the possible effect of higher fat absorption into the voids left by vaporized water, and about the general performance of the fresh LC in these conditions. The results are summarized in Figure 3.

The trend of decreased cooking loss with increasing LC content was observed in the whole 0–80% substitution range with both fresh and reconstituted LC; however, the decrease is very slight in the case of the fresh LC. Decreasing moisture content after cooking was also observed in the 0–40% LC content range. At higher replacement levels the LC had a reverse effect. The significantly higher moisture content of the raw mixtures with high LC content was the likely reason for the slower moisture removal upon the 8 min cooking.

In contrast, the fat content shows a reverse trend with a maximum corresponding to the moisture minimum. The increase in the fat content after cooking over that of the pure beef meat balls indicated some fat absorption by the fried meat ball from the oil bath. At higher LC levels, the drastic decrease of initial fat content in the raw mixture obviously could not be compensated for by the fat absorption from the oil bath. The mass transfer process might have been retarded also due to the much tighter structure of the raw meat ball. This would explain both the low fat content and high moisture content at the high LC substitution range.

Taste panel evaluation

In the first experiment (Table 2) the panelists were offered a different sample each day for 5 days. The samples, including an all-beef control, the experimental products with 10, 20 and 30% meat replaced by LC, and a 75% beef/25% TP mixture were fried for 6 min and served warm; the sampling sequence was at random. It appears that under these conditions all the samples were considered acceptable, although there is an obvious tendency towards lower scores in flavor, texture and overall quality with increasing LC addition. No spices, condiments or flavorings were added to any of the samples which undoubtedly contributed to the generally low level of acceptance. The main defect noted by panelists was dryness and crumbliness of the meat balls containing higher amounts of LC.

In conditions of direct comparison, the all-beef meat balls

Table 2—Mean taste panel scores[a] of the deep fried meat balls evaluated independently

Sample composition (% LC in mixture)	Sampling day	Appearance	Flavor	Texture	Overall quality
0	3	5.1 a,b	5.6 b,c	6.4	5.9 c
10	4	5.8 a,b	5.1 a,b	5.4 a	5.6 b,c
20	2	4.9 a,b	4.6 a	5.0 a	4.5 a
30	5	5.9 a	5.1 a,b	4.9 a	4.8 a,b
25[b]	1	4.8 b	6.1 c	5.4 a	6.1 c

[a] According to 9-point scale (9—like extremely, 5—neither like nor dislike, 1—dislike extremely). Means followed by the same letter are not significantly ($P \leq 0.05$) different.
[b] TP added instead of LC

Table 3—Mean taste panel scores[a] of the deep fried meat balls evaluated in comparison

Sample composition	Appearance	Flavor	Texture	Overall quality
All Beef	7.0 b	7.0 b	6.5 b	6.8
90% Beef/10% LC	6.4 a	5.5 a	5.6 a,b	5.7 a
90% Beef/10% TP	6.9 a,b	6.0 a,b	5.2 a	5.7 a

[a] According to 9-point scale (9—like extremely, 5—neither like nor dislike, 1—dislike extremely). Means followed by the same letter are not significantly ($P \leq 0.05$) different.

were generally preferable to either TP-beef or LC-beef products extended at 10% level (Table 3). This suggests that not more than 5–10% LC replacement might be used in pure ground beef without significantly changing its organoleptic quality.

DISCUSSION

ALTHOUGH NO EFFORT was made to optimize the flavor and the conclusion may thus be considered preliminary, it appears that certain amounts of the lactalbumin curd may be added to meat products without serious adverse effects. The main value of the curd would be in decreasing cost without appreciable effect on nutritive quality. Due to the high moisture content, the fresh curd would not be suitable as a water-binding agent; in fact, mixing with certain established dry meat additives, such as TP or NFDM, might be desirable to avoid the higher moisture losses and resulting dryness observed with the meat balls. Cooking methods other than deep frying may also help to diminish this problem. In addition, ground meats other than beef might be more suitable for extension by certain nonmeat additives, as indicated by a brief report on prototype development of meat-instant mashed potato mixtures (Sadler, 1974). The palatability of beef-containing products in this work was inferior to those made with ground lamb, pork, chicken or fish. Similarly, fresh pork sausage containing 80% ground pork and 20% of the reconstituted LC (30% TS) was rated as very acceptable, and no grittiness or dryness was noticed in the boiled sausages (Jelen, 1974).

The comparison of experiments with fresh vs reconstituted LC (Fig. 3) suggests that the fresh LC, despite its high moisture content, would perform satisfactorily in ground meats. The use of fresh LC in various meat products appears feasible especially within a limited geographic area, with smaller cheese processing plants supplying the local meat processors. In dried form, the LC powder is a good water-binding agent and it might be suitable as a functional meat product additive similar to the currently marketed undenatured whey protein products. Data of Berlin et al. (1973) suggest that the water-binding capacity of the whey proteins is not altered by heat denaturation. Thus, the heat-acid technology may be more economical than newer sophisticated methods in cases where protein insolubility would not be detrimental.

REFERENCES

Anonymous. 1973. Australian researchers develop a milk sausage. Fd. in Canada 33(6): 47.
Berlin, E., Kliman, P.G., Anderson, B.A. and Pallansch, M.J. 1973. Water binding in whey protein concentrates. J. Dairy Sci. 56: 984.
Inklaar, P.A. and Fortuin, J. 1969. Determining the emulsifying and emulsion stabilizing capacity of protein meat additives. Food Technol. 23: 103.
Jelen, P. 1974. New uses for the heat-acid whey fractionation products. Proceeding 3rd whey products conference, Chicago, Sept. 18—19, 1974. USDA, Philadelphia, Pa. (In press).
Jelen, P., Manning, P.B. and Coulter, S.T. 1973. Separation of nitrogenous residues from deproteinated whey for lactose crystallization. J. Dairy Sci. 56: 1492.
Judge, M.D., Haugh, C.G., Zachariah, G.L., Parmelee, L.E. and Fyle, R.L. 1974. Soya additives in beef patties. J. Food Sci. 39: 137.
Larmond, E. 1970. Methods for sensory evaluation of food. Publication 1284, Canada Dept. of Agric., Ottawa.
Mann, E.J. 1971. Lactalbumin and its uses. Dairy Ind. 36: 538.
Morr, C.V., Swenson, P.E. and Richter, R.L. 1973. Functional characteristics of whey protein concentrates. J. Food Sci. 38: 324.
Pien, J. 1971. Process for extracting proteins from whey. U.S. Patent 3,583,968, June 8.
Sadler, A. 1974. Instant foods. Can. Inst. Fd. Technol. J. 7(2): A28.
Scott, E.C. and McDonald, G.W. 1952. Manufacture of lactalbumin. U.S. Patent 2,497,420. Dairy Sci. Abstr. 14(2): 89.
Smith, G.C., Juhn, H., Carpenter, Z.L., Mattil, K.F. and Cater, C.M. 1973. Efficacy of protein additives as emulsion stabilizers in frankfurters. J. Food Sci. 38 849.
Thomas, M.A., Baumgartner, P.A., Board, P.N. and Gipps, P.G. 1973. Evaluation of some non-meat proteins for use in sausage. J. Food Technol. 8: 175.
Webb, B.H. 1970. Utilization of whey in foods and feeds. In: Proceedings, Whey Utilization Conference (University of Maryland), USDA, Philadelphia, Pa., p. 105.
Webb, B.H. and Hufnagel, C.F. 1946. The manufacture and use of condensed cheese whey and crude whey protein. Nat'l Butter Cheese J. 37(12): 34.
Wingerd, W.H. 1971. Lactalbumin as a food ingredient. J. Dairy Sci. 54: 1234.
Yeo, V., Wellington, G.H. and Steinkraus, K.H. 1974. Effects of soy curd on the acceptability and characteristics of beef patties. J. Fd. Sci. 39: 288.
Ms received 5/31/74; revised 5/13/75; accepted 5/20/75.

Financial assistance of the National Research Council of Canada for this work is gratefully acknowledged.

S. N. FALK,[1] R. L. HENRICKSON and R. D. MORRISON
Departments of Animal Sciences & Industry and Mathematics & Statistics
Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, OK 74074

EFFECT OF BONING BEEF CARCASSES PRIOR TO CHILLING ON MEAT TENDERNESS

INTRODUCTION

INCREASING PRODUCTION COSTS as a result of spiraling energy prices and wage rates, combined with a shift in consumer trends toward portion-controlled meat products, has strongly suggested a need for a more efficient method of fabricating the beef carcass. Heretofore, substantial effort has been given to hot or accelerated processing of the pork carcass as may be noted in a review by Falk (1974). It is generally recognized that the hot processing of the pork carcass may be initiated between 45 min to 1 hr postmortem without significant diminution in color, tenderness, water-holding capacity and flavor.

Hot boning of the beef carcass presents a more difficult problem when compared to pork because of the relatively slow rate of postmortem glycolysis. Marsh (1954) reported that all glycolytic processes in bovine carcasses should be completed within 36 hr postmortem. Jungk et al. (1967) reported that maximum isometric tension was attained within 3–16 hr after death. Busch et al. (1972) using a very sensitive measurement of isometric tension, illustrated that when bovine ST muscle strips were suspended in a 37°C dilute saline solution, tension development reached a maximum at 3–5 hr postmortem. The authors further observed that bovine muscle did not begin to develop isometric tension as early postmortem as did either rabbit or porcine muscle.

The comparatively slow rate of postmortem glycolysis in beef has resulted in a number of variations in procedure regarding the time at which the musculature was excised, as well as the subsequent treatment of the hot-boned muscle. Schmidt and Gilbert (1970) excised muscles from 6 carcasses at approximately 2 hr postmortem. To overcome the effects of muscle shortening and toughening, the hot muscle was aged for 24 or 48 hr postmortem at 15°C in gas impermeable bags and frozen at −14°C. Cold-boned muscles were excised from the remaining side of the carcass which was suspended for 24 hr at 9°C. Results indicated that hot-boned muscles aged for 24 hr were similar to the controls in tenderness, while hot-boned muscles aged for 48 hr were significantly more tender than muscle from the contralateral side. Kastner et al. (1973), using 18 animals, proposed a second method to alleviate the problems involved with rigor mortis by allowing the muscles to remain intact on one side of a carcass for either 2, 5 or 8 hr postmortem. The hot-boned side was vertically suspended from the rail in a 16°C cooler for the assigned treatment period. The remaining side was similarly suspended in a 2°C chill cooler for 48 hr postmortem, serving as the control or cold-boned side. Muscles boned (longissimus dorsi, biceps femoris, semimembranosus, and semitendinosus) at 2 and 5 hr postmortem were found to be significantly less tender than their corresponding controls. The 8 hr hot-boned muscles were not statistically different as compared to muscles from the control side. Although no statistical comparison was made between treatments, it is important to note that the difference in tenderness between hot and cold muscles was substantially less at the 5-hr treatment when compared with the 2-hr holding period. Additionally, no statistical evaluation was made on the individual test muscles. Schmidt and Keman (1974) evaluated the effect of hot processing on 6 Angus steer carcasses. In this procedure, the right side of each carcass was hot boned 1 hr after slaughter, whereas the left side was placed in a 1°C room. The boneless wholesale cuts from the right side were kept at 7°C for 4 hr and were subsequently placed in 1°C room overnight. The cuts were then vacuum packaged and replaced in the cold room for 7 days. The control side was boned after chilling for 8 days at 7°C. Taste panel evaluation as well as evaluation by Warner-Bratzler shear did not indicate a significant difference in tenderness between the two processing methods. Measurements of fiber diameter showed that most hot-boned muscles increased in fiber diameter when compared with the control.

Hot boning the bovine carcass has several potential advantages. Most notable is the fact that the procedure results in the removal of bone and excess fat prior to chilling, thereby reducing the amount of necessary cooling. Processors would benefit from increased efficiency resulting from the utilization of on-the-rail boning (Brasington and Hammons, 1971) which is enhanced by the fact that the muscle and fat are pliable and easily removed from the hot carcass. The boneless product would maximize refrigerative space and lend itself well to portion control.

This study was designed: (1) to evaluate the feasibility of hot boning the beef carcass at 3, 5 and 7 hr postmortem with respect to tenderness; and (2) to determine the minimum conditioning time before hot boning could be initiated.

MATERIALS & METHODS

30 CHOICE ANGUS STEERS ranging in weight from 405–555 kg, were randomly assigned to 3 treatment groups (3, 5 or 7 hr postmortem). Following the 24-hr shrinkage period, the steer was weighed and given antemortem Federal inspection. Care was exercised in handling the animals to avoid any adverse effect upon postmortem metabolic reactions, as well as ultimate product quality. Each animal was stunned, raised from the floor by both legs and bled. The time of death was recorded upon completion of exsanguination. During the conventional dressing operation extreme care was exercised to assure accurate splitting for even weight distribution on the vertically (tendon of Achilles) suspended sides. The slaughter and dressing operation proceeded as rapidly as possible so that Federal inspection was given within 45 min postmortem. Either the right or left side of the carcass was randomly assigned to one of two treatments: (1) removing the muscles at 3, 5 or 7 hr postmortem while the carcass was still warm (hot boning) or (2) removing the muscles after a 48 hr chilling period (cold boning) at 1°C.

Hot-boned side

Immediately after being weighed, the hot side was placed in a 16°C holding room. Thermocouples were inserted into the three test muscles, namely, the longissimus dorsi (LD), semimembranosus (SM) and semi-

[1] Present address: Dept. of Food Science & Nutrition, 1-74 Agriculture Bldg., University of Missouri, Columbia, MO 65201

tendinosus (ST). Each thermocouple was in turn connected to a Honeywell recording potentiometer. Following the expiration of a 3, 5 or 7-hr holding period, the side was fabricated into a streamlined hindquarter.

This fabrication consisted of chuck removal at the fifth thoracic vertebra with the flank and plate removed as in the commercial trade. After the streamlined hindquarter was weighed, on-the-rail dissection of muscles and muscle systems was initiated in the 16°C holding area.

The fat was carefully stripped from the muscle so that the epimysium remained intact. Excision of the muscles from the streamlined hindquarter was found to be the most efficient if they were removed in the following order: tensor fascia latae, sartorius, semimembranosus, semitendinosus, biceps femoris, quadriceps complex, psoas major, gluteus complex and longissimus dorsi. The remaining lean and small muscles were excised and utilized for lean trim. As soon as the excision of a given muscle was completed, it was placed into a Cry-O-Vac bag (S-507) (no vacuum was drawn) to prevent surface desiccation. Other components (bone, fat and lean trim) were treated in an identical manner. The dissection of the streamlined hindquarter was completed approximately 1 hr after initiation. The components were then removed to a 1°C cooler for the remaining portion of the 48-hr period (same cooler as the cold side).

Cold-boned side

Immediately after obtaining the hot weight, the side to be cold boned was placed into a 1°C chill cooler for 48 hr. Following the expiration of this time period, the cold-boned side was reweighed and broken down into a streamlined hindquarter. The side was then removed to the 16°C holding area and an identical procedure to that of the hot side was followed.

Sample preparation

Three test muscles (LD, SM, and ST) were selected for tenderness evaluation by both subjective and objective measures. Sample locations were the same for both the hot- and cold-boning treatment. During the sampling procedure the muscles were held at 1°C.

Steaks for the organoleptic evaluation and shear force were packaged, labeled and frozen (−10°C) for analysis at a later date. The steaks for histological examination were held fresh at 1°C until sampled.

pH Determination

Measurements of pH were taken from 1 through 5-hr postmortem and then again at 24 and 48-hr postmortem from the psoas major of both the hot and control sides. The psoas major was used in order to avoid cutting the test muscles which would result in tension loss and could possibly influence product tenderness. The procedure at each measurement period involved excising a freshly cut transverse section from the same general area on each side, using the skeleton as a guideline. 10g of finely minced muscle were weighed into a beaker containing 50 ml of distilled water. The solution was mixed thoroughly and allowed to stand for 1 min at which time mixing was repeated. The recorded pH was used as an index of the extent and rate of postmortem glycolysis in the hot- as compared to the cold-boned side.

Shear force

The test steaks were thawed at 1°C for 24 hr. Two steaks from each of the three test muscles were evaluated for both hot and cold boning; thus, 12 steaks were analyzed from each of the 30 carcasses. The thawed steaks were cooked in deep fat until an internal temperature of 72°C was reached. The cooked steaks were chilled for 24 hr at 1°C to provide adequate firmness and insure uniform cores (Kastner and Henrickson, 1969). Each steak yielded three, 1.90 cm diam cores and each core was sheared three times by the Warner-Bratzler shear instrument. A 1.90 cm bore was preferred to a 2.54 cm bore as it facilitated the removal of three cores from each steak. Paul and Bratzler (1955) found that there was close agreement between shears of 1.27 and 2.54 cm in diameter and suggested that either size may be used to measure shear force. Therefore, no loss of accuracy was anticipated as a result of using the smaller diameter bore.

Histological evaluation

Fiber diameter and kinkiness score. One, 1.27 cm core was removed from the center of corresponding steaks of the hot and cold LD, SM and ST. Cores from the two steaks of a given muscle and process (hot or cold) were placed in 10% buffered formalin and stored at 1°C until the muscle fibers were isolated. The formalin was changed after 24 hr to insure adequate fixation. The fiber diameter was observed at the same time as the kinkiness score (Cagle and Henrickson, 1970).

Sarcomere length. Because of the difficulty of measuring sarcomere length, a limited study was accomplished using the previously discussed fibers isolated from hot and cold LD muscles of the first four carcasses in each holding period (3, 5 or 7 hr). The suspension of fibers contained in a sample vial was placed in a Waring Blendor jar with reversed blades and blended at high speed (100 on the rheostat) for 1 min to isolate the myofibrils. The suspension was then returned to the glass jar. Five myofibrils per jar were randomly photographed using a Zeiss phase-contrast microscope equipped with a Polaroid camera and Zeiss light meter at 1500 magnifications. Thus a total of 120 myofibrils (20 hot and 20 cold for each holding period) were photographed. The individual myofibril on each photomicrograph was evaluated by measuring the length of ten sarcomeres by use of a caliper. The caliper was placed on a metal ruler with 1/100-in. graduations, and the value was converted to mm. Sarcomere length was calculated as follows:

$$\frac{A}{(B)\,(C)} = \text{Sarcomere length in microns}$$

where A = Number of millimeters measured; B = Number of sarcomeres measured = 10; and C = Magnification of myofibril = 1500

Organoleptic evaluation (tenderness panel)

The LD and SM muscles were utilized for appraisal by a tenderness panel. Because of its size, the ST was not used. Two steaks from the LD and SM muscles of both right and left sides of the carcass were thawed for 24 hr at 1°C. Six trained panel members were used for each trial, although all six members were not the same from trial to trial. The judges consisted of both men and women of different ages selected from the employees of the Meat Science Laboratory.

The duo-trio test (Kramer and Twigg, 1966; Amerine et al., 1965) was used to determine whether differences in tenderness existed between hot and cold boning. Each judge was asked to choose which of the unknowns best matched the reference sample. Randomization was used to determine which process (hot or cold) would serve as the pair (reference and corresponding unknown) and which would be designated as the single (odd sample). The steaks from a given muscle were weighed and then cooked in Frymax oil at 135°C to an internal temperature of 72°C. After cooking, the steaks were blotted, reweighed, and prepared for sample extraction. A 1.27 cm diameter bore was used to remove two cores from the steak used as the pair and one core from the steak serving as the single.

Care was exercised to avoid extraneous variation in response by control of sample position, labeling, randomization, temperature, lighting and odors.

The duo-trio test was performed first, then each judge was asked to indicate a preference for one of the two unknowns. If there were no preference, the judge was asked to flip a coin. Lastly, each judge assigned to each unknown a level of acceptability based on a six-point scale (the larger the number, the higher the level of acceptability).

The duo-trio panel was evaluated by means of Table 85 as shown in Kramer and Twigg (1966) such that 73/120 correct responses were required for significance at the 0.05 level. Analysis of the preference selection was accomplished by assigning the preferred treatment a value of 2 and the remaining treatment a value of 1. The hedonic scale was evaluated similarly such that the treatment receiving the higher level of acceptability was ranked with a 2 and the remaining treatment with a value of 1. In case of a tie, each treatment received a value of 1.50. Both the preference and hedonic evaluation were analyzed by the Freidman test (Conover, 1971).

Statistical analysis

The SAS computer programming system (Service, 1972) was used to analyze all data presented in this study. Organoleptic panels were evaluated by using the ranking procedure described by Conover (1971) in conjunction with the Chi-square test. The analysis of variance was used in the remainder of the statistical evaluations. F-tests concerned with the main unit analysis utilized the animal × process mean square with nine degrees of freedom as the error term. The subunit analysis F-tests were conducted using the pooled animal × steak plus animal × process × steak mean square with 18 degrees of freedom. Furthermore, it is important to note that each holding period was considered as a separate experiment. Thus no statistical comparison was made between the 3, 5 and 7-hr treatments.

RESULTS & DISCUSSION

AS SHOWN IN TABLE 1, differences in shear force values between hot vs cold boned muscle were small, averaging less than 0.90 kg. Although not important from a practical stand-

point, shear force values were statistically different ($P < 0.05$) only at the 5-hr holding period for the LD and at the 7-hr holding period for the SM. Statistical evaluation of the interaction as a result of sampling location was nonsignificant ($P > 0.05$) in the three test muscles at all holding periods, indicating that steaks from a given location responded similarly regardless of processing method.

As shown in Table 2, sarcomere length was slightly, but not significantly, smaller ($P > 0.05$) in hot-processed LD as compared to the 48-hr control at all holding periods indicating that the hot muscle had not shortened appreciably. Differences in the fiber diameter were significant ($P < 0.05$) in the LD, SM and ST muscles at the 3-hr holding period (Table 3). However, the largest difference between the two processes was only 7.0 μm (3 hr ST), representing an 11% decrease in fiber length as compared to the control side. A decrease in length of this magnitude is not of consequence when one considers that a muscle may shorten by 20% with little or no decrease in tenderness (Marsh and Leet, 1966). Fiber diameter was slightly greater for hot-processed LD and ST as compared to the control at the 3, 5 and 7-hr holding periods. It is interesting to note that the reverse situation occurred in the SM at all boning periods. A large amount of shortening was not anticipated in the SM as studies by Herring et al. (1965) and Hostetler et al. (1970; 1972; 1973) demonstrated that suspension of sides by the Achilles tendon does not exert a stretching effect on the SM; therefore, removal of the intact muscle should not adversely influence its tenderness.

Kinkiness scores for the hot-boned LD (Table 4) were somewhat greater ($P < 0.05$) than the corresponding control at all three holding periods. Differences in kinkiness scores for hot vs cold-boned SM and ST muscles (Table 4) were nonsignificant ($P > 0.05$) for all holding periods. It is of further importance to note that kinkiness scores were in the range of wavy to twisted (3–5), giving further evidence that the hot-boned muscles had not undergone a substantial degree of shortening.

Subjective evaluation via the duo-trio test revealed that the judges were able to distinguish difference ($P < 0.05$) between the tenderness of hot and cold processed LD only at the 7-hr holding period (Table 5). As shown in Table 6, rank analysis of preference frequency showed no statistical difference in preference between the two processes at the 3 and 7-hr holding periods. A significant difference ($P < 0.05$) was indicated for the LD at the 5-hr holding period as the panelists preferred the cold-boned sample 62% of the time whereas the hot-boned muscle was preferred 38% of the time. Hot-boned LD was

Table 1—Shear values (kg) of hot vs cold-boned LD, SM and ST muscles

Muscle	Holding period[a] (hr)	Mean "hot" shear force	Mean "cold" shear force	Std. error of treatment mean
LD	3	7.46	6.86	0.21
	5	6.92	6.10*	0.20
	7	6.62	6.50	0.21
SM	3	9.40	8.91	0.20
	5	9.08	8.75	0.15
	7	9.72	8.96*	0.21
ST	3	9.83	9.48	0.15
	5	9.63	9.62	0.12
	7	10.18	9.87	0.14

[a] Postmortem holding period for hot-boned side
* Significant difference ($P < 0.05$) between hot- and cold-boned sides

Table 2—Sarcomere length (μm) in hot vs cold-boned LD

Holding period[a] (hr)	Mean "hot" sarcomere length	Mean "cold" sarcomere length	Std. error of treatment mean
3	2.04	2.15	0.05
5	2.00	2.06	0.02
7	2.14	2.25	0.03

[a] Postmortem holding period for hot-boned side

Table 3—Fiber diameter (μm) in hot vs cold-boned LD, SM and ST muscles

Muscle	Holding period[a] (hr)	Mean "hot" fiber diameter	Mean "cold" fiber diameter	Std. error of treatment mean
LD	3	69.84	63.72*	1.52
	5	67.16	63.52	1.34
	7	66.04	64.48	1.88
SM	3	58.00	62.00*	0.69
	5	61.16	62.24	1.48
	7	60.04	61.36	1.09
ST	3	65.12	58.64*	1.64
	5	61.00	59.48	2.05
	7	54.88	54.76	1.28

[a] Postmortem holding period for hot-boned side
* Significant difference ($P < 0.05$) between hot- and cold-boned sides

Table 4—Kinkiness score in hot vs cold-boned LD, SM and ST

Muscle	Holding period[a]	Mean "hot" kinkiness score	Mean "cold" kinkiness score	Std. error of treatment mean
LD	3	4.90	3.54*	0.35
	5	5.16	3.52*	0.33
	7	4.46	3.18*	0.28
SM	3	3.98	4.66	0.29
	5	4.38	4.30	0.27
	7	4.47	4.91	0.25
ST	3	3.00	3.62	0.26
	5	3.01	3.63	0.27
	7	3.27	3.88	0.28

[a] Postmortem holding period for hot-boned side
* Significant difference ($P < 0.05$) between hot- and cold-boned steaks

Table 5—Paired comparison analyses for the LD and SM muscles

Muscle	Holding period[a] (hr)	Total number of paired comparisons	Total number identifying pair
LD	3	120	63
	5	120	62
	7	120	73*
SM	3	120	60
	5	120	51
	7	120	59

[a] Postmortem holding period for hot-boned side
* Significant difference ($P < 0.05$) between hot- and cold-boned steaks

slightly preferred (52%) to the 48-hr control at the 7-hr holding period. Differences in the frequency of assigning one process or higher level of acceptability over another were nonsignificant ($P > 0.05$) at the 5 and 7-hr holding periods (Table 7), but a significant difference ($P < 0.05$) did occur at the 3-hr holding period as the hot-boned LD was given a higher level of acceptability 41% of the time. Comparison of Table 1 with Tables 6 and 7 illustrates that the subjective tenderness evaluation of the LD mirrored the shear force analysis. As may be noted, the hot-boned muscle showed a slight decrease in both preference and acceptability comparing the 3 and 7-hr holding periods to the 5-hr holding period. The author questions the significance of the duo-trio test at the 7-hr holding period (Table 5) because of the small differences in actual hedonic scores and preference ranking between hot and cold LD (Tables 6 and 7). Significant differences in acceptability and preference scores at the 3 and 5-hr holding periods (Tables 6 and 7), respectively, were greatly diminished by the fact that the judges could not differentiate between the two processes in the duo-trio test. This statement is supported by the similarity in actual hedonic scores. Therefore, the apparent lack of agreement of the duo-trio test with the preference and acceptability analyses was further indication that the differences in tenderness within steaks of a given process were no greater than differences between processes.

The duo-trio test for the SM (Table 5) revealed that the judges could not distinguish differences ($P > 0.05$) in tenderness between processes at any holding period. Rank analysis (Table 6) showed a greater preference frequency (59% vs 51%) for the cold-boned muscle as compared to the hot-boned SM at 3 hr; however, the difference in frequency was nonsignificant ($P > 0.05$) at the 5 and 7-hr holding periods. At all holding periods, panelists assigned a higher level of acceptability (Table 7) to the cold-boned sample more frequently than to the SM which was hot boned. As may be noted, actual hedonic scores averaged slightly acceptable at all holding periods. Comparison of Table 1 with Tables 6 and 7 again illustrates that the subjective evaluation mirrored the objective analysis by Warner-Bratzler shear. No significant interactions ($P > 0.05$) were noted with respect to sampling location in either the LD or SM muscles. The somewhat low levels of acceptability, with particular reference to the LD, were due to the fact that the meat was not aged. The results of the tenderness panel further reinforced the slight differences exhibited by Warner-Bratzler shear and the histological evaluation were of little practical importance. It is generally accepted that a minimum difference of at least 0.5 kg or more (Khan et al., 1973) must exist in shear force before detection is possible by sensory panel evaluation. Furthermore, Sharrah et al. (1965) postulated that sensory discrimination among samples was more acute within a lower range of shear force values than within a higher range.

The feasibility of hot boning bovine muscle as early as 3 hr postmortem was further supported by both the pH and temperature data. As shown in Figure 1, the pH of hot-boned psoas major (PM) was always lower from the 2 through the 24-hr measurement period as compared with the 48-hr control. The somewhat more rapid rate of pH decline was a result of conditioning the hot side in a 16°C cooler, allowing the muscles to maintain their temperature (37°C) for a longer period of time.

As may be noted in Figure 1, the pH curves of hot and cold-boned sides at the 3-hr holding period indicated that the rate of pH decline was somewhat greater from 1–3 hr postmortem as compared to the same time period for sides assigned to either the 5- or 7-hr conditioning period. However, from 3–48 hr postmortem, only minor variations in pH among

Table 6—Preference rank analyses for the LD and SM muscles

Muscle	Holding period[a] (hr)	Mean rank of hot-boned steaks[b]	Mean rank of cold-boned steaks[b]
LD	3	1.40	1.60
	5	1.38	1.62*
	7	1.52	1.48
SM	3	1.41	1.59*
	5	1.46	1.54
	7	1.47	1.53

[a] Postmortem holding period for hot-boned side
[b] Larger value denotes increased preference
* Significant difference ($P < 0.05$) between hot- and cold-boned steaks

Table 7—Hedonic scale score rank analyses for the LD and SM muscles

Muscle	Holding period[a] (hr)	Mean "hot" hedonic score[b]	Mean "cold" hedonic score[b]	Mean "hot" ranked score[b]	Mean "cold" ranked score
LD	3	4.14	4.42	1.41	1.59*
	5	4.42	4.68	1.41	1.59
	7	4.47	4.41	1.52	1.48
SM	3	3.96	4.08	1.46	1.54
	5	3.91	4.19	1.42	1.58
	7	3.76	4.07	1.43	1.57

[a] Postmortem holding period for hot-boned side
[b] Larger value denotes increased preference
* Significant difference ($P < 0.05$) between hot- and cold-boned steaks

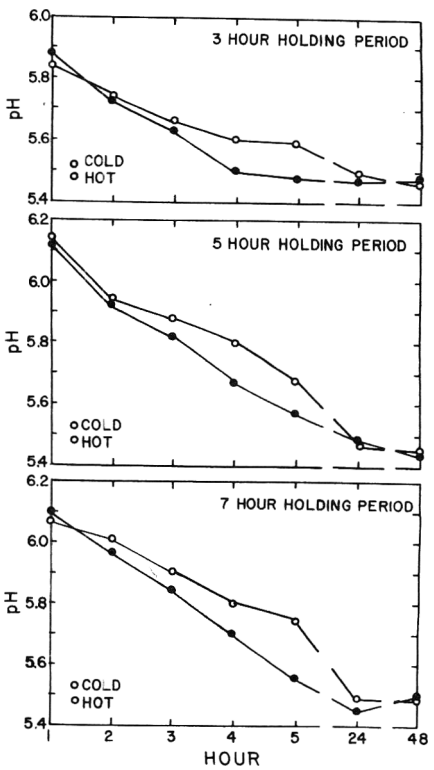


Fig. 1—pH decline in hot vs cold-boned psoas major at the 3, 5 and 7-hr holding period.

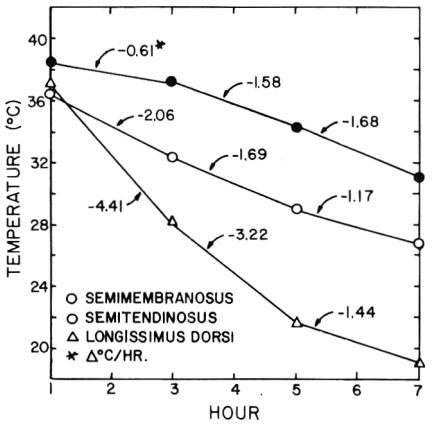


Fig. 2—Temperature decline of test muscles in the 7-hr hot-boned side.

conditioning periods occurred. Mean pH for all hot sides at 3 hr postmortem was 5.76 ± 0.07.

Figure 2 revealed that both the LD and ST muscles had attained their maximum rates of temperature decline from 1–3 hr postmortem, whereas, the SM muscle showed almost no temperature decrease during this interval. Considering the mass and location of the three muscles studied, one would expect the most rapid temperature decline in the LD, followed by the ST, and lastly the SM. These data gain importance when one considers that postmortem glycolysis will result in substantial heat production and therefore, one would not expect maximal decreases in temperature until there was a concomitant decrease in glycolytic rate, as demonstrated by the pH data in Figure 1. Although only temperature data from the 7-hr holding period are presented, similar trends were followed in the test muscles from both the 3 and 5-hr conditioning periods.

These data provide evidence that the hot-boned muscle had begun to proceed into the onset phase of rigor mortis before excision was initiated. Furthermore, there does not appear to be a requisite for muscle to attain its ultimate pH prior to excision in order to minimize the extensive muscle shortening which is deleterious to tenderness. Bouton et al. (1972) pointed to the fact that with the many conflicting factors which may influence tenderness in "normal animals" having ultimate pH values of less than 6.0, it would be surprising that there were always a direct relationship between pH and tenderness. There does, however, seem to be the requirement for muscle to have just begun to proceed into the onset phase of rigor mortis before being boned. This point may be illustrated by Kastner et al. (1973) who found that muscle boned at 2 hr postmortem was still in the delay phase and therefore shortened and toughened appreciably because of the lack of restrainment during the initial stages of the onset phase. Thus, the problems incurred by fabricating hot carcasses at 2 hr postmortem may be overcome by restraining the muscle on the carcass for an additional hour before boning.

CONCLUSIONS

RESULTS from both the Warner-Bratzler shear and histological evaluation of tenderness demonstrated that only very minor changes in contraction state are to be anticipated if muscle is treated as described in this report. Furthermore, the small differences in contraction state resulted in minor differences in tenderness when evaluated by sensory panel. One must also consider that no attempt was made to age the hot-boned muscle. This fact becomes particularly important when one realizes that fresh meat normally requires 7–10 days to pass through distribution channels and may have been tenderized mechanically. In view of these facts, the slight differences in tenderness between muscle which is hot boned at 3 hr postmortem and that which is allowed to remain on the suspended carcass for 48 hr are not practically significant.

REFERENCES

Amerine, M.A., Pangborn, R.M. and Roessler, E.B. 1965. "Principles of Sensory Evaluation of Food," p. 333. Academic Press, New York.
Bouton, P.E., Carroll, F.D., Fisher, A.L., Harris, P.V. and Shorthose, W.R. 1973. Effect of altering ultimate pH on bovine muscle tenderness. J. Food Sci. 38: 816.
Brasington, C.F. and Hammons, D.R. 1971. Boning carcass beef on the rail. ARS 52—63 U.S. Dept. of Agriculture.
Busch, W.A., Goll, D.E. and Parrish, F.C. Jr. 1972. Molecular properties of postmortem muscle. Isometric tension and decline in bovine, porcine and rabbit muscle. J. Food Sci. 37: 289.
Cagle, E.D. and Henrickson, R.L. 1970. Influence of slicing warm porcine muscle on fiber diameter, kinkiness and shear force. J. Food Sci. 35: 270.
Conover, W.J. 1971. "Practical Nonparametric Statistics," p. 264. John Wiley & Son, Inc., New York.
Falk, S.N. 1974. Feasibility of "hot" processing the bovine carcass. Ph.D. thesis, Oklahoma State University, Stillwater, Okla.
Herring, H.K., Cassens, R.G. and Briskey, E.J. 1965. Further studies on bovine muscle tenderness as influenced by carcass position, sarcomere length, and fiber diameter. J. Food Sci. 30: 1049:
Hosteler, R.L., Landmann, W.A., Link, B.A. and Fitzhugh, H.A. Jr. 1970. Influence of carcass position during rigor mortis on tenderness of beef muscles: Comparison of two treatments. J. Animal Sci. 31: 47.
Hostetler, R.L., Link, B.A., Landmann, W.A. and Fitzhugh, H.A. Jr. 1972. Effect of carcass suspension on sarcomere length and shear force of some major bovine muscles. J. Food Sci. 37: 132.
Hostetler, R.L., Link, B.A., Landmann, W.A. and Fitzhugh, H.A. Jr. 1973. Effect of carcass suspension on sensory panel scores from some major bovine muscles. J. Food Sci. 38: 264.
Jungk, R.A., Snyder, H.E., Goll, D.E. and McConnell, ??. 1967. Isometric tension changes and shortening in muscle strips during postmortem ageing. J. Food Sci. 32: 158.
Kastner, C.L. and Henrickson, R.L. 1969. Providing uniform meat cores for mechanical shear force measurement. J. Food Sci. 34: 603.
Kastner, C.L., Henrickson, R.L. and Morrison, R.D. 1973. Characteristics of hot boned bovine muscle. J. Animal Sci. 36: 484.
Khan, A.W., Lentz, C.P. and van den Berg, L. 1973. Relation between shear force and tenderness of beef. J. Food Sci. 38: 1258.
Kramer, A. and Twigg, B.A. 1966. "Fundamentals of Quality Control for the Food Industry," p. 141. Avi Publishing Co., Westport, Conn.
Marsh, B.B. 1954. Rigor mortis in beef. J. Food Sci. Agr. 2: 70.
Marsh, B.B. and Leet, N.G. 1966. Studies in meat tenderness. 3. The effects of cold shortening on tenderness. J. Food Sci. 31: 450.
Paul, P.C. and Bratzler, L.J. 1955. Studies on tenderness of beef. 3. Size of shear cores: End-to-end variation in the semimembranosus and adductor. Food Res. 20: 635.
Schmidt, G.R. and Gilbert, K.V. 1970. The effect of muscle excision before the onset of rigor mortis on the palatability of beef. J. Food Technol. 5: 331.
Schmidt, G.R. and Keman, S. 1974. Hot boning and vacuum packaging of eight major bovine muscles. J. Food Sci. 39: 141.
Service, J. 1972. "A User's Guide to the Statistical Analysis System." Students Supply Stores, North Carolina State University, Raleigh, NC.
Sharrah, N., Kunze, M.S. and Pangborn, R.M. 1965. Beef tenderness: Comparison of sensory methods with the Warner-Bratzler and L.E.E.-Kramer shear presses. Food Technol. 19: 238.
Ms received 1/4/75; revised 3/21/75; accepted 3/22/75.

Journal Series Paper No. 1941 of the Oklahoma Agricultural Experiment Station. Supported in part by the Meat Science Research Laboratory, ARS, USDA, Cooperative Agreement 12-14-100-10, 867 (51).

PARIMAL CHATTOPADHYAY, B. C. RAYCHAUDHURI[1] and A. N. BOSE[2]
Dept. of Food Technology & Biochemical Engineering, Jadavpur University, Calcutta 700 032, India

PREDICTION OF TEMPERATURE OF ICED FISH

INTRODUCTION

THE MAXIMUM TIME of transport of iced fish in India varies from 72–80 hr. The temperature of fish should not exceed 5°C during the period of transport. In a tropical country like India where the ambient temperature may be as high as 40°C during summer, it becomes extremely difficult to transport fish at 5°C without using large quantities of ice or re-icing the consignment at intermediate points enroute (Chattopadhyay et al., 1974a, b). For effective and economical utilization of ice, it is necessary to know the time required for a quantity of ice to melt as well as the temperature of the fish kept inside a container. Automatic recording of temperature by means of thermocouples positioned at different points inside a container is a difficult task when fish is transported in nonrefrigerated rail wagon along with other commodities.

Numerous methods are available for predicting food temperatures during various processing treatments (Charm, 1961, 1963; Hayakawa, 1971, 1972; Hayakawa and Ball, 1971; Kopelman and Pflug, 1968; Lenz and Lund, 1973; Pflug et al., 1965). Analytical solutions are also available in the field of heat conduction but these are mostly relevant to simpler problems (Arpaci, 1966; Carslaw and Jaeger, 1959; Luikov, 1968). Transient temperatures are also predicted from Laplace transformation (Lund et al., 1972) and by numerical methods (Wadsworth and Spadaro, 1970). But no attempt has yet been made to predict the fish temperature in ice and fish-packed container.

It has thus been found necessary to derive a computational technique for the determination of time of melting of ice and subsequent rise of fish temperature in iced fish containers to ascertain the total time required for the fish at 0°C to rise over 5°C during transportation. This problem may be divided into two parts: (1) Ascertaining the time taken for the ice at 0°C to melt completely assuming the fish temperature remains constant at 0°C during the entire period of melting of ice; and (2) Ascertaining the additional time taken for the fish to rise from 0°C to 5°C after the complete melting of the ice.

In this investigation, (a) an error function solution of inverse Laplace transformation is used to determine the time required for the melting of the ice and (b) a numerical method was used to determine the temperature rise of fish in the container after the melting of ice is complete, to ascertain the time required for the fish to rise to 5°C.

Development of computational procedure

The problem, although, a three dimensional one, is considered in this paper, for simplicity, as one dimensional assuming a symmetrical central plane.

Further, heat flow has been assumed to take place in a two-layer composite: (a) one layer of insulation and another layer of ice in constant contact for the determination of time required for complete melting of ice; (b) one layer of insulation and one layer of fish in constant contact (considering the same as a solid layer) while determining the temperature rise in fish after melting of ice. The following assumptions were also made: (a) in melting, ice shifts to the rear, and (b) that water from the molten ice has completely drained off.

Time required for complete melting of ice used in a fish container

The heat conduction equation in nondimensional form to be solved is:

$$\frac{\partial \theta'_1}{\partial t'} = \alpha'_1 \frac{\partial^2 \theta'_1}{\partial x'^2}$$

where $\theta'_1 = \frac{\theta_1}{V}$, $t' = \frac{t}{T}$, $x' = \frac{x}{L}$ and $\alpha'_1 = \frac{\alpha_1 T}{L^2}$

Let us consider the section of the fish container represented by Figure 1.

The initial condition is,

$$\theta'_1 = \theta'_2 = 0 \text{ at } 0 \leq x' \leq \frac{l_2}{L} \text{ and } t' \leq 0$$

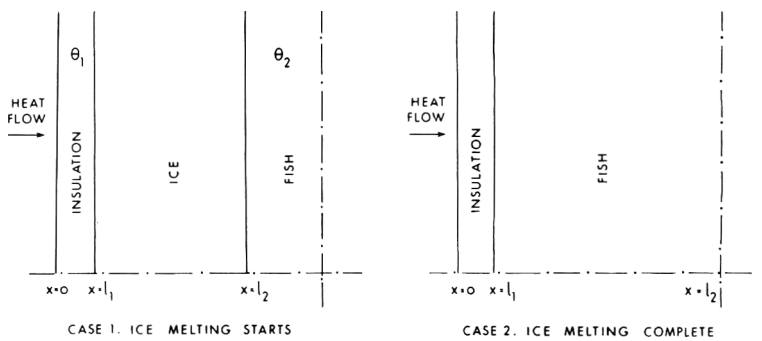


Fig. 1–One dimensional heat flow in fish container packed with ice and fish.

[1] Central Mechanical Engineering Research Institute, Durgapur 713 209, India
[2] Vice-Chancellor, Jadavpur University, Calcutta 700 032, India

where, $\theta'_2 = \frac{\theta_2}{V}$. Boundary conditions are:

$\theta'_1 = 1$ at $x' = 0$ and $t' > 0$ as h_s is very large

$\theta'_1 = \theta'_2$ at $x' = \frac{\ell_1}{L}$ and $t' > 0$

$\theta'_2 = 0$ at $\frac{\ell_1}{L} \leqslant x' \leqslant \frac{\ell_2}{L}$ and $t' > 0$

and $-\int_0^{t'} K_1 \frac{\partial \theta'_1}{\partial x'} dt' = \frac{L_T \rho L^2}{VT} \int_0^{t'} \frac{\partial \epsilon'}{\partial t'} dt'$ at $x' = \frac{\ell_1}{L}$ and $t' > 0$

where, $\epsilon' = \frac{\epsilon}{L}$ and ϵ is the elementary width of the ice layer which has been melted in time dt. Now the heat conduction equation can be solved with the above initial and boundary conditions. The error function solution for inverse Laplace transformation may be applied (Carslaw and Jaeger, 1959) as:

$$\theta'_1 = \sum_{n=0}^{\infty} \operatorname{erfc}\left[\frac{(x' + \frac{2n\ell_1}{L})}{2\sqrt{\alpha'_1 t'}}\right] - \sum_{n=0}^{\infty} \operatorname{erfc}\left[\frac{(x' - \frac{2n\ell_1}{L})}{2\sqrt{\alpha'_1 t'}}\right] \quad (1)$$

The first derivative of Eq. (1) becomes:

$$\frac{\partial \theta'_1}{\partial x'} = \frac{2}{\sqrt{\pi}} \sum_{n=0}^{\infty} \left[e^{-\frac{(x' + \frac{2n\ell_1}{L})^2}{4\alpha'_1 t'}} - e^{-\frac{(x' - \frac{2n\ell_1}{L})^2}{4\alpha'_1 t'}} \right] \quad (2)$$

If a total heat flux $\int_0^t - K_1 \frac{\partial \theta}{\partial x} dt$ is required to melt completely a layer of ice with a thickness $(\ell_2 - \ell_1)$ and density ρ as shown in Figure 1, we can write,

$$-\int_0^t K_1 \frac{\partial \theta}{\partial x} dt = L_T (\ell_2 - \ell_1)\rho \quad (3)$$

where K_1 is the thermal conductivity of the insulated material. Again, writing the dimensionless variables as,

$$\theta'_1 = \frac{\theta_1}{V}, \; x' = \frac{x}{L} \text{ and } t' = \frac{t}{T}$$

we have,

$$\frac{\partial \theta}{\partial x} = \frac{V}{L} \cdot \frac{\partial \theta'_1}{\partial x'} \quad (4)$$

and

$$dt = T \, dt' \quad (5)$$

From Eq. (3), (4) and (5) we get,

$$-\int_0^{t'} \frac{\partial \theta'_1}{\partial x'} dt' = L_T (\ell_2 - \ell_1)\rho \left[\frac{L}{K_1 VT}\right] \quad (6)$$

From Eq. (2) and (6) we can write,

$$-\frac{2}{\sqrt{\pi}} \int_0^{t'} \sum_{n=0}^{\infty} \left[e^{-\frac{(x' + \frac{2n\ell_1}{L})^2}{4\alpha'_1 t'}} - e^{-\frac{(x' - \frac{2n\ell_1}{L})^2}{4\alpha'_1 t'}} \right] dt' = L_T (\ell_2 - \ell_1)\rho \left[\frac{L}{K_1 VT}\right] \quad (7)$$

The integral Eq. (7) was solved numerically for t' (or t, the time required for ice melting) using the trapezoidal rule in a Model IBM 1132 Computer (McCracken and Dorn, 1965; McCormick and Salvadori, 1964). A subroutine was prepared to represent the error function and a program was developed to carry out the summation of the left-hand side of Eq. (7). All factors on the right-hand side of Eq. (7) were available from the thermo-physical properties of materials (Chattopadhyay et al., 1974a).

Temperature of fish after complete melting of ice

The basic one-dimensional heat conduction equation can be represented in the following finite difference form (Gerald, 1970) after neglecting the error terms,

$$\theta_{i+1,j} = \frac{\alpha_1 k}{h^2} (\theta_{i,j+1} + \theta_{i,j-1}) + (1 - \frac{2\alpha_2 k}{h^2})\theta_{i,j} \quad (8)$$

The solution for Eq. (8) can be obtained by imposing the following boundary conditions,

$$\theta_1 (\ell_1, t_i) = \theta_2 (\ell_1, t_i)$$

$$\theta_2 (x_j, t_0) = 0$$

$$\theta_2 (\ell_2 - h, t_i) = \theta_2 (\ell_2 + h, t_i)$$

where, t_0 = time taken for the complete melting of ice.

θ_1 and θ_2 denote the temperature in the insulation and fish layers, respectively (Fig. 1). A separate program was developed to solve Eq. (8) in a Model IBM 1132 computer as the time required for complete melting of ice is already determined from Eq. (7).

EXPERIMENTAL

A PLYWOOD BOX lined inside with 400 gauge polyethylene film, a plywood box lined inside with 1 cm thick expanded polystyrene (BASF India Ltd.) and a 3-ply moisture-proof double-walled corrugated fiberboard box were packed with ice and fish (*Tilapia mosambica*). Three different ice-to-fish ratios (1:1, 2:1 and 3:1 by weight) were used in this study. The dimensions of the experimental boxes were 30.48 cm × 30.48 cm × 30.48 cm. Fish was kept at the middle of the box and was surrounded by crushed ice on all sides. The thickness of ice layer was the same on all sides and varied depending upon the ice-to-fish ratio. All boxes were kept in a room maintained at 31°C, and the time required for complete melting of ice was noted. Triplicate experiments were conducted with each box using a particular ice-to-fish ratio.

The fish temperature along the symmetrical central plane of these containers after complete melting of ice was recorded by a Negretti and Zambra automatic recorder (Bestobell India Ltd.) using an ice-to-fish ratio of 1:1 by weight and following the icing process as described earlier. The temperature sensor was placed at the geometric center of the container. Triplicate experiments were conducted with each container.

The procedures for the determination of the values of the thermophysical constants of the materials are described in our earlier work (Chattopadhyay et al., 1974a).

RESULTS & DISCUSSION

THE NUMBERICAL VALUES of the physical constants and boundary conditions used for solving Eq. (7) and (8) are given in Table 1. The ambient temperature (V) was 31°C and the latent heat of fusion of ice (L_T) was taken as 80 Kcal/kg.

The accuracy of the solution of the integral Eq. (7) by the numerical method depends on the numerical magnitude of x' and t'. After careful examination of the experimental results it was found that the desired accuracy can be obtained if both x' and t' are made equal to unity or, in other words, $x' = \frac{\ell_1}{L} = 1$

and $t' = \frac{t}{T} = 1$. The values, close to the experimental results, have to be assumed for L and T, so that $\ell_1 = L$ and $t = T$. It was observed that the summation in Eq. (7) can be carried out within the values of n = 1–5 in order to obtain a finite solution with reasonable accuracy. Eq. (7) was solved numerically in this study. The three experimental values of t (time required for ice melting) for different ice-to-fish ratios used in different containers are given in Table 2. The differences in experimental and predicted values, although insignificant, were ob-

Table 1–Numerical values of the physical constants and boundary conditions used in the mathematical models

Material	Thickness (ℓ) (m)	Thermal conductivity (K) (Kcal/m hr °C)	Density (ρ) (kg/m^3)	Specific heat (Cp) (Kcal/kg °C)	Thermal diffusity (α) (m^2/hr)
Plywood[a]	0.0040	0.0376	480	0.65	1.20×10^{-4}
Polyethylene[a]	0.0001	0.0280	940	0.55	0.41×10^{-4}
Expanded polystyrene[a]	0.0100	0.0280	15	0.24	77.00×10^{-4}
3-ply moisture proof corrugated board[a]	0.0032	0.0544	310	0.32	5.50×10^{-4}
Fish[b]	c	0.4740	842	0.80	1.50×10^{-4}
Ice[d]	c	0.9100	916	0.49	42.54×10^{-4}

[a] Chattopadhyay et al. (1974a)
[b] Cutting (1969)
[c] Thickness of ice and fish layers depends upon icing technique, ratio of ice to fish and dimension of the container.
[d] FAO (1968).

Table 2–Time required for melting of entire amount of ice used in different fish containers at 31°C

Container no.	Material	Container description cubic: 30.48 cm X 30.48 cm X 30.48 cm	Run no.	Ratio of ice to fish	Thickness of ice layer (m)	Thickness of fish layer (m)	Time required for ice melting (hr)	
							Experimental	Predicted from Eq. 7
1	Plywood	Plywood box lined inside with polyethylene film	1	1:1	0.09	0.06	39.5 40.0 40.5	38
			2	2:1	0.12	0.03	50.0 52.5 53.5	50
			3	3:1	0.14	0.01	60.0 59.0 61.0	59
2	Plywood	Plywood box lined inside with 1 cm thick expanded polystyrene in polyethylene bag	1	1:1	0.09	0.06	64.0 63.5 64.5	61
			2	2:1	0.12	0.03	83.5 84.5 84.0	82
			3	3:1	0.14	0.01	96.0 96.0 96.0	93
3	Paper	3-ply double walled corrugated moisture-proof board with wood/wool insulation in between two walls	1	1:1	0.09	0.06	50.5 49.5 50.0	49
			2	2:1	0.12	0.03	63.5 64.0 64.5	62
			3	3:1	0.14	0.01	75.0 75.0 75.0	74

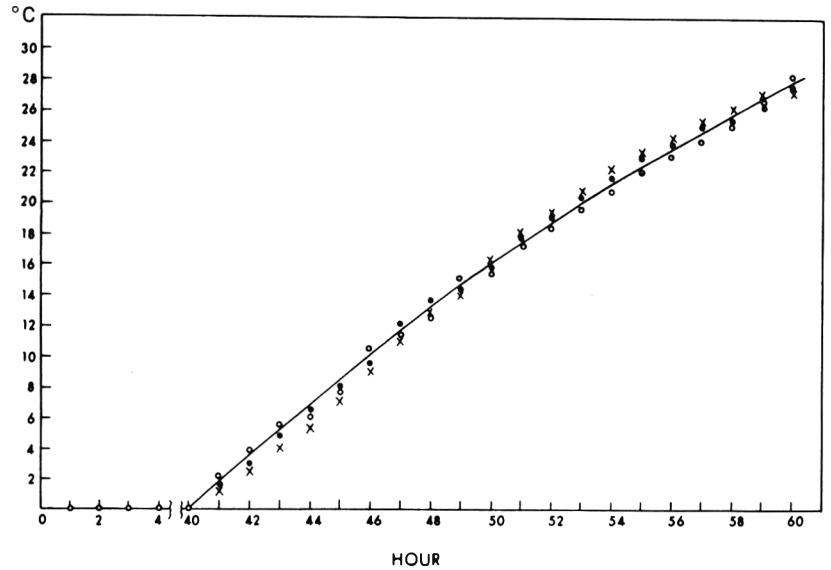


Fig. 2—Temperature history curve of iced fish in container 1 (Run no. 1) after complete melting of ice. Line represents predicted values; open circles, closed circles and crosses represent experimental results of triplicate experiments. Ambient temperature = 31°C.

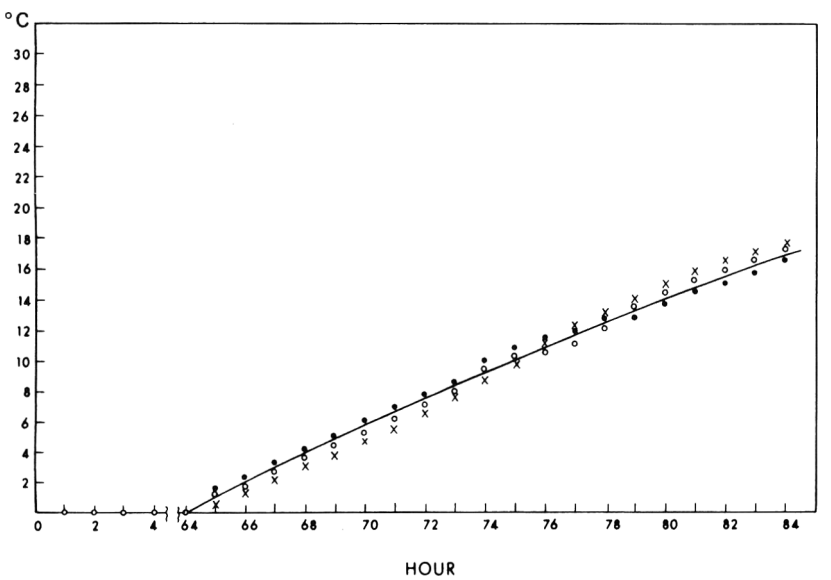


Fig. 3—Temperature history curve of iced fish in container 2 (Run no. 1) after complete melting of ice. Line represents predicted values; open circles, closed circles and crosses represent experimental results of triplicate experiments. Ambient temperature = 31°C.

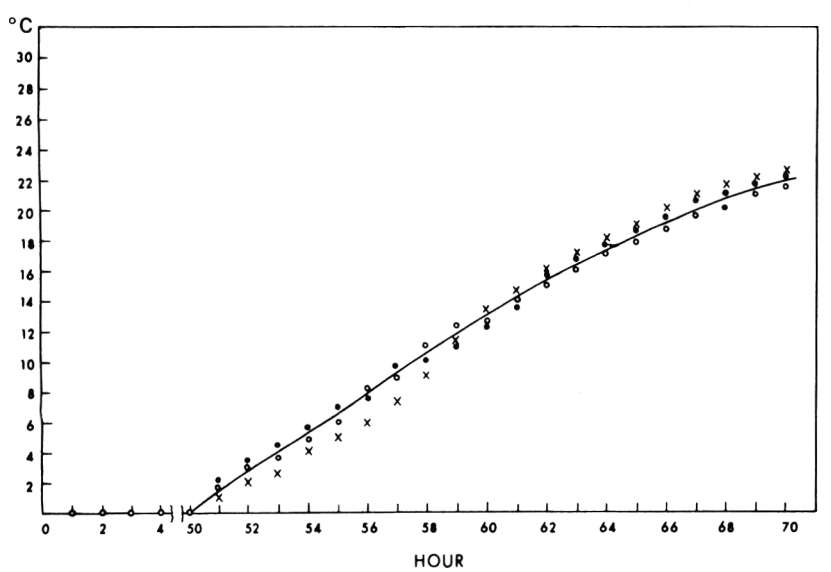


Fig. 4—Temperature history curve of iced fish in container 3 (Run no. 1) after complete melting of ice. Line represents predicted values; open circles, closed circles and crosses represent experimental results of triplicate experiments. Ambient temperature = 31°C.

served as the summation in Eq. (7) was carried out within the values of n = 1–5. It may be noted here that the thicknesses of ice and fish layers depend on the ratio of ice to fish as well as on the dimension of the fish container.

If all the conditions are symmetrical around the mid-plane, a one dimensional heat flow condition may be assumed. The fish temperatures along the symmetrical central plane after melting of ice, were determined from Eq. (8) at various time intervals. The most suitable mesh length was found to be h = 0.01 and k = 0.0001. The computer-predicted temperature values are compared with triplicate experimental values in Figures 2 to 4. It can be seen that for most of the cases there is reasonable agreement between the experimental and predicted temperature values (these two values became almost equal during the 10th and 11th hour).

When all sides of the experimental (cubical) box are exposed to the same external excitations, it would possibly cause the ice layer to melt uniformly from all sides provided the effect of edges and corners are neglected. Complete melting will be necessary to cause any rise in the fish temperature which can be computed by the method just described. But if the sides are dissimilar or have different excitations, then the depth of melting of ice layer in different walls will be different. In that case the fish temperature may start rising from the one side where ice layer has been completely melted.

CONCLUSIONS

ANALYTICAL METHODS can be applied to predict the time required for complete melting of ice used to transport iced food materials. A numerical technique can be used to predict the temperature history of the food material to ascertain the deterioration in quality due to a rise in temperature during transportation. If the thermo-physical properties and boundary conditions are known, these methods can be applied to containers of any dimension, capacity or material with reasonable accuracy.

NOMENCLATURE

V	outside air temperature (°C)
θ'	dimensionless temperature
T	representative time (hr)
t	time required for complete melting of ice (hr)
t'	dimensionless time
L	representative thickness (m)
x'	dimensionless thickness
ℓ_1	actual thickness (m)
α'	$= \frac{\alpha T}{L^2}$ = dimensionless thermal diffusivity
α	$= K/\rho c_p$ = thermal diffusivity (m^2/hr)
ρ	density (kg/m^3)
C_p	specific heat (Kcal/kg °C)
K	thermal conductivity (Kcal/m hr °C)
L_T	latent heat of fusion of ice (Kcal/kg)
i	time index
j	position index
θ_1	temperature in container wall (°C)
θ_2	temperature in fish layer (°C)
h	spacing for variable "x"
ϵ	elementary width of the ice layer (m)
k	spacing for variable "t"
h_s	surface heat transfer coefficient (Kcal/m^2 hr °C)

REFERENCES

Arpaci, V.S. 1966. "Conduction Heat Transfer." Addison-Wesley Publishing Co., Reading, Mass.

Carslaw, H.S. and Jaeger, J.C. 1959. "Conduction of Heat in Solids." Oxford University Press, London.

Charm, S.E. 1961. A method for calculating the temperature distribution and mass average temperature in conduction-heated canned foods during water cooling. Food Technol. 16: 248.

Charm, S.E. 1963. "The Fundamentals of Food Engineering." The Avi Publishing Co., Inc., Westport, Conn.

Chattopadhyay, P., Raychaudhuri, B.C. and Bose, A.N. 1974a. Computation of insulation efficiencies of fish transport containers. J. Food Sci. 39: 1006.

Chattopadhyay, P., Raychaudhuri, B.C. and Bose, A.N. 1974b. Computer simulation of thermal characteristics of fish containers. Presented at the Fourth International Congress of Food Science & Technology, September 22–27, at Madrid, Spain.

Cutting, C.L. 1969. Fish processing. In "Food Industries Manual," 20th ed, p. 230. Leonard Hill. London.

FAO. 1968. Ice in fisheries. Fish Rep. (59): 68.

Gerald, C.F. 1970. "Applied Numerical Analysis." Addison-Wesley Publishing Co., California.

Hayakawa, K. 1970. Experimental formulas for accurate estimation of transient temperature of food and their application to thermal process evaluation. Food Technol. 24: 1407.

Hayakawa, K. 1971. Estimating food temperatures during various processing or handling treatments. J. Food Sci. 36: 378.

Hayakawa, K. and Ball, C.O. 1971. Theoretical formulas for temperatures in cans of solid food and for evaluating various heat processes. J. Food Sci. 36: 306.

Hayakawa, K. 1972. Estimating temperatures of food during various heating or cooling treatments. ASHRAE Journal 14(9): 65.

Kopelman, I.J. and Pflug, I.J. 1968. The relationship of the surface, mass average and geometric center temperatures in transient conduction heat flow. Food Technol. 22(6): 141.

Lenz, M.K. and Lund, D.B. 1973. Temperature distribution during heat/hold processing of food. J. Food Sci. 38: 630.

Luikov, A.V. 1968. "Analytical Heat Conduction Theory." Academic Press, Inc., New York.

Lund, D.B., Bruin, S. Jr. and Lazar, M.E. 1972. Internal temperature distribution during individual quick blanching. J. Food Sci. 37: 167.

McCormick, J.M. and Salvadori, M.G. 1964. "Numerical Methods in FORTRAN." Prentice Hall, N.J.

McCracken, D.D. and Dorn, W.S. 1965. "Numerical Methods and FORTRAN Programming." John Wiley & Sons, New York.

Pflug, I.J., Blaisdell, J.L. and Kopelman, I.J. 1965. Developing temperature time curves for objects that can be approximated by a sphere, infinite plate, or infinite cylinder. ASHRAE Trans. 71: 238.

Wadsworth, J.I. and Spadaro, J.J. 1970. Transient temperature distribution in whole sweet potato roots during immersion heating. 2. Computer simulation. Food Technol. 24: 913.

Ms received 1/15/75; revised 4/19/75; accepted 4/22/75.

This investigation was supported financially by the Indian Council of Agricultural Research, New Delhi. The authors thank Dr. S.K. Majumdar and Mr. M.K. Roy, Computer Centre, Jadavpur University, for their assistance.

JFS RESEARCH NOTES

M. W. BENSON, R. H. KURTZMAN JR., W. U. HALBROOK and R. M. McCREADY
USDA Western Regional Research Laboratory, ARS, Berkeley, CA 94710

A Research Note
AFLATOXIN PRODUCTION ON SOME FEEDS AND FOODS

INTRODUCTION

TESTS for aflatoxin production have been reported on some cereal grains and oil seed commodities which are known to be hosts of *Aspergillus flavus* Link (Armbrecht et al., 1963; Mayne et al., 1966), and on forages (Hesseltine et al., 1968; Smalley et al., 1972). *A. flavus* was reported to infect pecans (Lillard et al., 1970), corn (Koehler and Woodworth, 1938), seeds of *Citrus* spp. (Durbin, 1959) and *Phaseolus* spp. (Seymore, 1929). Borker et al. (1966) reported corn, peas and potatoes samples have been found contaminated with aflatoxin. *A. flavus* might grow occasionally on foods and feeds, therefore, its growth and production of aflatoxins on some selected hydrated commodities was investigated.

EXPERIMENTAL

Growth of *Aspergillus flavus*

Meals, pulps and some other materials, 25g of solid commodity was mixed with 50 ml of water, or 40g of fresh or frozen commodity was mixed with 40 ml of water and sterilized by autoclaving for 20 min at 15 psi. After cooling, the sterile hydrated materials were inoculated with an FDA isolate of *A. flavus* and allowed to grow for up to 21 days at 25°C.

Dried pasteurized whole eggs and whites were placed directly into sterile petri dishes with sterile distilled water, and then inoculated.

Replicates of cultures of feed meals which would not support the production of aflatoxins by the FDA isolate of *A. flavus* were inoculated with a second aflatoxin-producing strain of *A. flavus* to see if the lack of production was due to the one isolate.

Assay of cultures for aflatoxins (Wiley, 1966)

Cultures were extracted with 70% acetone-water, filtered, treated with neutral lead acetate and centrifuged. Aflatoxins were extracted with chloroform, dried with anhydrous sodium sulfate and evaporated to dryness. The residue was transferred with chloroform to a vial evaporated under nitrogen and diluted to 100 µl with chloroform. Half the aflatoxin-extract was spotted across one thin-layer chromatography plate (1:1 silica gel and cellulose) along with an internal standard (sample and aflatoxin spotted together, the aflatoxin standard was obtained from Dr. L. Goldblatt, Southern Regional Research Laboratory, USDA, New Orleans, La.).

The TLC plates were developed in anhydrous methyl acetate, dried, viewed under UV light and marked. The band even with the standard aflatoxin (B_1) was scraped off, eluted with acetone, dried, extracted with chloroform, dried and dissolved in 50 µl chloroform. Ten µl of this solution (sample), an internal standard and standards were spotted on a TLC plate and developed in 2% v/v methanol chloroform. The aflatoxin spots viewed under UV illumination were estimated nearest to the appropriate standard of 50, 250 and 500 ppb.

Confirmation of aflatoxin

The production of aflatoxin B_1 by the isolate of *A. flavus* used was confirmed chemically with acetic acid and thionyl chloride, and with formic acid and thionyl chloride. The products using TLC were visually identical with those obtained by reacting authentic B_1 in the same way (Andrellos and Reid, 1964).

Table 1—Growth of Aspergillus flavus and production of aflatoxin on hydrated, autoclaved commodities

Commodity	Days incubated	Growth	Aflatoxin (ppb)
Alfalfa, meal	15	+++	0
Apples, dried	18	+++	50
Apricots, dried	16	++	50
Castor, meal	14	+++	0
Celery, sliced	11	+++	50
Dates, dried	8	+++	250
Egg White, dried[a]	19	+	50
Egg, whole, dried[a]	12	++	250
Figs, dried	8	+++	50
Onion, sliced	7	+++	250
Peaches, dried	16	+++	50
Pecans, chopped	8	+++	50
Potatoes, sliced	8	+++	500
Prunes, dried	21	+++	50
Raisins, dried	12	++	50
Split Peas, dried	5	+++	500
Sugar Beets, ground	18	+++	250

[a] Egg samples were pasteurized and not autoclaved.

RESULTS & DISCUSSION

SELECTED COMMODITIES such as seeds, grains and pieces were surface sterilized by soaking in 2% hydrogen peroxide–1% ethanol for 2 min, washed, 20% water added and inoculated with *A. flavus*. The experiments are not reported here in table form because poor growth of *A. flavus* occurred probably because insufficient water was used for hydration in some cases and because some unidentified phycomycetes outgrew the *A. flavus*. When *A. flavus* grew in a period of 8 days, nuts, grains and fresh vegetables showed high contamination with aflatoxin. When the commodities tested were autoclaved, sterilization was complete and the materials were uniformly hydrated.

The results in Table 1 show hydrated commodities tested here contain nutrients which support the growth of *A. flavus* and aflatoxin is produced. In the case of alfalfa and castor meal growth of *A. flavus* occurred but the medium became alkaline. No aflatoxin was extracted from the alkaline medium. When the medium was acidified with mineral acid to pH 3 or below, condition where the lactone ring would form, no aflatoxin could be extracted from the acidified medium.

Most of the hydrated, sterilized foods and feeds tested here contain nutrients to support the growth of *A. flavus* and produce aflatoxin. However, modern food handling and processing procedures do not include storage of high moisture foods except at freezing temperatures. Only in exceptional or accidental isolated instances would danger of aflatoxin contamination of foods be likely.

REFERENCES

Andrellos, P.J. and Reid, G.R. 1964. Confirmatory tests for aflatoxin B_1. J. Assoc. Off. Agr. Chem. 47: 805.

Armbrecht, B.H., Hodges, F.A., Smith, H.R. and Nelson, A.A. 1963. Mycotoxins. 1. Studies on aflatoxin derived from contaminated peanut meal and certain strains of Aspergillus flavus. J. Assoc. Off. Agr. Chem. 46: 805.

Borker, E., Insalata, N.F., Levi, C.P. and Witzeman, J.S. 1966. Mycotoxins in feed and foods. Adv. Appl. Microbiol. 8: 315.

Durbin, R.D. 1959. The possible relationship between Aspergillus flavus and albanism in citrus. Plant Disease Reptr. 43: 922.

Hesseltine, C.W., Shotwell, O.L., Smith, M.L., Shannon, G.M., Vandegraft, E.E. and Goulden, M.L. 1968. Laboratory studies on the formation of aflatoxin in forages. Mycologia 60: 304.

Koehler, B. and Woodworth, C.M. 1938. Corn-seedling virescence caused by Aspergillus flavus and Aspergillus tamarii. Phytopathology 28: 811.

Lillard, H.S., Hanlin, R.T. and Lillard, D.A. 1970. Aflatoxigenic isolates of Aspergillus flavus from pecans. Appl. Microbiol. 19: 128.

Mayne, R.Y., Pons, W.A. Jr., Franz, A.O. Jr. and Goldblatt, L.A. 1966. Elaboration of aflatoxin on cottonseed products by Aspergillus flavus. J. Amer. Oil Chem. Soc. 43: 251.

Smalley, E.B., Marasas, W.F.O. and Daugherty, S. 1972. Development of Aspergillus flavus and aflatoxin in harvested forage. University of Wisconsin, Research Bull. R2412. 24 p.

Seymour, A.B. 1929. Host Index of the Fungi of North America, 732 p. Harvard University Press, Cambridge.

Wiley, M. 1966. Note on the analysis for aflatoxins. J. Assoc. Off. Anal. Chem. 49: 1223.

Ms received 3/24/75; accepted 5/14/75.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Dept. of Agriculture to the exclusion of others that may be suitable.

MUNEHIKO TANAKA, DARUNEE THANANUNKUL, TUNG-CHING LEE and C. O. CHICHESTER
Dept. of Food & Resource Chemistry, University of Rhode Island, Kingston, RI 02881

A Research Note

A SIMPLIFIED METHOD FOR THE QUANTITATIVE DETERMINATION OF SUCROSE, RAFFINOSE AND STACHYOSE IN LEGUME SEEDS

INTRODUCTION

ALTHOUGH LEGUME SEEDS have high nutritional value among vegetable proteins, they contain a considerable amount of oligosaccharides which have been implicated as factors responsible for flatulence (Steggerda, 1968; Steggerda and Dimmick, 1968; Hellendoorn, 1969). The flatus produced by fermentation of dietary carbohydrates in the lower intestine may cause nausea, cramps, diarrhea and discomfort. The flatus-forming factor is mainly found in the low molecular weight carbohydrate fractions of legumes (Rackis et al., 1970), which contain primarily sucrose, raffinose and stachyose.

An aqueous or alcoholic extract of legume seeds is usually prepared, clarified, then chromatographed for the analysis of its sugar content. Quantitative determination of sugars by paper chromatography was reported by Tanusi et al. (1972). Sugimoto and Van Buren (1970) separated monosaccharides, sucrose, raffinose, stachyose and verbascose in the soybean milk on a cellulose sheet by an ascending thin layer chromatography. Their quantities were determined colorimetrically after elution from a cut-out spot on a paper or thin-layer chromatogram. A rapid gas-liquid chromatography method was developed recently by Delente and Ladenburg (1972) to analyze oligosaccharides in defatted soybean meal. Kim et al. (1973) employed liquid chromatography for the rapid determination of monosaccharides, disaccharides and stachyose and raffinose in soybeans, but absolute values for the quantity of the different sugars were not obtained by this method.

The present work describes an analytical procedure for determining the amounts of sucrose, raffinose and stachyose in the whole legume seeds by using the thiobarbituric acid reaction (Percheron, 1962). The present method is fast and does not require the use of expensive analytical equipment such as gas chromatography or liquid chromatography units.

EXPERIMENTAL

SOYBEANS, green mung beans, adzuki beans and white beans were purchased from a local grocery store. Whole dry beans (10g) were ground to 20 mesh and suspended in 100 ml of 80% ethanol (Kawamura et al., 1966). The suspension was refluxed for 1 hr and filtered through a Whatman No. 1 filter paper. The residue was stirred in 100 ml of distilled water for 30 min and filtered again, then washed with water until the filtrate gave negative triphenyltetrazolium chloride reaction of the washing (Horn et al., 1968). The extracts and washings were combined and concentrated to 100 ml under vacuum below 50°C.

The amount of 20 μl of the concentrated sugar extract was applied on an Eastman Chromagram sheet (catalog number 6064, cellulose) and developed by ascending chromatography using a mixture of n-propanol, ethylacetate and water (6:1:3, v/v). The modified α-naphthol reagent (Albon and Gross, 1950) was sprayed to locate fructose-containing sugars. The guide-strip technique was used to determine the amounts of sucrose, raffinose and stachyose. A sugar spot on a chromatogram was scraped off and the sugar was extracted with 2 ml of distilled water in a test tube overnight at room temperature. One ml of eluent (containing 10–70 μg of sugar) was mixed with 1 ml of 0.02M thiobarbituric acid and 1 ml of concentrated hydrochloric acid. The mixture was heated in a boiling water bath for exactly 6 min, then cooled under running water (Percheron, 1962). The yellow color produced was read at 432.5 nm in a Beckman type DB-G spectrophotometer. The concentration of sugar was calculated from working standards, ranging from 10–100 μg sugar per 20 μl.

RESULTS & DISCUSSION

A SOLVENT SYSTEM (n-propanol, ethylacetate and water, 6:1:3, v/v) was developed to provide a good separation of sucrose, raffinose and stachyose on a cellulose thin-layer chromagram sheet. For the determination of sugars, the thiobarbituric acid reaction was chosen because not only is this reaction specific to fructose, but also the interference of glucose, galactose, lipids and proteins is negligible under the conditions of the test. Therefore, the removal of lipids and proteins from legume seeds is not necessary for our procedure. This is in contrast to the phenol-sulfuric acid method used by Sugimoto and Van Buren (1970), Tanusi et al. (1972) and Kim et al. (1973) where their measurements were found to be interfered with in the presence of lipids or proteins. Oligosaccharides tested in the concentration range of 0–100 μg showed highly significant linear correlations with the absorbance at 432.5 nm.

The recovery tests with a series of ternary mixtures containing varying amounts of sucrose, raffinose and stachyose showed the high reliability of our procedure (Table 1). Raffi-

Table 1—The recovery of sugars from ternary mixture

	Sucrose – mg/100 ml			Raffinose – mg/100 ml			Stachyose – mg/100 ml		
Mixture	Added	Found	Recovery (%)	Added	Found	Recovery (%)	Added	Found	Recovery (%)
1	250	252	100.8	375	379	101.1	187.5	187.5	100
2	187.5	190	101.3	125	136	108.8	250	249.5	99.8
3	125	125	100	250	237	94.8	125	135	108
4	62.5	65	104	187.5	185	98.7	125	130	104
Average			101.5			100.8			102.9

Table 2–Oligosaccharides in legume seeds

Seeds	Procedure	Weight in g/100g seeds		
		Sucrose	Raffinose	Stachyose
Soybeans	ours	4.01	1.25	3.80
	Hymowitz et al. (1972)	5.06–6.78	0.65–0.95	2.23–3.05
	Tanusi et al. (1972)	4.2	1.3	2.7
	Kawamura et al. (1966)	5.0	1.1	3.8
	Kawamura (1954)	3.7	1.0	3.2
Mung beans	ours	0.93	0.44	1.96
	Tanusi et al. (1972)	0.85	0.49	1.71
Adzuki beans	ours	0.74	0.25	4.06
	Tanusi et al. (1972)	0.62	0.25	2.80
White beans	ours	2.61	0.66	3.39
	Becker et al. (1974)		0.4	3.7

nose and stachyose determinations had somewhat more variation than sucrose. Although our procedure includes the use of thin-layer chromatography, extraction and colorimetry, the average overall recovery of the added sugars was satisfactory.

Table 2 shows the contents of oligosaccharides of different types of legume seeds analyzed by our method, along with those previously reported. It is known that the level of the oligosaccharides in the legume seeds is different among varieties and strains and also rises during the maturation of the seeds (Hymowitz et al., 1972). However, the relative levels found in our study were in agreement with those presented by other workers.

Furthermore, some of the procedures used in this study can be modified if one desires to have a rapid determination. The water extraction procedure used by Delente and Ladenburg (1972) can replace the 80% ethanol extraction procedure. However, the sample should be fully defatted by this method. Sugars separated on a chromatogram can be eluted by water in a test tube at 70°C for 2 hr with occasional shakings (Sugimoto and Van Buren, 1970).

REFERENCES

Albon, N. and Gross, D. 1950. The chromatographic determination of raffinose in raw sugars. Analyst 75: 454.

Becker, R., Olson, A.C., Frederick, D.P., Kon, S., Gumbmann, M.R. and Wagner, J.R. 1974. Conditions for the autolysis of alpha-galactosides and phytic acid in California small white beans. J. Food Sci. 39: 766.

Delente, J. and Ladenburg, K. 1972. Quantitative determination of oligosaccharides in defatted soybean meal by gas-liquid chromatography. J. Food Sci. 37: 372.

Hellendoorn, E.W. 1969. Intestinal effects following ingestion of beans. Food Technol. 23: 87.

Horn, M.J., Lichtenstein, H. and Womack, M. 1968. Availability of amino acids. A methionine-fructose compound and its availability to microorganisms and rats. J. Agr. Food Chem. 16: 741.

Hymowitz, T., Walker, W.M., Collins, F.I. and Panczner, J. 1972. Stability of sugar content in soybean strains. Commun. Soil Sci. Plant Anal. 3: 367.

Kawamura, S. 1954. Studies on soybean carbohydrates. 4. Determination of oligosaccharides in soybeans. Nippon Nogei Kagaku Kaishi 28: 851.

Kawamura, S., Tada, M. and Narasaki, T. 1966. Sugars of the cotyledon, hull and hypocotyl of soybeans. J. Jap. Soc. Food & Nutr. 19: 268.

Kim, W.J., Smit, C.J.B. and Nakayama, T.O.M. 1973. The removal of oligosaccharides from soybeans. Lebensm.-Wiss. u. Technol. 6: 201.

Percheron, F. 1962. Colorimetric determination of fructose and fructofuranosides by the thiobarbituric acid reaction. Compt. Rend. 255: 2521.

Rackis, J.J., Honig, D.H., Sessa, D.J. and Steggerda, F.R. 1970. Flavor and flatulence factors in soybean protein products. J. Agr. Food Chem. 18: 977.

Steggerda, F.R. 1968. Gastrointestinal gas following food consumption. Ann. New York Acad. Sci. 150: 57.

Steggerda, F.R. and Dimmick, J.F. 1968. Effects of bean diets on concentration of carbon dioxide in flatus. Am. J. Clin. Nutr. 19: 120.

Sugimoto, H. and Van Buren, J.P. 1970. Removal of oligosaccharides from soy milk by an enzyme from Aspergillus saitoi. J. Food Sci. 35: 655.

Tanusi, S. 1972. Changes of carbohydrate contents of the soybean seed during growth. J. Jap. Soc. Food & Nutr. 25: 89.

Tanusi, S., Kasai, T. and Kawamura, S. 1972. Determination of oligosaccharides in some edible legume seeds. J. Jap. Soc. Food & Nutr. 25: 25.

Ms received 2/21/75; revised 4/7/75; accepted 4/15/75.

Agricultural Experiment Station, University of Rhode Island Contribution No. 1594.

THOMAS PHILIP and JAMES W. BERRY
Dept. of Agricultural Biochemistry, The University of Arizona, Tucson, AZ 85721

A Research Note

NATURE OF LUTEIN ACYLATION IN MARIGOLD (Tagetes erecta) FLOWERS

INTRODUCTION

THE MAJOR CAROTENOID in marigold (*Tagetes erecta*) flowers is lutein which occurs naturally acylated with fatty acids (Quackenbush and Miller, 1972). Alam et al. (1968) reported that the xanthophylls in marigold flowers are acylated with lauric, myristic, palmitic and stearic acids. Dried marigold flowers and carotenoid extracts from them are used as a chicken feed supplement to enhance the color of egg yolks. Guenthner et al. (1973) compared the egg coloring efficiency of pure lutein with a number of natural extracts containing lutein and concluded that natural extracts contained unknown factors that aided the utilization of lutein by chickens. This investigation was undertaken to study the nature of lutein acylation in marigold flowers and to evaluate the potential of lutein esters as food colorants.

MATERIALS & METHODS

THE MARIGOLD (*Tagetes erecta*) flower extracts were obtained from INEXA Industria Extractora, Quito, Ecuador in May, 1974.

Instrumental analysis

Visible spectrum: Perkin-Elmer 202 UV-Visible Spectrophotometer
IR spectrum: Perkin-Elmer 137 IR Spectrophotomer (KBr disc)
NMR spectrum: Varian T60 NMR Spectrometer (CCl_4)
Gas chromatography: Wilkens Instrument and Research Inc. Model 600 C with FID.

Thin-layer chromatography

Adsorbent: Silica gel G
Solvent 1: Acetone + petroleum ether (30–60°C), 5 + 95.
Solvent 2: Acetone + benzene + petroleum ether (30–60°C), 20 + 10 + 70.

Quantitative analysis

The marigold extract (1.0g) was saponified using the method of Quackenbush (1973). The saponified carotenoids were taken up in carbon disulfide (100 ml) and the absorbance measured at 475 nm (total carotenoids). An aliquot (1.0 ml) of saponified carotenoids was applied as a thin strip on silica gel G plates and developed with solvent 2. The lutein band was quantitatively scraped off, eluted with carbon disulfide and absorbance measured at 475 nm after appropriate dilution. The total carotenoids were calculated as lutein based on an $E_{1\%}^{1cm}$ value of 2160 at 475 nm in carbon disulfide (Davies, 1965).

The concentration of lutein esters in the extract was calculated as follows: A chloroform solution of the extract was applied as a thin strip on silica gel G plates and developed using solvent 1. The lutein ester bands were quantitatively scraped off, eluted and the absorbance measured at 443 nm(λ_{max}) in hexane. The percentage of lutein esters was calculated based on the relative absorbance at 443 nm in hexane.

Purification of lutein esters

The marigold flower extract was subjected to a preliminary purification by column chromatography on alumina by the method of Philip (1973). A preliminary separation of xanthophyll esters on silica gel G thin layers using solvent 1, revealed the presence of three major ester bands and several minor ester bands. Based on the visible spectra, the three major bands (Bands A, B and C) were recognized as lutein esters. They were isolated in large quantities by preparative TLC using solvent 1. The bands A and B contained traces of short-chain triglyceride impurities and were treated as follows: To a solution of esters in benzene (5 mg/1 ml) in a screw cap bottle, 5 ml of 0.4N sodium methylate in methanol was added. The bottle was then immersed in a water bath at 65°C for 3 min with the screw cap securely applied. The bottle was removed, cooled and the contents transferred to a separatory funnel containing petroleum ether (50 ml). The lower layer was drained and the petroleum ether layer washed with distilled water. The petroleum ether layer was evaporated and the ester purified by TLC using solvent 1. Under these conditions, very little hydrolysis of esters took place. On the other hand, the triglyceride impurities were converted to fatty acid methyl esters (Philip et al., 1971). The methyl esters moved with the solvent front during TLC purification using solvent 1.

Fatty acid analysis

The fatty acid analyses were carried out according to the method of Alam et al (1968).

Preparation of lutein esters

Lutein (5 mg) isolated from marigold flower extracts and crystallized from methanol, was dissolved in pyridine (10 ml) and the solution was transferred to a separatory funnel. Acyl chloride (10 drops) was added dropwise and the mixture shaken vigorously for 5 min. Petroleum ether (50 ml) was then added, and the mixture after shaking, washed with dil HCl (1 + 2) and distilled water. The petroleum ether layer was evaporated to dryness and the ester was used for comparison of R_f values.

Solubility of lutein esters

A weighed amount of lutein esters (a mixture of dipalmitate and dimyristate isolated from marigold flower extracts) was slowly added with stirring to commercial corn oil (1.0g) contained in a beaker maintained at 80°C on a water bath. The oil was occasionally cooled to room temperature to observe precipitation of esters and addition of ester was continued until saturation. The solubility was calculated on a weight per weight basis.

RESULTS & DISCUSSION

THE EXTRACT of marigold (*Tagetes erecta*) flowers contained 11.9% total xanthophylls. The major xanthophyll of the extract readily crystallized from methanol and had the following physico-chemical properties: λ_{max} in hexane 421, 444 and 476 nm; λ_{max} in carbon disulfide 447, 475 and 506 nm; NMR signals at 8.03, 8.28, 8.30, 8.94, 9.02 and 9.17τ in the ratio 4:1:1:2:1:1 and R_f value 0.30 in solvent 2. The spectral shape indicated the presence of a chromophore similar to zeaxanthin, and the NMR bands at 9.02, 9.17 and 8.94τ showed the presence of both α- and β-end groups (Barber et al., 1960). Based on these properties, the major xanthophyll of the marigold flower extract was identified as lutein. Lutein accounted for 60% of the total xanthophylls.

The epiphasic nature of unsaponified xanthophylls of marigold flowers indicated that their hydroxyl groups are engaged in acylation with fatty acids. The unsaponified xanthophylls readily separated on silica gel G thin layers when developed with a nonpolar solvent (solvent 1). Based on the visible absorption spectrum (λ_{max} in hexane 421, 444 and 474 nm), three major bands (bands A, B and C) were recognized as lutein esters. The properties of lutein esters are given in Table 1. The infrared spectra of bands A, B and C showed strong ester carbonyl vibrations at 1725 cm^{-1} confirming the nature of lutein-fatty acid bond. The absence of hydroxyl vibrations in the infrared spectra of bands A and B indicated that they were lutein diesters. The low R_f value of band C in solvent 1 and the presence of hydroxyl vibration (33-3600 cm^{-1}) in its

Table 1—Physico-chemical properties of lutein esters

Band	%	R_f value (Solvent 1)	λ_{max} in hexane (nm)	IR bands		Fatty acid	Identity[a]
				-OH	ester C = 0		
A	56	0.45	421,444,474	–	+	palmitic acid	Lutein dipalmitate
B	36	0.44	421,444,474	–	+	myristic acid	Lutein dimyristate
C	8	0.10	420,443,473	+	+	myristic acid	Lutein monomyristate

[a] The identity of bands A and B was confirmed by comparing R_f values with synthetic lutein dipalmitate and lutein dimyristate.

infrared spectrum indicated that band C is a monester. Band A gave palmitic acid, and bands B and C gave myristic acid on hydrolysis. Based on these data, the lutein esters were identified as lutein dipalmitate (band A), lutein dimyristate (band B) and lutein monomyristate (band C). The identities of lutein dipalmitate and lutein dimyristate were confirmed by comparison of their R_f values with synthesized esters.

A mixture of lutein dipalmitate and lutein dimyristate are soluble in hot corn oil (80°C) to the extent of 25% (w/w) and forms a red homogeneous solution. The solubility of lutein ester in cold corn oil was less than 25%.

CONCLUSION

THE OCCURRENCE of lutein as esters in marigold flower extracts and the increased solubility of lutein esters in lipids may be responsible for the observation by Guenthner et al. (1973) that natural extracts containing lutein are better utilized by chicken compared to pure lutein. The effect of lutein acylation on egg coloring efficiency is under investigation in this laboratory.

Marigold flowers are the most concentrated common source of xanthophylls (Scott et al., 1968) and lutein accounts for 60% of the total carotenoids. Lutein and lutein-fatty acid esters are common carotenoids in edible plants. These parameters are important in the evaluation of marigold flower extract as a potential food colorant for human consumption. The high solubility of lutein esters in vegetable oil (25%) compared to the poor oil solubility of FDA approved synthetic carotenoids (Bunnell and Bauernfeind, 1962) is a very important factor in the commercial application of xanthophyll esters for coloring foods.

REFERENCES

Alam, A-U, Couch, J.R. and Creger, C.R. 1968. Fatty acid composition of the xanthophyll esters of Tagetes erecta petals. Lipids 3(2): 183.
Barber, M.S., Davis, J.B., Jackman, L.M. and Weedon, B.C.L. 1960. Studies in Nuclear magnetic resonance. Part 1. Methyl groups of carotenoids and related compounds. J. Chem. Soc. 1960: 2870.
Bunnell, R.H. and Bauernfeind, J.C. 1962. Chemistry, uses and properties of carotenoids in foods. Food Technol. 16(7): 36.
Davies, B.H. 1965. Analysis of carotenoid pigments. In "Chemistry and Biochemistry of Plant Pigments," Ed. Goodwin, T.W., p. 489. Academic Press, N.Y.
Guenthner, E., Carlson, C.W., Olson, O.E., Kohler, G.O. and Livingston, A.L. 1973. Pigmentation of egg yolks by xanthophylls from corn, marigold, alfalfa and synthetic sources. Poultry Sci. 52: 1787.
Philip, T. 1973. The nature of carotenoid esterification in citrus fruits. J. Agric. Food Chem. 21: 964.
Philip, T., Nawar, W.W. and Francis, F.J. 1971. The nature of fatty acids and capsanthin esters in paprika. J. Food Sci. 36: 98.
Quackenbush, F.W. and Miller, S. 1972. Composition and analysis of carotenoids in marigold petals. J.A.O.A.C. 55: 617.
Quackenbush, F.W. 1973. Use of heat to saponify xanthophyll esters and speed analysis for carotenoids in feed materials. J.A.O.A.C. 56: 748.
Scott, M.L., Ascarelli, I. and Olson, G. 1968. Studies of egg yolk pigmentation. Poultry Sci. 47: 863.
Ms received 2/11/75; accepted 3/22/75.

Presented at the 35th Annual Meeting of the Institute of Food Technologists, Chicago, June 8—11, 1975.

The authors are grateful to Dr. L.W. Levy, for providing samples of marigold flower extracts.

C. E. LYON and B. G. LYON
USDA Animal Products Utilization and Marketing Laboratory, ARS, P.O. Box 5677, Athens, GA 30604

A Research Note
DONENESS OF COMMERCIALLY COOKED BROILER THIGHS AS INDICATED BY AN OBJECTIVE COLOR METHOD

INTRODUCTION

MANY FURTHER processed broiler products are cooked, frozen, stored and reheated prior to consumption. These products are labeled "cooked" to indicate they meet USDA inspection requirements of at least 71.1°C internal temperature to insure destruction of microorganisms (Meat and Poultry Inspection Reg., 1973). However, most companies attempt to cook their products to an adequate degree of doneness (i.e., higher than 71.1°C) so that additional recommended heating from the frozen state merely warms the products sufficiently for consumption.

Methods to estimate doneness of cooked poultry have included "spearing" of leg and breast muscles to determine tenderness and "working" of wing and leg joints to detect softening of tendons (Alexander, 1941). Subjective evaluations of color, taste and/or general appearance as measures of doneness have also been employed (Mostert and Stadelman, 1964; Stone and May, 1969). The most common method of estimating doneness of commercially cooked broiler parts is to subjectively examine a small number of thigh pieces for objectionable color (pink or red) near the bone.

The work of Lyon et al. (1975) established that Hunter a_L values (degree of redness) could be used to estimate doneness of laboratory-prepared water-cooked broiler thighs. The objective of this study was to determine the range of Hunter a_L values of various types of commercially cooked broiler thigh products and to relate these data to the findings of Lyon et al. (1975) and to determine whether reheating procedures significantly affected final yield of these products.

MATERIALS & METHODS

FROZEN COOKED broiler thighs, from four commercial poultry processing firms, are defined in Table 1. Processors A and B used water-cooking methods exclusively. Processor C used a water-cooking method for product 1 and a steam-cooking method for product 2. Processor D used a deep-fat fry and infra-red oven combination cooking procedure. All products were stored at −30°C until ready for doneness (color) examination with the Hunter Color and Color Difference Meter. Determinations were made on thawed products and on products reheated directly from the frozen state.

For examination of the thawed products, five thigh pieces of each of the seven product types were removed from frozen storage and thawed at room temperature for 4 hr. For examination of reheated products five thigh pieces of each product type were removed from frozen storage, weighed, and reheated from the frozen state according to each processing firm's instructions for reheating (Table 1). Reheating pieces by deep-fat frying (Frymax oil) was done in a Hotpoint deep-fat fryer. A Despatch rotary reel oven was used for oven reheating. Immediately after the specified reheating period, a copper-constantan thermocouple probe was inserted into meat adjacent to the femur in two of five thigh pieces and the maximum internal temperature reached was recorded with a Honeywell potentiometer.

Samples were prepared for color examination using the procedure of Lyon et al. (1975). The entire procedure was replicated twice thus

Table 1—Hunter a_L values, reheated yields and end-point reheating temperatures of commercially cooked broiler thighs

Processor and product	Reheat procedure	Hunter a_L values[a]		Reheated yield[b]	End-point temp[c] (°C)
		Thawed	Reheated		
A - 1 (Precooked and breaded)	Deep-fat fry 6 min. 162.7°C	5.9a	6.0a	83 ± 1.0%d	59.4 ± 1.3bc
2 (Precooked and browned)	Oven 30 min. 176.6°C	6.5a	6.7a	94 ± 0.4%a	68.8 ± 6.2ab
B - 1 (Precooked and breaded)	Deep-fat fry 6 min. 176.6°C	5.2a	5.6a	86.5 ± 0.4%c	47.7 ± 1.6c
2 (Precooked and battered)	Oven 30 min. 176.6°C	4.9a	4.7a	94 ± 0.6%a	75.2 ± 1.4a
C - 1 (Precooked and breaded)	Deep-fat fry 6 min. 162.7°C	4.5a	5.0a	89 ± 1.0%b	46.9 ± 0.6c
2 (Precooked and battered)	Deep-fat fry 6 min. 176.6°C	4.8a	4.6a	72 ± 1.0%e	46.1 ± 2.1c
D - 1 (Precooked and breaded)	Oven 35 min. 190.5°C	7.2a	5.1b	85 ± 0.5cd	82.2 ± 4.3a

[a] Each Hunter a_L value is a mean of 10 observations. Values within rows followed by different letters differ significantly ($P < 0.05$) according to Duncan's Multiple Range Test.

[b] Weights were taken prior to and after reheating. Each number is a mean value of 10 observations with the standard error of the mean. Values within a column followed by the same letter are not significantly different ($P < 0.05$) according to Dundan's Multiple Range Test.

[c] Temperatures represent highest internal temperature reached. Each number is the mean of four observations with the standard error of the mean. Values within a column followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's Multiple Range Test.

Table 2—Ranges of Hunter a_L values which correspond to panel terms of doneness[a]

	Doneness Terms			
	Very underdone	Moderately underdone	Slightly underdone	Done
Panel 1	>8.9	8.9–7.3	7.2–5.6	<5.6
Panel 2	>7.0	7.0–5.9	5.8–4.6	<4.6

[a] Ranges of Hunter a_L values extrapolated from doneness regression lines of best fit according to data from the study by Lyon et al. (1975).

providing data for 10 thawed (unheated) and 10 reheated thighs of each of the seven product types. Significant differences between means were determined by using Duncan's Multiple Range Test (Steel and Torrie, 1960).

RESULTS & DISCUSSION

HUNTER a_L values for thawed and reheated samples are shown in Table 1. The a_L values can be converted to subjective estimates of doneness by use of the relation between Hunter a_L values and panel doneness scores established by Lyon et al. (1975). In that study two highly trained sight panels each established ranges of doneness based on evaluation of color differences in water-cooked broiler thighs. The two panels differed in their responses to the same change in a_L values and established different doneness ranges for cooked samples (Table 2).

All a_L values of the commercially cooked thighs (Table 1) are within the "slightly underdone" to "done" range as established by Panel 1 (Table 2). The highest a_L value for thawed samples was for the precooked and breaded product of Processor D (7.2). This a_L value is on the upper limit of the "slightly underdone" range (Panel 1). However, the a_L value of this product reheated (5.1) was in the "done" range. When a_L values for the commercially cooked thighs (thawed and reheated) were compared to the a_L ranges for doneness for Panel 2 (Table 2), one a_L value was in the "very underdone" range (thawed product, Processor D), four were in the "moderately underdone" range, eight were in the "slightly underdone" range, and one was in the "done" range.

Differences in a_L values between thawed and reheated samples were significant only for Processor D (Table 1). The change in this value of 7.2 to 5.1 upon reheating corresponds to change from "slightly underdone" to "done" (Panel 1) or "very underdone" to "slightly underdone" (Panel 2).

End-point temperatures of all oven reheated products were significantly higher than those of deep-fat fry reheated products except for the products of Processor A. There were no significant differences in end-point temperature within methods for reheating. As expected, the reheating method (oven 190.5°C–35 min) recommended by Processor D gave the highest end-point product temperature (82.2°C) and the only significant reduction in a_L value due to reheating (Table 1). This indicated that Processor D's initial cooking procedure and compliance with reheating instructions would insure a done product. Reheating methods recommended by the other processors for their respective products did not significantly reduce a_L values, indicating that reheating did not increase degree of doneness of these products.

Recommended reheating procedure also significantly affected yield, which ranged from 72–94% (Table 1). The higher oven temperature and longer exposure time recommended for Processor D's product resulted in a significantly lower yield (85%) than the other two oven reheated products (94%). All yields of deep-fat fry reheated products were significantly different from each other and were generally lower than yields of oven reheated products.

In summary, average Hunter a_L values of seven types of commercially cooked broiler thighs ranged from 4.5–7.2 when products were examined thawed and from 4.6–6.7 when the products were reheated from the frozen state. Reheating produced a significant reduction in a_L value in only one of the seven products. These data can be compared to previous work, but definite statements on adequate doneness of these products cannot be made until there is more information on correlations of consumer acceptance criteria with objective color measurements as planned by these authors.

REFERENCES

Alexander, L.M. 1941. Poultry cooking. USDA Farmers' Bull. 1888.
Lyon, B.G., Lyon, C.E. and Klose, A.A. 1975. Subjective and objective methods for estimating doneness in water-cooked broiler thighs. J. Food Sci. 40: 133.
Meat and Poultry Inspection Regulations. 1973. Book 11, Poultry Inspection Regulations, Title 9, Chap. 111 Subchapter C Sec. 381.50. Code of Federal Regulations, U.S. Govt. Printing Office, Washington, D.C.
Mostert, G.C. and Stadelman, W.J. 1964. Effect of method of cookery on shrinkage, moisture, and ether extractable content of broiler legs and thighs. Poultry Sci. 43: 896.
Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Co., Inc.
Stone, E.W. Jr. and May, K.N. 1969. The effect of time and temperature of cooking on quality of freeze-dehydrated chicken. Poultry Sci. 48: 813.
Ms received 3/19/75; revised 5/17/75; accepted 5/20/75.

Use of company or product name by the Department does not imply approval or recommendations of the product to the exclusion of other products which may also be suitable.

J. O. REAGAN,[1] T. R. DUTSON, Z. L. CARPENTER and G. C. SMITH
Meats & Meat Chemistry Section, Dept. of Animal Science
Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843

A Research Note

MUSCLE FRAGMENTATION INDICES FOR PREDICTING COOKED BEEF TENDERNESS

INTRODUCTION

THE NEED to relate beef quality attributes directly to methods for carcass classification, suggests that the development of a quick and accurate method for tenderness evaluations using raw tissues would be of immense value. Takahashi et al. (1967) studied postmortem changes in the pectoral muscles of chicken breasts and developed a fragmentation index to determine the percent of myofibrils broken during postmortem aging as an indication of muscle tenderness. These researchers reported a definite relationship between myofibril fragmentation and time postmortem, although no difference in fragmentation was observed between "tender" and "less tender" pectoral muscles (Takahashi et al., 1967). In a similar study, Sayre (1970) concluded that the mechanical breaking of myofibrils upon homogenization was an indication of the structural weakening that occurs with increases in tenderness.

Berry (1972) developed a fragmentation index which involved determinations of the size of myofibril fragments and the number of sarcomeres per myofibril, following muscle homogenization. The results of the latter research indicated that longer sarcomeres and shorter myofibril fragments were closely associated with higher sensory panel scores for tenderness (Berry, 1972). Dutson and Lawrie (1974), studying the effect of postmortem aging on bovine muscle tenderness, observed that as time postmortem increased, the protein content of the supernatant from homogenized muscle tissue increased and the muscles became more tender. Thus, it seems feasible that a method, similar to that used by Dutson and Lawrie (1974), might be identified which would be useful for predicting tenderness by use of raw muscle samples.

EXPERIMENTAL

SEMIMEMBRANOSUS MUSCLES were excised from the intact left side of 20 bovine carcasses following a 7-day aging period at 2°C. The muscles were wrapped in two layers of polyethylene-coated freezer paper and frozen in a −34°C blast freezer. After freezing, four steaks (2.5 cm in thickness) were removed from the center section of each muscle, double wrapped in polyethylene-coated freezer paper and stored in a −23°C freezer. The two center steaks were utilized for shear force determinations and sensory panel evaluations and the remaining two steaks (steaks 1 and 4) were utilized for fragmentation measurements. Steaks for sensory panel analyses and shear force determinations were cooked in a 175°C electric oven for 50 min to an approximate internal temperature of 72°C. A six-member trained sensory panel evaluated each sample for three tenderness traits (initial tenderness, muscle fiber tenderness rating and amount of connective tissue rating) as described by Cross et al. (1973). Shear force values were determined using five 1.3 cm cores taken from each sample. Ten semimembranosus muscles with shear force values less than 5.33 kg ("tender") and ten semimembranosus muscles with shear force values greater than 8.88 kg ("tough") were selected for use in developing a fragmentation index to predict muscle tenderness. The raw muscle samples were ground through a 4.8 mm plate and blended in a Virtis homogenizer at high speed for the selected blending times. Two sample sizes (5g and 10g) and two blending times (30 sec and 60 sec) were compared to identify the optimum procedure. After blending, the homogenate was filtered through one layer of cheesecloth (Curtiss No. 50). A 20 ml aliquot of the filtrate was placed in a preweighed, 50 ml polyethylene centrifuge tube and centrifuged at 31,300 × G for 10 min. The supernatant was decanted, leaving the myofibrillar protein residue in the centrifuge tube. The remaining fat particles were removed from the inside of the centrifuge tube using a metal spatula wrapped with cheesecloth. The tube containing the residue was again weighed and the total weight of the residue determined. Residue weight (muscle fragment weight) was used as an index of fragmentation.

RESULTS & DISCUSSION

SIMPLE CORRELATION COEFFICIENTS for shear force values and muscle fragment weights for each of the four fragmentation methods are presented in Table 1. Three of the four indices were significantly ($P < 0.01$) correlated with shear force values. The fragmentation index employing a 10-g sample and 60 sec of blending time exhibited the highest coefficient of correlation and was selected for subsequent evaluations.

Means and standard deviations for muscle fragment weights and shear force values for the "tough" and "tender" groups of muscles are reported in Table 2. The average weight of the centrifuged pellet, resulting from the use of a 10-g sample blended for 60 sec obtained from the muscle samples classified as tender, was significantly ($P < 0.01$) greater than that obtained from the tough muscles. These findings support the theory (Sayre, 1970) that as muscle tenderness increases, the degree of fragmentation which occurs when a muscle is blended also increases. Shear force values differed significantly ($P < 0.01$) between the two groups of muscles utilized in the present study.

Sensory panel evaluations for subjective measures of tenderness of the semimembranosus muscle were not significantly

Table 1—Simple correlation coefficients between shear force values and muscle fragment weights

Fragmentation method[a]	Sample size (g)	Blending time (sec)	Correlation between muscle fragment weights and shear force values (r)
1	5	30	−0.64**
2	5	60	−0.42
3	10	30	−0.66**
4	10	60	−0.79**

[a] n = 20 muscle samples per fragmentation method
** $F < 0.01$

[1] Present address: Dept. of Food Science, University of Georgia, Athens, Ga.

Table 2—Means and standard deviations for muscle fragment weights and shear force values for tough and tender samples of muscle

Muscle tenderness group	Muscle fragment weight[a]			Shear force, kg	
	n	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Tough	10	1.16[b]	0.17	9.2[b]	1.13
Tender	10	1.94[c]	0.28	4.6[c]	0.64

[a] The weight (g) of the centrifuged myofibrillar pellet obtained from the cheesecloth filtered fraction of a 10-g meat sample blended for 60 sec.

[b,c] Means in the same column bearing different superscripts differ significantly ($P < 0.01$).

correlated with the muscle fragmentation index (initial tenderness, r = 0.43; muscle fiber tenderness ratings, r = 0.41; and amount of connective tissue rating, r = 0.31). The relatively small number of samples used may have been partially responsible for this finding. An experiment using additional muscles and greater numbers of samples would strengthen the evaluation of this concept for predicting tenderness. Fragmentation index values were closely associated ($P < 0.01$) with shear force values (r = −0.79) indicating that the index developed in the present study measured the same tenderness components that were evaluated by the Warner-Bratzler shear machine.

The results of the present study do not provide conclusive evidence regarding the effectiveness of the fragmentation index as an objective indicator of muscle tenderness. However, the significant ($P < 0.01$) association between the fragmentation index and shear force values suggests that the development of a method for measuring muscle fragmentation, similar to the one utilized in the present study, might result in a feasible method for prediction of ultimate tenderness of cooked meat.

In conclusion, determinations of fragmentation index by use of a small quantity of raw muscle sample and a short period of homogenization could provide a potential method for selection of carcasses according to expected tenderness and, depending upon the projected tenderness level, other treatments such as aging, mechanical treatments or enzymatic tenderization could be used to improve the palatability of less desirable carcasses.

REFERENCES

Berry, B.W. 1972. Characteristics of bovine muscle, cartilage and bone as influenced by physiological maturity. Ph.D. thesis, Texas A&M University, College Station, Texas.

Cross, H.R., Carpenter, Z.L. and Smith, G.C. 1973. Effects of intramuscular collagen and elastin on bovine muscle tenderness. J. Food Sci. 38: 998.

Dutson, T.R. and Lawrie, R.A. 1974. Release of lysosomal enzymes during postmortem conditioning and their relationship to tenderness. J. Food Technol. 9: 43.

Sayre, R.N. 1970. Chicken myofibril fragmentation in relation to factors influencing tenderness. J. Food Sci. 35: 7.

Takahashi, K., Fukazawa, T. and Yasui, T. 1967. Formation of myofibrillar fragments and reversible contraction of sarcomeres in chicken pectoral muscle. J. Food Sci. 32: 409.

Ms received 3/10/75; revised 4/14/75; accepted 4/22/75.

T.A. 11773, Texas Agricultural Experiment Station. This study was partially supported by a grant from King Ranch, Inc., Kingsville, Texas and the American Brahman Breeders Association, Houston, Texas.

MAURICE R. BERRY, JR. and ROGER W. DICKERSON, JR.
Department of Health, Education and Welfare, Food and Drug Administration, Bureau of Foods
Division of Food Technology, Food Engineering Branch, Cincinnati, OH 45226

A Research Note
APPARATUS FOR MAINTAINING HOLDING TEMPERATURE WHILE SERVING CAFETERIA ROUNDS OF BEEF

INTRODUCTION

LARGE BEEF ROASTS (30–40 lb) are routinely held on conventional cafeteria serving lines and may remain on the serving line for as long as 10 hr. In the absence of proper temperature control, microbial growth can lead to food-borne illness. Under the best existing operating conditions, the roast is held under infrared lamps on a heated steam table. The exclusive use of one or more infrared lamps is also a common method of operation; however, a bacteriological survey (Bryan and Kilpatrick, 1971) has demonstrated that this practice yields holding temperatures in the range that support growth of *Clostridium perfringens* (65–122°F).

The recommended temperature for holding hot foods in food-service operations is 140°F (USPHS, 1962), but proprietors contend that holding these large rounds of beef on serving lines for extended periods at conditions necessary to maintain 140°F tends to dry the meat, leaving it undesirable for their customers. Such drying is due primarily to the lack of proper equipment to hold the large size rounds at uniform temperature and prevent overheating of certain portions of the meat.

The purpose of this work was to develop an apparatus for holding cafeteria rounds of beef on serving lines so that the roast beef could be hand-sliced and presented to the customer.

MATERIALS & METHODS

USDA CHOICE cafeteria rounds of beef (33-lb boneless, rolled and tied, rump and shank off, knuckle removed; Item number 166, Meat Buyer's Guide, NAMP, 1961) were selected for the experiments as being representative of roasts used commercially. A total of 13 rounds were placed on racks in a roasting pan and cooked in a commercial oven (Vulcan-Hart, Model 6751A) at low (160–225°F) or moderate (300°F) oven temperatures. The roasts were removed from the oven so that residual cooking effects yielded a final center temperature of approximately 140°F (rare). The instrumentation was left in position and the roasts were placed directly on the serving table.

Meat temperatures were recorded during cooking and holding by a multipoint recorder (Dynamaster, Bristol Co.) using copper-constantan thermocouples (O.F. Ecklund, Customer Thermocouples). Three sets of three thermocouples were placed near the surface (1/4 in. above and 1/16 and 1-1/2 in. below) on the top, side, and bottom surfaces of the roast. Temperatures at 1/16 in. below the surface were considered as surface temperature. A temperature profile was measured along the horizontal axis with three additional thermocouples located at 3 and 4-1/2 in. below the surface and at the center of the roast. Oven temperatures were recorded in four corners of the oven and the average was considered as oven temperature.

A portable roast beef serving table (Seco Co., Model B-2-RB) was modified to develop a system for maintaining holding temperature in rounds of beef on cafeteria serving lines. The major modification to the standard serving table consisted of a stainless steel deep-well serving pan which was installed in the wet-well opening in the serving table and provided thermal protection for the sides of the roast (Fig. 1). The deep-well encloses most of the roast exposing only enough of the top surface to facilitate slicing. The well is 16 in. in diameter by 8 in. deep. The serving pan is equipped with an adjustable rack so that the entire roast can be raised in 1-in. increments as the top surface is sliced away. Steam in the wet-well surrounded the deep-well enclosure so that the temperature in the deep-well of the pan could be controlled by controlling the water temperature in the wet-well (±1/2°F). Ten holding tests were conducted with the roast extending approximately 3 in. above the top surface of the serving pan. Various heat lamp arrangements were used with wet-well temperatures between 190 and 212°F.

In addition to the holding tests, three runs were conducted where the roast was raised and sliced at the rate of 1 in. each hour. A wet-well temperature of 195°F and two infrared lamps 16 in. above the cutting surface were used. For the slicing tests, thermocouples were positioned at five locations on the top and side surfaces and at 1-in. intervals below the slicing surface along the vertical axis of the roast.

RESULTS & DISCUSSION

ANGELOTTI et al. (1961, 1963) and Bryan and Kilpatrick (1971) indicate that hot foods should be maintained at 140°F to insure the prevention of outbreaks of illness from food-poisoning organisms (*C. perfringens*, salmonella, staphylococci and streptococci). The recent work of Brown and Twedt (1972) suggests that after a beef roast is heated to 140°F, *C. perfringens, Salmonella enteritidis* and *Staphylococcus aureus* continue to be inactivated at any temperature above 128°F.

Fig. 1—Serving system for cafeteria rounds of beef.

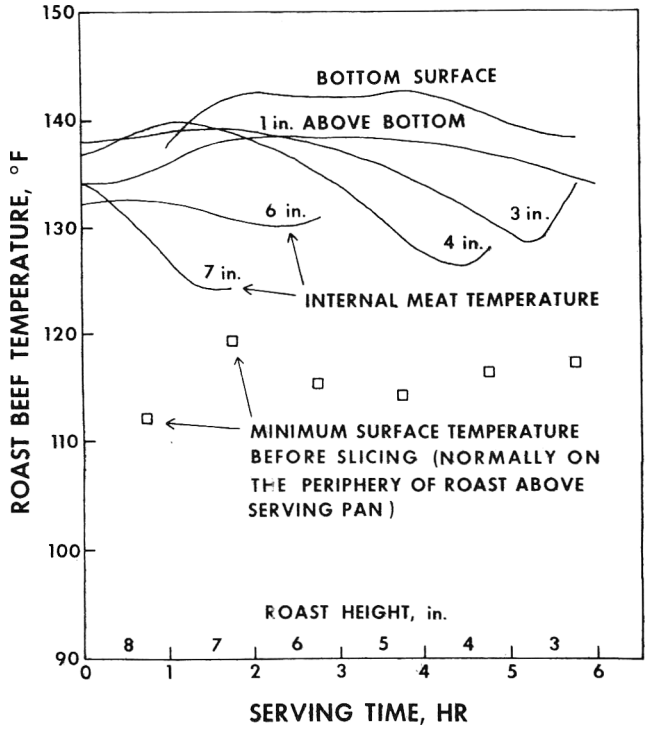


Fig. 2—Roast beef serving in a deep-well pan at the rate of 1-in. per hour.

Based on these data, the criteria established for the design of a long-term holding and serving system for cut-to-order roast beef was a mass average internal temperature of at least 140°F. To prevent the possibility of recontamination on the surface of the roast, the temperature should not drop below 128°F for extended periods.

One test was conducted to simulate current commercial techniques. A roast, cooked to a center temperature of 135°F, was placed on the perforated drip rack of the portable serving table, with most of the surface of the roast exposed to ambient conditions. With the 1,000-watt wet-well heater operating continuously, the wet-well water temperature was maintained at 212°F. One infrared heat lamp was directed onto the top surface of the roast from a distance of 11 in. Within 15 min, the surface temperatures around the sides of the roast were less than 90°F. By the end of the 8-hr holding period, equilibrium conditions were attained with internal temperatures falling below 122°F. The average temperature was 110°F, some 30°F less than the recommended holding temperature.

These data show that additional thermal assistance is needed to maintain roasts at the required holding temperatures, and the modified serving table, with the deep-well enclosure that protected the sides of the roast, performed well. With the wet-well temperature held at 206°F, and one infrared lamp 14 in. above the top of the roast, surface temperatures remained above 130°F for the entire 8-hr holding period with internal meat temperatures ranging from 142–148°F.

Maintaining wet-well temperature is important. A test was conducted identical to the above except that wet-well temperature was held at 190°F, and surface temperatures on the sides of the roast dropped to 117°F.

Other factors affected the required wet-well temperature: (1) A 200°F wet-well temperature was sufficient for a roast cooked in a 300°F oven. Higher cooking temperatures yield higher meat temperatures near the surface for the same center temperature, and this leads to a smaller energy demand from the holding system. (2) Two infrared lamps located 16 in. above the cutting surface of the roast, with a wet-well temperature of 195°F, were sufficient to maintain surface temperatures of the roast above 134°F. (3) For one test, the infrared lamps were replaced with 150-watt incandescent flood lamps. The surface temperatures dropped to 121°F indicating that flood lamps cannot supply adequate heating to the top surface. (4) Also, roasts not placed on serving lines immediately after cooking require a higher wet-well temperature or additional heat lamps to bring them within safe limits in a short period of time.

Results of a typical slicing test are shown in Figure 2. Locations indicated on the figure are measured above the lower surface of the roast and indicate where continuous temperature measurements were recorded. The trend shows that the temperature at a point within the roast remains relatively constant until it reaches a point about 1–2 in. below the cutting surface. Then the temperature begins to fall until the effect of the heat lamps is felt as the point moves closer to the cutting surface. These data indicate that the heat loss to the surroundings occurs on the periphery of the roast between the cutting surface and the top of the deep-well serving pan. This distance should therefore be kept at the minimum practical level that will not impair efficient slicing operations. Whereas the minimum surface temperature before slicing (Fig. 2), which occurs on the side surface above the deep-well pan, tended to fall below the minimum of 128°F, the time spent at these temperatures is too brief for any significant microbial growth (about 1 hr). During periods of reduced slicing activity, the roast can be lowered completely into the deep-well serving pan and covered with a lid.

Based on the results of this investigation, a roast beef must have thermal protection for its side surfaces in order to satisfy the holding criteria established. The apparatus described in this note provides such protection and satisfies the minimum temperature requirements with a wet-well temperature of 206°F and one infrared lamp 14 in. above the cutting surface, or with two lamps at 16 in. and a 195°F wet-well temperature.

REFERENCES

Angelotti, R., Foter, M.J. and Lewis, K.H. 1961. Time-temperature effects on salmonellae and staphylococci in foods. Appl. Microbiol. 9: 308.

Angelotti, R., Lewis, K.H. and Foter, M.J. 1963. Fecal streptococci in foods and at warm-holding temperatures. J. Milk Food Technol. 26: 296.

Brown, D.F. and Twedt, R.M. 1972. Assessment of the sanitary effectivness of holding temperatures on beef cooked at low temperature. Appl. Microbiol. 24: 599.

Bryan, F.L. and Kilpatrick, E.G. 1971. Clostridium perfringens related to roast beef cooking, storage, and contamination in a fast food service restaurant. Am J. Public Health 61: 1869.

NAMP. 1961. Meat buyer's guide to standardized meat cuts. National Association of Meat Purveyors, Chicago, IL 60606.

USPHS. 1962. "Food Service Sanitation Manual." U.S. Public Health Service, Publication No. 934. U.S. Government Printing Office, Washington, D.C.

Ms received 4/2/75; revised 5/16/75; accepted 5/20/75.

Presented at the 34th Annual Meeting of the Institute of Food Technologists, New Orleans, La., May 12–15, 1974.

Mention of commercial products does not imply endorsement by the Food and Drug Administration.

R. G. CASSENS,[1] R. N. TERRELL[2] and C. COUCH[1]
Dept. of Meat & Animal Science, University of Wisconsin and Griffith Labs, Chicago

A Research Note

THE EFFECT OF TEXTURED SOY FLOUR PARTICLES ON THE MICROSCOPIC MORPHOLOGY OF FRANKFURTERS

INTRODUCTION

CURRENT DIRECTION in the manufacture of meat foods is to balance quality and quantity of protein against manufacturing functionality, nutritional value and cost. A variety of non-meat ingredients have been used as fillers, binders and extenders in comminuted, cooked, cured meat food sausages. These have been used in formulations at a 3–4% level based on weight of meat (edible flesh). When higher levels are incorporated, problems are often encountered with emulsion stability, textural properties and flavor acceptance. Textured soy flours (extrusion expanded, puffed) have been used successfully in products such as ground meat casseroles, chili and stews where a coarse textural property is desired. The use of textured soy flours in finely or coarsely comminuted meat food mixes, to be used in sausage formulations, is now under strong consideration.

This note describes the morphological characteristics of frankfurter-type products into which textured soy flour particles were incorporated.

MATERIALS & METHODS

THREE BATCHES of frankfurters (Table 1) were prepared at three different times. Batch #1 was a typical all meat frankfurter formulation, whereas Batch #2 had 9.9% finely divided soy flour added, and Batch #3 had the same amount of coarsely divided textured soy flour added. The product was manufactured in a conventional Silent Cutter, and after comminution, was stuffed into 22 mm cellulose casings and heat processed in a conventional smokehouse for 1–1½ hr. Finished internal product temperature of 70°C was reached, and then the product was cold showered with water for 5 min and placed overnight in a 0°C cooler before peeling and vacuum packaging the following day.

The coarsely divided textured soy flour was a commercial product ("Promate") manufactured by The Griffith Laboratories, and had an approximate size of 1/4 × 1/8 × 1/8 in. To make finely divided material, this coarse product was ground through a lab model Raymond Hammermill so that it would be retained on a 100 mesh screen.

Microscopic evaluation was conducted on the finished product stored from 1–6 wk at cooler temperature or frozen for the same length of time. Whole slices of frankfurters were frozen in liquid nitrogen and sectioned at approximately 10μ thickness in a cryostat. Sections were stained with Oil red O and Harris hematoxylin.

RESULTS & DISCUSSION

A PHOTOMICROGRAPH representative of the control sample is shown in Figure 1a. The lipid globules are present as spherical or oval deposits, and have a size distribution ranging from about 160μ diameter down to very small globules at the limit of resolution with the light microscope. The lipid globules are more or less uniformly dispersed throughout the protein water matrix. These results are similar to previous reports on the morphology of finely comminuted meat products (Hanson, 1960; Swift et al., 1961; Helmer and Saffle, 1963; Carpenter and Saffle, 1964; Meyer et al., 1964; Borchert et al., 1967).

Artifacts are present and do present a problem in interpretation. The most serious of these is the situation created during sectioning, which causes lipid to "roll out" of its place. This results in some empty spaces, formerly occupied by lipid, and produces small lipid globules which are deposited elsewhere. It is therefore difficult to decide whether the open white areas in the photomicrographs are sites previously occupied by lipid or if they are air pockets formed during manufacture of the frankfurter product. The spherical lipid droplets, indicated as artifacts in Figure 1a and b, are almost certainly displaced from elsewhere. Such displaced lipid globules are often on the surface of the section and can be confirmed by careful focusing. Fixation prior to sectioning did not improve the problem.

The chunks of textured soy flour are detected easily in Figure 1b and c, by their characteristic morphology. This is best described as a loosely connected network of "fibers" which form or enclose long, oval shaped openings or interstices. Specific histochemical tests for identification of soy protein products in meat foods have been described (Linke, 1969; Coomaraswamy and Flint, 1973).

Two potentially important morphological changes were observed in products to which textured soy flour had been added. First, the lipid globules sometimes assumed an irregular or angular shape when they occurred next to a chunk of textured soy flour. Lipid globules not located adjacent to textured soy flour appeared spherical or oval shaped, similar to those in the control samples. Second, lipid was apparently not incorporated into the interstices of the textured soy flour. Examples of these two observations are identified in Figure 1.

Table 1—Frankfurter formulations using finely divided and coarsely divided textured soy flour[a,b]

	Batch number		
Ingredient	1	2	3
Edible flesh	74.4	49.9	49.9
Ice/water	19.1	33.9	33.9
Finely divided TSP[c]		9.9	
Coarsely divided TSP[c]			9.9
Seasoning/sugar	4.5	4.5	4.6
Salt	2.0	1.7	1.7

[a] Ingredients expressed as percent
[b] The equivalent of 1/4 oz sodium nitrite, 1/8 oz sodium erythorbate and 7 oz sodium acid pyrophosphate per 100 lb batch was added.
[c] Textured soy flour

[1] Dept. of Animal Science, University of Wisconsin, Madison, WI 53706
[2] The Griffith Laboratories, 1415 W. 37th St., Chicago, IL

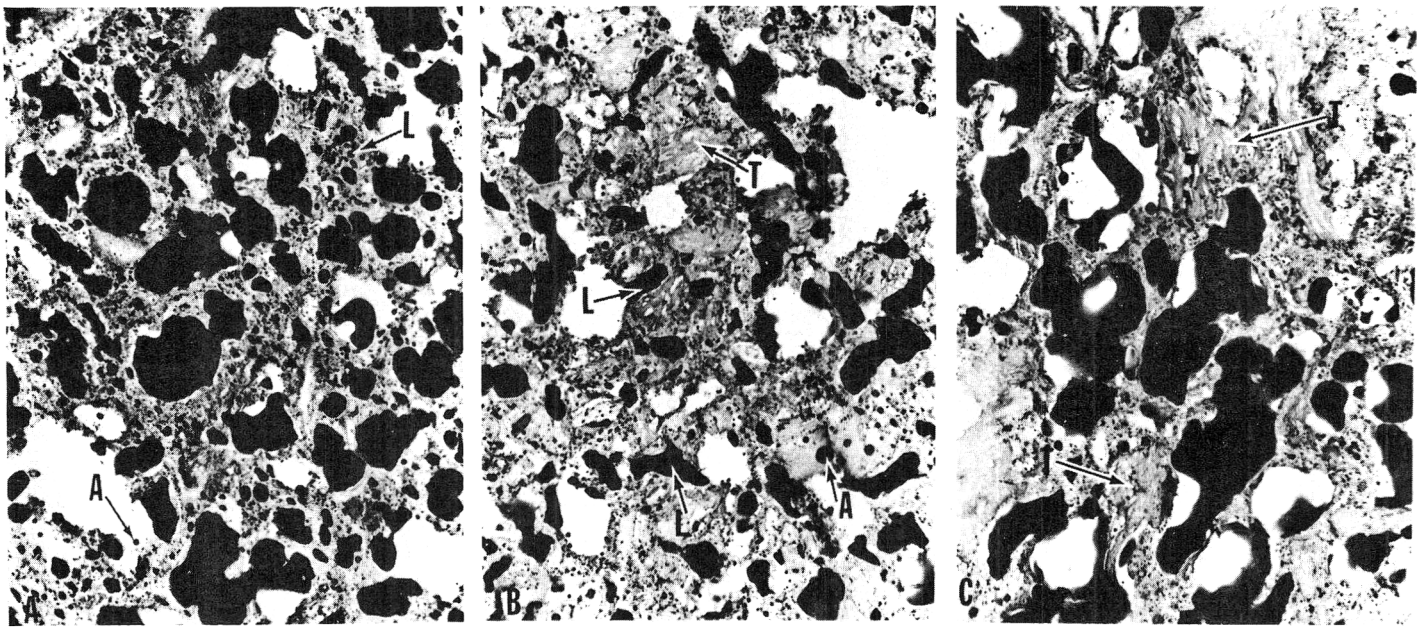


Fig. 1–Fresh frozen sections (10μ thick) made from frankfurters and stained with Oil red O and Harris hematoxylin (Mag. 72X). Lipid has been stained black, the protein-water matrix is gray and open areas are white. "A" (left) is from a control all meat frankfurter, "B" (center) contains finely divided textured soy flour and "C" (right) contains coarsely divided textured soy flour. Lipid droplets are identified as "L," textured soy flour is identified as "T" and artifacts are identified as "A."

Two irregularly shaped lipid globules are identified in Figure 1b and the chunks of textured soy flour seen in Figure 1b and c do not show evidence of incorporated lipid.

The significance of the morphological findings that added textured soy flour chunks do not contain histochemically detectable fat and that the added chunks cause, in some instance, the fat globule to conform to the straight or angular shape of the textured soy flour particle is not yet known. Chemical analysis revealed that the control batches contained 28–32% lipid, while the batches containing textured soy flour had a lipid content ranging from 20–25%. This is a well-known effect when using textured soy flours in frankfurter formulations (lipid is diluted to a lower level and protein is increased substantially).

We believe, on the basis of our morphological study and in view of the different sizes of textured soy flour particles used, that the effect is primarily physical with the added particles merely taking up space in the matrix, and thereby limiting the amount of fat which can be dispersed and held in the product.

REFERENCES

Borchert, L.L., Greaser, M.L., Bard, J.C., Cassens, R.G. and Briskey, E.J. 1967. Electron microscopy of a meat emulsion. J. Food Sci. 32: 419.

Carpenter, J.A. and Saffle, R.L. 1964. A simple method of estimating the emulsifying capacity of various sausage meats. J. Food Sci. 29: 774.

Coomaraswamy, M. and Flint, F.O. 1973. The histochemical detection of soya "novel proteins" in comminuted meat products. Analyst 98: 542.

Hanson, L.J. 1960. Emulsion formation in finely comminuted sausage. Food Technol. 14: 565.

Helmer, R.L. and Saffle, R.L. 1963. Effect of chopping temperature on the stability of sausage emulsions. Food Technol. 17: 115.

Linke, H. 1969. Histologischer Nachweis von "TVP," Die Fleischwirtschaft 4: 469.

Meyer, J.A., Brown, W.L., Giltner, N.E. and Guinn, J.R. 1964. Effect of emulsifiers on the stability of sausage emulsions. Food Technol. 18: 1796.

Swift, C.E., Lockett, C. and Fryar, A.J. 1961. Comminuted meat emulsions. The capacity of meats for emulsifying fat. Food Technol. 15: 468.

Ms received 4/18/75; accepted 5/6/75.

Dept. of Meat & Animal Science Ms. No. 83. Research supported by the College of Agricultural & Life Sciences, University of Wisconsin, Madison.

LUH C. TAO
Dept. of Chemical Engineering, University of Nebraska, Lincoln, NE 68508

A Research Note

EFFECT OF LINEAR APPROXIMATION OF ENTHALPY-TEMPERATURE CURVE IN SIMULATING HEAT TRANSFER DURING FREEZING

INTRODUCTION

SIMULATION of heat transfer during a freezing operation was recently made by integrating numerically a set of differential equations on a digital computer (Joshi and Tao, 1974). Among the inputs required for such computations is the enthalpy-temperature relationship of the system to be frozen. Above the freezing temperature, heat capacities of most systems usually have only minor temperature dependency and the enthalpy would be linear. For the temperature below the freezing point, enthalpy of a mixture such as foods is a nonlinear function of temperature because the major contribution to the enthalpy change is the latent heat of fusion of water, and the fractional amount of water frozen is a nonlinear function of temperature. This nonlinear function is used to compute the enthalpies of all grid points below the freezing temperature in each repetitive computation cycle and any simplification such as linearization may thus effect a saving of computation time. This note presents the freezing curves obtained by the simulation program of Joshi and Tao (1974) for the nonlinear as well as the two linearized enthalpy functions.

EXPERIMENTAL

THE SYSTEM SIMULATED is an apple slab of 2-in. thickness. Originally at 77.5°F (T* = 1.70), it was suddenly dipped into a well stirred cooling bath so that the slab surface was maintained constantly at −39°F (T* = 0). The nonlinear enthalpy function was obtained by using Riedel's work (1951) of apple juice to be adjusted with more solids content for the apple slab.

RESULTS

LINEARIZATION of this enthalpy curve may be made in terms of many piecewise-continuous linear segments and the simplest would be to use only two segments. In this study two arbitrarily chosen patterns of two lines are shown in Figure 1. The dash lines are tangents, one at the freezing point and the other at −40°F. This linearization emphasizes a good approximation near the freezing point temperature and the surface temperature. The dotted lines represent a linearization for good approximations in the region of −40 to 20°F. Certainly other lines can also be drawn for observation if necessary. This linearization in terms of two lines reduced about 20% of the computation time of the same system with the nonlinear enthalpy-temperature function.

These computed freezing curves of the same system with the nonlinear and two linearized enthalpy-temperature functions are shown in Figure 2. These results display graphically the sensitivity of freezing curves toward the changes of enthalpy function in simulation calculations. Also, they lead to the following observations:

1. Linear approximations of the enthalpy curve used here affect very little the freezing curve of the slab center prior to its reaching the freezing temperature (0–15 min) even though other parts are already frozen.

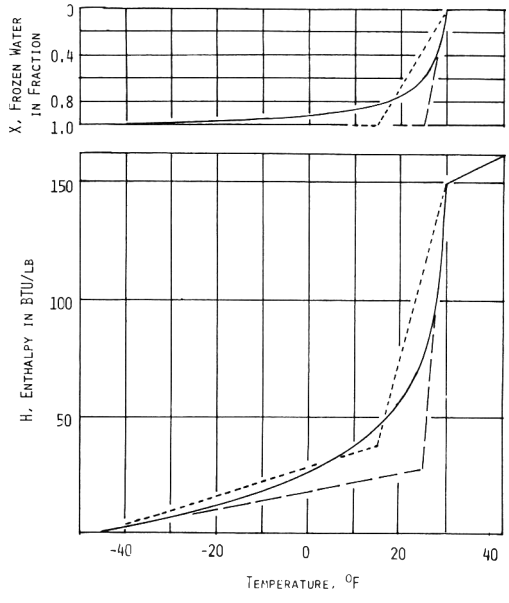


Fig. 1—Three enthalpy-temperature functions are related ice fractions.

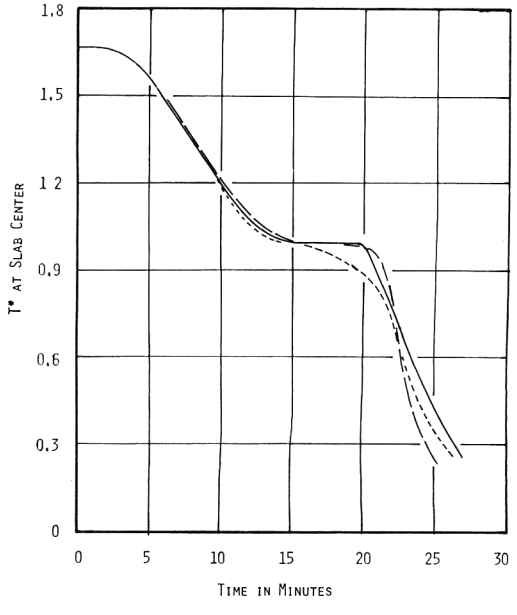


Fig. 2—Calculated freezing curves at a slab center for three different enthaloy functions.

2. The enthalpy curve or line immediately below the freezing temperature appears to have major influence on the plateau (15–20 min) of the freezing curve. A more vertical enthalpy line effects a longer plateau portion.
3. For the time range of 0–22 min here, the freezing curve based on the use of two tangent segments is very close to that using the nonlinear enthalpy function. This offers a useful guide for making the linear approximation of enthalpy-temperature data in simulation computations.

A corollary of item 1 is that the linear approximation of enthalpy data appears to affect very little the computed time requirement for the center to reach the freezing temperature.

REFERENCES

Joshi, C. and Tao, L.C. 1974. A numerical method of simulating the axisymmetrical freezing of food systems. J. Food Sci. 39: 623.
Ridel, L. 1951. The refrigerating effect required to freeze fruits and vegetables. Refrig. Engr. 59: 670.
Ms received 3/6/75; revised 4/14/75; accepted 4/22/75.

CHAN T. DU and F. J. FRANCIS
Dept. of Food Science & Nutrition, University of Massachusetts, Amherst, MA 01002

A Research Note
ANTHOCYANINS OF GARLIC (Allium sativum L.)

INTRODUCTION

THE DRIED inner scale leaves of garlic (*Allium sativum* L.) are colored, but the types of pigments present and their composition have not been reported. Another Allium species, notably the Spanish red onion, *A. cepa* L. was known to contain anthocyanins which were responsible for its color. This study reports isolation and partial identification of the major pigments in garlic.

EXPERIMENTAL

FRESH GARLIC CLOVES (*Allium sativum* L.) were obtained from a local food market. The dry outer scale leaves were peeled and discarded, while the inner colored dry scale leaves were collected, peeled, and macerated in a blender with several different concentrations of HCl/methanol (0.05%, 0.5%, 1%, 3%) to observe the effect of mineral acid on the composition of anthocyanins during extraction. For isolating individual pigments, 0.05% HCl/methanol was used as the extracting solvent. The extract was concentrated to a small volume, washed with hexane and ethyl acetate respectively, further concentrated and purified on CG-50 Amberlite cation exchange resin to give a partially purified pigment extract (Fuleki and Francis, 1968).

Isolation of pure garlic anthocyanins

The partially purified pigment extract was purified by paper chromatography as described by Du et al. (1975).

Identification of garlic anthocyanins

The identification of pigments followed in general the spectroscopic and chromatographic methods described by Harborne (1967). Details of solvent systems, spectral measurements, mobility, partial and complete acid hydrolysis, peroxide hydrolysis, alkali hydrolysis, etc. were reported previously (Du et al., 1975). In order to detect acid labile intermediates, a solution of pigment in 0.5% HCl/methanol was heated in a boiling water bath for about 5 min, after which 2N HCl was added dropwise at 1/2 min intervals for 5 min, and finally an equal volume of 2N HCl to effect complete hydrolysis. Aliquots were withdrawn at 1/2-min intervals during the early stages of acid hydrolysis and spotted on Whatman No. 1 paper along with authentic standards and developed in BFW and 15% HAc.

RESULTS & DISCUSSIONS

PROLONGED CHROMATOGRAPHY of garlic pigment extract in BFW (2 days) showed the presence of seven pigment bands (Table 1). Pigment bands G4, G5, G6, G7 were present in trace quantities and appeared to be contaminated with other plant constituents. Isolation and identification of these bands was not attempted. Pigments G1, G2 and G3 were the major bands. The mineral acid strength of the extracting solvent has a great effect on the relative amounts of pigments of bands G1, G2 and G3. 1–3% HCl/methanol resulted in great reduction of the intensity of G1 and G2 and increased G3, while the use of 0.05% HCl/methanol as the extracting solvent revealed that G1 and G2 were present in much greater concentration, but G3 was still the major pigment. Alkali hydrolysis of the purified pigment extract resulted in the complete disappearance of G1 and G2 but not G3, suggesting that G1 and G2 were possibly acylated anthocyanins.

G1, G2 and G3 showed a positive, blue color reaction with lead acetate (Fuleki and Francis, 1967) typical of cyanidin pigments. Complete acid hydrolysis of purified fractions of these three pigments indicated that cyanidin was the only aglycone and glucose the only sugar. Hydrogen peroxide hydrolysis of G1, G2 and G3 yielded only glucose.

G3 exhibited the typical spectral properties of a Cy-3-glycoside (Evis. max = 528 nm, Euv. max = 280 nm, E440 nm/Evis. max = 22%, E320 nm/Euv. max = 22%, positive $AlCl_3$ shift) with nonaromatic acid acylation. It also yielded no detectable hydrolyzed intermediates on partial acid hydrolysis. Based on identical chromatographic mobility of G3 to that of authentic pigment (Table 2), G3 was positively identified as cyanidin-3-glucoside, the predominant pigment in garlic.

Though readily alkali hydrolyzed to form Cy-3-G, G2 showed no aromatic acylation and exhibited a typical Cy-3-glycoside spectrum (Evis. max = 527 nm, Euv. max = 280 nm, E440 nm/Evis. max = 22%, E320 nm/Euv. max = 21%, positive $AlCl_3$ shift). Upon partial acid hydrolysis under mild conditions, G2 formed two hydrolyzed intermediates: one cor-

Table 1—Separation of garlic pigments in BFW

Pigments bands	Relative quantity	$R_{Cy\text{-}3\text{-}G}$ X 100[a]
G1	++	230
G2	++	163
G3	++++	100
G4	trace	87
G5	trace	56
G6	+	36
G7	trace	19

[a] Average values determined on Whatman No. 1 paper at band fronts after 2 days separation in BFW

Table 2—Chromatographic mobility of anthocyanins from garlic

	Solvent systems			
	BFW	1% HCl	HAc-HCl	
Pigments	Cy-3-G X 100	Rf X 100		15% HAc
G1	220	15	49	67
G2	162	10	38	55
G3	101	8	32	50
Authentic markers				
Cy-3-G (Raspberry, Francis, 1972)	100	7	32	51
Cy-3-laminariobioside (Red orion, Du et al., 1975)	45	9	34	53

responding to Cy-3-G as the major hydrolyzed compound, and another intermediate with fast mobility in BFW ($R_{Cy\text{-}3\text{-}G}$ = 236). The latter intermediate was readily detected at the early stages of acid hydrolysis, and it did not correspond to any known anthocyanidins or anthocyanins. From the above evidence, the pigment was postulated to be Cy-3-G-$(U)_2$ or Cy-3-G$<^{U}_{U}$ where U is an unknown aliphatic acyl residue. Occurrence of double aromatic acylation has been reported in *Solanum guineese* (Francis and Harborne, 1966), and nonaromatic acyl residues were noted more recently in Zea mays (Harborne and Garvazzi, 1969), apples (Timberlake and Bridle, 1971), and grapes (Anderson et al., 1970). In the case of grapes, the acyl group was reported to be acetic acid. Bloom and Geissman (1973) reported that the acyl moiety of cyanidin-3-glucoside from *Mimulus Lutens* was malonic acid.

G1 also indicated no cinnamoyl absorption and gave a typical Cy-3-glycoside spectrum (Evis. max = 528 nm, Euv. max = 280 nm, E440 nm/Evis. max = 21%, E320 nm/Euv. max = 26%, positive $AlCl_3$ shift). Upon partial acid hydrolysis, it yielded Cy-3-G and G2 as the major intermediates, and in addition, two minor intermediates; one corresponded to the hydrolyzed intermediate found with G2 and another highly mobile one ($R_{Cy\text{-}3\text{-}G}$ = 290) which was not identified. Thus, G1 was tentatively identified as Cy-3-G$<^{U}_{U\text{-}U}$. The two postulated structures for the acylated Cy-3-G compounds would fit the chromatographic mobility and partial hydrolysis data but conclusive proof of structure will depend on the identification of the type and amount of the acyl moiety.

The results in this work showed that acylated anthocyanins might occur more often than generally recognized, especially those of aliphatic acyl groups which might have escaped detection due to their lability in mineral acids and lack of absorption in the cinnamoyl region of ultraviolet spectra. Our preliminary survey on red onion and ornamental cherries indicated that acylated anthocyanins existed in the extract of these plant tissues. Thus extracting solvents containing a high concentration of mineral acid should be avoided to prevent loss of deacylation. Use of organic acids (Anderson et al., 1970) and low amounts of HCl in the extracting solvent are preferable. We have found that the use of 0.1–0.5% HCl/methanol as a pigment streaking solution has the advantage of giving much greater band resolution during separation in BFW than a pigment solution in acetic acid. Deacylation of anthocyanins at this HCl concentration was negligible during the chromatographic separation.

REFERENCES

Anderson, D.W., Gueffrey, D.E., Webb, A.D. and Kepner, R.E. 1970. Identification of acetic acid as an acylating agent in grapes. Phytochem. 9: 1579.
Bloom, M. and Geissman, T.A. 1973. Malonic acid: The acyl moiety of the Mimulus Luteus anthocyanin. Phytochem. 12: 2005.
Du, C.T., Wang, P.L., and Francis, F.J. 1975. Cyanidin-3-laminariobioside in Spanish red onions. J. Food Sci. 39: 1265.
Francis, F.J. 1972. Anthocyanins of Durham and Heritage raspberries. HortSci. 7: 398.
Francis, F.J. and Harborne, J.B. 1966. Anthocyanins of the garden huckleberry Solanum guineese. J. Food. Sci. 31: 524.
Fuleki, T. and Francis, F.J. 1967. Lead acetate as a chromogenic reagent for anthocyanins. Phytochem. 6: 1161.
Fuleki, T. and Francis, F.J. 1968. Quantitative methods for anthocyanins. 3. Purification of cranberry anthocyanins. J. Food Sci. 33: 266.
Harborne, J.B. 1967. "Comparative Biochemistry of the Flavonoids." Academic Press. N.Y.
Harborne, J.B. and Garvazzi, G. 1969. Effect of Pr and pr alleles on anthocyanin biosynthesis in Zea mays. Phytochem. 8: 999.
Timberlake, C.F. and Bridle, P. 1971. The anthocyanins of apples and pears, the occurrence of acyl derivatives. J. Sci. Fd. Agr. 22: 509.
Ms received 3/19/75; accepted 4/28/75.

S. D. SENTER and B. G. LYON
USDA Richard B. Russell Agricultural Research Center, ARS, Athens, GA 30604
and B. D. HORTON
USDA Southeastern Fruit & Tree Nut Research Station, ARS, Byron, GA 31008

A Research Note

EFFECTS OF DIFFERENT CONCENTRATIONS OF SUCCINIC ACID-2,2-DIMETHYLHYDRAZIDE ON THE FLAVOR OF PUREE FROM FRESH AND CANNED FREESTONE PEACHES

INTRODUCTION

DEMAND for canned peaches in the United States is predicted to reach 1,857.7 million pounds by 1980, a 43% increase over consumption in 1968 (USDA, 1971). The expected demand, in conjunction with labor shortage and the increased costs of peach production, has influenced research with plant growth regulators to increase yields and quality, and to shorten the ripening period to facilitate once-over mechanical harvesting. Considerable success has been achieved with succinic acid-2,2-dimethylhydrazide (SADH) applied to peach trees prior to completion of pit-hardening.

Application of SADH to peach trees hastens the ripening of fruit (Byers and Emerson, 1973). SADH treated fruit abcise more readily from the stem and leave fewer fruit on the tree after mechanical harvest (Gambrell et al., 1967). Red and yellow skin colors are increased by SADH (Byers and Emerson, 1969), and better internal color is apparent (Sims et al., 1971; Baumgardner et al., 1972) perhaps as a result of increased carotene biosynthesis. Other parameters such as firmness, titratable acidity, soluble solids and pH, which are used as indices of quality, tend to vary with peach cultivars. However, these variations have not appeared to be detrimental to the quality or acceptability of the treated fruit.

Daniell (1969) indicated that no detrimental effects were observed on the odor and flavor of mid-ripe fruit that had been treated with SADH. Preliminary findings by Senter (1974) indicated that application of 1500 ppm of SADH to 'Loring' and 'Elberta' peach trees enhanced the flavor of fresh, ripe fruit and significantly increased acceptability. Information is not available concerning the effects of SADH on the flavor of canned peaches.

Because of the commercial acceptance of SADH for application to peach trees, the present study was designed to further evaluate the effects of SADH on the flavor of fresh freestone peaches, and to determine if the differences were apparent in canned peaches.

EXPERIMENTAL

Samples

'Dixiland' peach trees in their 7th year were selected from a 0.8 hectare plot at the USDA Research Station at Byron, Ga. for uniformity of fruit-set and vigor. The 25 trees were organized into five replications in a randomized complete block design and their identities were maintained through the evaluation of the processed peaches. SADH at concentrations of 0, 1000, 1500, 2000 and 2500 ppm was applied to single-tree plots at the beginning of pit-hardening.

About 34 kg (1.5 bu) of fruit was harvested from each tree on July 17. Fruits were selected randomly from each tree for the firm-ripe stage of maturity. The harvest was made at daybreak and fruits were transported in an air conditioned vehicle to the laboratory. They were stored within 2 hr from harvest at 4°C until the fruit were evaluated for fresh quality and the remainder was processed for evaluations of canned quality.

24 peaches were selected from each sample for uniform size (7 cm diam ± 1 cm) and ripeness (firm-ripe). Firmness, measured by the Magnus-Taylor pressure tester (0.47 cm tip) on the pared cheeks of peaches, was used as the index for ripeness. Selections were limited to fruit having average readings of 3–6 lb force. Fresh fruit analyses and canning were completed within 24 hr from harvest.

Fresh fruit

Four peaches were randomly chosen from the 24 selected from each sample and composited on a treatment basis. They were then washed in cold water and quartered. Sections from each of the 20 fruit were then blended at low speed for 15 sec. Portions of the puree thus formed were used for sensory and chemical evaluations of the five treatments.

Aliquants of the puree were poured into coded, 4-oz styrofoam cups. The cups were capped and left at room temperature for 15 min to allow the volatile components of the puree to equilibrate. The samples were presented to panelists in a sensory evaluation room that had controlled lighting (green), positive air pressure, and booths. Multiple comparison evaluations were made on the five randomly arranged samples using a nontreated sample as reference. Panelists were instructed to indicate their appraisal of each sample on a 7-point scale which ranged from "very superior to reference" (Score = 7) to "very inferior to reference" (Score = 1). In addition to multiple comparison analysis, the samples were rated on a hedonic scale of 9 (like very much) to 1 (dislike very much). 36 evaluations were made by 12 panelists in 24 hr (11:00 a.m., 3:00 p.m. and 11:00 a.m.). Each panel was run on quarter-section aliquots of the same 20 peaches per treatment that had been maintained at 4°C.

Soluble solids, titratable acidity and pH were measured on puree from composites of the remaining quarter sections of the 20 fruit per treatment. A 100-g sample from each composite was taken for pH measurement with glass electrodes and for titratable acidity determinations. Titratable acidity was expressed as ml of 0.1N NaOH required to titrate the 100-g sample to pH 8.1. Soluble solids of filtered juice, expressed as % sucrose, was determined with an Abbé refractometer.

Canned fruit

The 20 peaches remaining from the original 24 selected from each tree were maintained throughout canning operations and sensory evaluations of canned fruit on a "per tree" basis. Commercial canning procedures were used. The peaches were washed in cold water, pitted with a commercial freestone peach pitter, peeled with hot sodium hydroxide (2% NaOH at 90°C), and washed with high pressure water spray. The prepared peach halves were retained in a 1% ascorbic acid solution until packed. The halves were packed in No. 2 cans with a 25% solution of sucrose (Van Blaricom, 1948) and exhausted to an internal temperature of 65°C. The cans were then sealed and cooked at 116°C for 7 min in a rotary cooker that had been modified for laboratory purposes. The cans were cooled, dried and then stored at ambient temperatures for 8 wk.

Five panels, composed of the 12 members who rated fresh fruit, evaluated the flavor of the canned peaches. Each panel was held at mid-morning on consecutive days. These evaluations were conducted as before using multiple comparison analyses and hedonic ratings of puree from the five treatments. Three cans of peaches were randomly selected from each sample. For each of the five panels, samples from different

trees within treatments were evaluated. For each panel, three cans from each treatment were opened, the fruit was composited, drained for 45 min and pureed.

Peach halves from each treatment were washed, blotted dry and pureed for determination of per cent soluble solids. Measurements were made on filtered juice with the refractometer and found to be 17.4%.

RESULTS

Fresh fruit

Table 1 reports the means of Magnus Taylor pressure tests for firmness of peaches selected from each treatment and the corresponding values for pH, titratable acidity and soluble solids. Analysis of variance indicated no significant differences ($P < 0.05$) between nor within treatments.

Flavor scores were treated by analysis of variance and Duncan's multiple range test (Duncan, 1955). Preference was significant ($P < 0.05$) in both the multiple comparison test and the hedonic ratings for peaches treated with 2500 ppm SADH. Generally, no significant differences were found among the other treatments, nor between these treatments and the control (Table 2). Differences among panelists and panel sessions were not significant. Further analysis of these data by the Friedman test was significant ($K = 3.15$), indicating the probability was < 0.001 that these judgments happened by chance.

Canned fruit

Analysis of data from multiple comparison and hedonic ratings of canned peaches indicated significant difference among the ratings of the 12 panel members. The ratings of each member were then analyzed by analysis of variance to determine consistency of the panelists and their ability to discriminate (Amerine, 1965). The variance ratios (F Values) of six members were found to be significant at the 10% level, indicating their ability to discriminate. Further examination of the ratings of these members in the multiple comparison tests showed they had consistently identified the untreated sample. The ratings from these six panel members were then analyzed, and results indicated differences between the scores for treated and nontreated peaches were significant ($P < 0.05$). Peaches treated with 2500 ppm SADH were significantly preferred to other treated fruit (Table 2). Scores assigned to the samples by 5 of the panel members increased with increasing concentrations of SADH, but scores assigned by the 6th member decreased with increasing concentrations of SADH.

Comments from the five panelists who rated treated samples higher indicated they had based their preference on a sweeter, more pleasant flavor in the samples they had rated high and the absence of an odor-flavor that was reminiscent of peach pits, or benzaldehyde, that was apparent in the samples they had rated lower.

Table 1—Mean value of select physicochemical properties of SADH treated 'Dixiland' peaches

ppm SADH	Firmness[a]	pH[b]	Titratable acidity[bc]	Soluble solids[bd]
Control	4.56[e]	3.66[e]	79.3[e]	11.4[e]
1000	4.08[e]	3.72[e]	78.8[e]	11.2[e]
1500	3.54[e]	3.77[e]	81.0[e]	11.2[e]
2000	4.33[e]	3.77[e]	80.2[e]	10.7[e]
2500	3.88[e]	3.75[e]	78.4[e]	11.4[e]

[a] Pounds force, Magnus Taylor pressure tester (0.47 cm tip). Means based on 120 observations per treatment.
[b] Analyses made on samples composed of composited quarter-sections from 20 peaches per treatment.
[c] ml 0.1N NaOH required to titrate 100-g sample to pH 8.1.
[d] Expressed as % sucrose
[e] Mean separation in columns by Duncan's multiple-range test, 5% level

Table 2—Mean flavor scores of puree from fresh and canned 'Dixiland' peaches as affected by various concentrations of SADH

	Fresh[a]		Canned[b]	
ppm SADH	Multiple[c] comparison	Hedonic[c] rating	Multiple[c] comparison	Hedonic[c] rating
Control	3.89[d]	5.28[d]	4.00[d]	5.60[d]
1000	4.25[d]	5.72[d]	4.60[e]	6.47[e]
1500	3.97[d]	5.33[d]	4.37[ed]	6.07[e]
2000	3.69[d]	5.11[d]	4.80[e]	6.60[e]
2500	4.97[e]	6.94[e]	5.46[f]	7.47[f]

[a] Means of 36 observations per treatment (12 panelists, three sessions)
[b] Means of 30 observations per treatment (six discriminating panelists, five sessions).
[c] Multiple Comparison, 1–7, 7 = very superior to reference. Hedonic rating, 1–9, 9 = like very much.
[d-f] Mean separation in columns by Duncan's Multiple Range Test, 5% level

DISCUSSION

THE PREFERENCE SHOWN for SADH-treated peaches in this study cannot be attributed to a variation in those factors normally associated with the sweetness or acidity of a peach (Table 1). Therefore, it is suggested that preference for the SADH treated peaches was caused by an increase in organic constituents comprising the flavor of peaches (Do et al., 1969), or a decrease in those constituents that would tend to mask the true peach flavor. This is partially substantiated by the benzaldehyde odor that appeared to be stronger in the untreated peaches, and was objectionable to five of six discriminating panelists.

Further research in different years is needed to determine the effects of SADH on the flavor of different cultivars of freestone and clingstone peaches before this practice can be recommended for improving the flavor quality of fresh and canned peaches. This study does indicate, however, that within limits, changes in peach flavor caused by SADH are desirable.

REFERENCES

Amerine, M.A., Pangborn, R.M. and Roessler, E.B. 1965. "Principles of Sensory Evaluation of Food," p. 285. Academic Press, New York.
Baumgardner, R.A., Stembridge, G.E., Van Blaricom, L.O. and Gambrell, C.E. 1972. Effects of succinic acid-2,2-dimethylhydrazide on the color, firmness, and uniformity of processing peaches. J. Amer. Soc. Hort. Sci. 97: 485.
Byers, R.E. and Emerson, F.H. 1969. Effects of succinamic acid-2,2-dimethylhydrazide (Alar) on peach fruit maturation and tree growth. J. Amer. Soc. Hort. Sci. 94: 641.
Byers, R.E. and Emerson, F.H. 1973. Effects of SADH and ethephon on peach fruit growth and maturation. HortScience 8: 48.
Daniell, J.W. 1969. The effects of Alar on ripening and quality of peach fruits. Proc. Assoc. Southern Agr. Workers 66: 195.
Do, J.Y., Salunke, D.K. and Olson, L.E. 1969. Isolation, identification, and comparison of the volatiles of peach fruit as related to harvest maturity and artificial ripening. J. Food Sci. 34: 618.
Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11: 1.
Gambrell, C.E. Jr., Rhodes, W.H. and Sims, E.T. Jr. 1967. Some effects of four growth regulators on thinning, yield, size and maturity of 'Ranger' peaches when applied for one year and two years in succession. S.C. Agr. Expt. Sta. Hort. Res. Series 101, p. 1.
Senter, S.D. 1974. Effects of SADH on flavor and select physicochemical properties of freestone peaches. HortScience 9(1): 30.
Sims, E.T., Gambrell, C.E. Jr. and McClary, J.T. 1971. The influence of succinic acid-2,2-dimethylhydrazide on peach quality. J. Amer. Soc. Hort. Sci. 96: 527.
Van Blaricom, L.O. 1948. Effect of syrup density on the flavor rating of canned peaches. J. Amer. Soc. Hort. Sci. 50: 229.
USDA. 1971. U.S. peach industry. Part 1. Structures, trends and consumption projections to 1980. Report No. 200. Agric. Res. Ser. and Economic Res. Ser.

Ms received 1/27/75; revised 4/4/75; accepted 4/8/75.

M. PELEG and L. GÓMEZ BRITO
Instituto Venezolano de Investigaciones Tecnológicas e Industriales—INVESTI
Apartado 7668, Carmelitas, Caracas 101, Venezuela

A Research Note

THE RED COMPONENT OF THE EXTERNAL COLOR AS A MATURITY INDEX OF PAPAYA FRUITS

INTRODUCTION

EXTERNAL COLOR has traditionally been used as an index for the harvesting of papayas. The ripening of the papaya fruits is accompanied by the appearance of light longitudinal bands which turn yellow as ripening proceeds. However, the yellow coloration pattern is not necessarily restricted to the bands pattern only and yellow colored sites can appear almost anywhere on the papaya skin. Under field conditions the estimation of the coloration degree is usually done by personal judgment and therefore personal experience might introduce a crucial factor into the quality of post harvest ripening fruits. Guiding criteria offered by Akamine and Goo (1971) for the Solo variety suggest that fruits with initial coloration covering almost 6% of the outer surface area develop their full total soluble solids content. In a previous communication (Peleg and Gómez Brito, 1974), it was reported that the initial maximum yellow color intensity in terms of Hunter "b" units might provide an indication about the percentage and time of normal ripening of the Venezuelan papaya fruits. The mentioned indices, however, as well as other criteria offered for papayas (Papayas in Hawaii, 1970; Brukner and Kinch, 1968) require either a subjective estimation of the coloration degree, or the application of relatively sophisticated instrumental techniques which are hardly suitable under field conditions. Furthermore, the Carica papaya family is characterized by the phenomenon that on the same plant, fruits at every possible maturity stage—from flowers to full or over ripeness—can be found. Therefore, a sampling program and maturity evaluation of samples by sophisticated methods might be ruled out under practical conditions and every fruit ought to be inspected individually when picked from the plant or graded in a later stage.

A feasible way to minimize personal judgment and at the same time avoid the necessity of sophisticated equipment would be the use of a set of color standards for maturity grading of the papaya fruits. For their development, however, it would be necessary to determine the relationship between initial external color and post harvest ripening patterns. As the yellow component and lightness of the initial color (Peleg and Gómez Brito, 1974) could provide only part of the desired information, we studied the possibility of relating the maturity grades of papayas to the initial red component of their external color.

EXPERIMENTAL

Raw material

Fresh Venezuelan papaya fruits were collected in commercial plantations. Elongated, hermaphrodite-type of fruits ranging in weight between 3 and 6 kg were selected. External color of the fruit ranged from the stage where yellow bands were barely apparent up to the stage of completely yellow skin. The fruits were washed and packed in wooden cases with cloth shreds to avoid mechanical damage during the transport from the field to the laboratory. Initial color evaluation started about 24 hr after harvest and the fruits were left to ripen at room temperature (22–24°C).

Color evaluation

Color was evaluated by a Hunterlab Color Difference Meter model D25D2 calibrated with a standard yellow tile (L = 78.1; a = −2.2; b = 22.6). A plastic sheet having a round aperture of 20 mm diameter was placed in a way that its aperture was located in a central position with regard to the original aperture of the Hunter instrument. The Hunter color components (L, a and b) of various yellow color standards (Federal Standard No. 595) were measured with and without the above mentioned cover. From these data calibration equations were derived which enabled the calculation of the color components in terms of Hunter units from color measurements taken at the restricted sites.

The external color of the papayas was evaluated by placing the intact fruit under the sheet with the 20 mm aperture in a position in which the most apparently yellow field would be under test. Two to four fields were examined on each fruit and for each field, the fruit was moved slightly until maximum values were obtained on the digital controller of the Hunter instrument.

The internal color of the stored fruits was evaluated using longitudinal flesh slices. Maximum Hunter "a" values were determined using the full aperture of the Hunter instrument (Peleg and Gómez Brito, 1974).

Ripeness classification

Fruits after softening or after 14–15 days storage were analyzed for total soluble solids content by a refractometer, maximum red coloration of flesh by the Hunter instrument, and textural strength by penetration with a 5/16-in. plunger mounted on the moving arm of an Instron Universal Testing Machine Model TM. The penetration speed was 20 cm./min.

Fruits were classified according to Peleg and Gómez Brito (1974) to three ripeness levels: ripe, almost ripe and not ripe. The latter were based on combined levels of the TSS content, flesh color development and texture softness.

RESULTS & DISCUSSION

Determination of the external color of papaya

Yellow color determination of papaya with the standard Hunterlab Color Difference Meter represents certain difficulties. The major problem is that the yellow-colored sites, at least in the very initial stages of the color development, seldom occupy an area big enough to be wholly circumscribed by the original aperture of the standard Hunter instrument. Furthermore, the colored sites themselves might vary to a great extent with regard to size, shape and color uniformity; therefore, the evaluation of the various factors contributing to the total color measurement is extremely difficult.

In order to minimize such errors and to enable color measurements within restricted sites on the papaya skin, experiments were carried out using various plastic sheets having apertures down to 7 mm in diameter. However, the smaller the aperture, the less sensitive the measurements were, resulting in greater possible experimental errors. Due to these antagonistic factors a technical compromise was necessary and the 20 mm aperture was selected, enabling fairly sensitive measurements on one hand and complete coverage of the yellow sites for most fruits on the other.

Color measurements at same sites on the intact fruit and subsequently on a separated plane form showed that the curva-

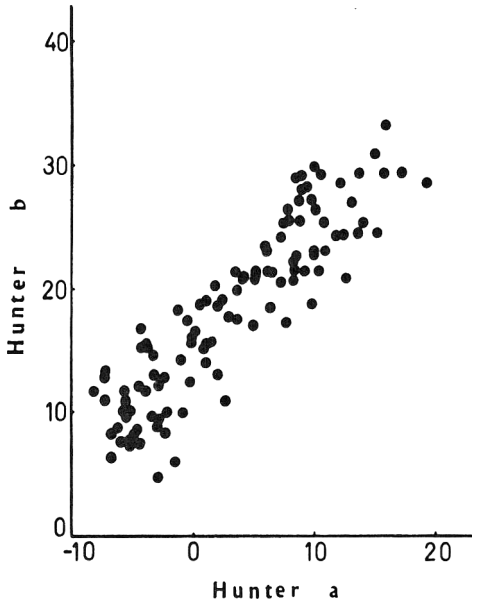


Fig. 1—Relationship between Hunter "a" and "b" values of papayas external color, determined at sites 20 mm in diameter.

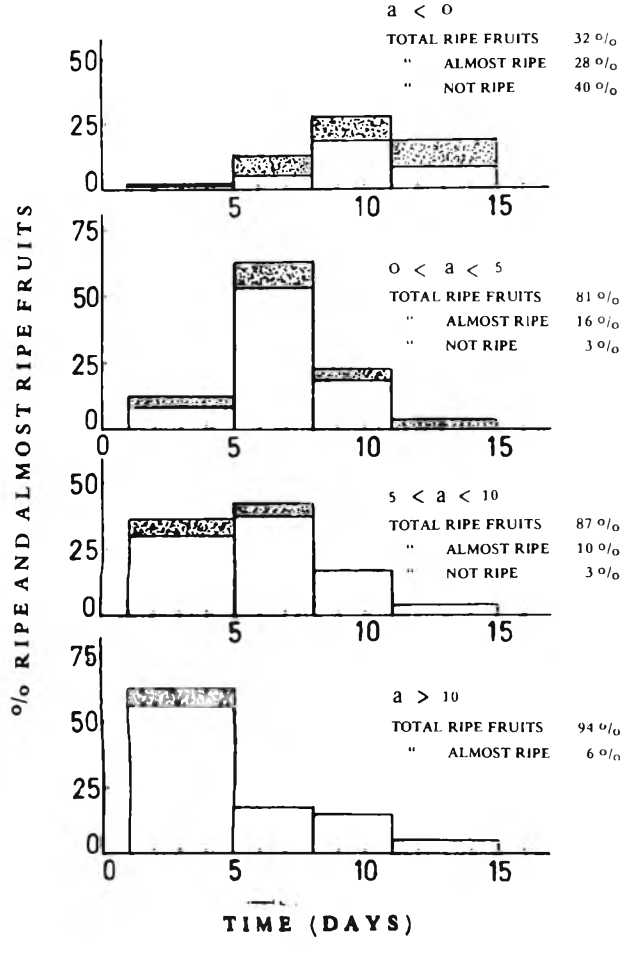


Fig. 2—Ripening of papaya fruits as a function of their initial red color component. (Light portions of the columns represent ripe fruits; dotted portions represent almost ripe fruits.)

ture of the fruit could affect the measurements in the order of up to 0.5 units and therefore could be considered a minor cause of experimental error.

Fruits at various stages of maturity—from the green unripe stage to fully ripe—were analyzed to their maximum yellow coloration. As could be expected (Peleg and Gómez Brito, 1974), the intensification of the yellow component "b" was accompanied by a lighter overall color which was represented by an increase of the Hunter "L" values.

The relationship between the red component "a" and the yellow component "b" in the 20 mm sites is demonstrated in Figure 1. Unlike the case of using the full aperture of the Hunter instrument (Peleg and Gómez Brito, 1974) it can be seen that the yellow coloration was accompanied by the introduction of a red component which gave it an apparent orange hue. The measurements, however, showed considerable dispersion which might be explained by color variations within the sites under test. It was especially noticed in the presence of small green spots which affected the "a" readings to a great extent. Because the Hunter "a" values represent by definition the color component on the Green-Red axis, even a minor contribution of a green element could considerably reduce the overall measured "a" values. It seems, therefore, that some of the sites which are represented in Figure 1 had apparently more intense orange hue than the experimental data showed.

Ripening and the initial red component

More than 200 fruits were classified according to their calculated Hunter "a" and "b" components at the most yellow sites and left to ripen at room temperature. When in a daily inspection it was observed that a fruit started to soften, it was left for another day and then classified to its maturity grade by the criteria suggested by Peleg and Gómez Brito (1974). After 14—15 days the remaining fruits were also analyzed in the same way. Results of the percentage and time of ripening as a function of the initial red component "a" in the most yellow sites are shown in Figure 2. It could be observed that the function was very similar in shape to the relationship based on the "b" values as previously reported. It could be seen that the higher the initial Hunter values, the greater the portion of fruits ripened normally. It was also observed that the time until ripening for the major part of the fruits was shorter for those having initially higher Hunter "a" values. Results also showed that initial positive Hunter "a" values (i.e., the presence of an apparent red component on the peel) were well correlated with normal ripening in terms of percentage of ripe fruits. Therefore, the level of the initial Hunter "a" value could be a fairly good criterion for safe harvesting of the fruits. With regard to ripening time, however, the distribution of the relationship was fairly wide. Though a clear shift towards shorter ripening time could be observed for the majority of fruits having higher initial Hunter "a" values, it was also observed that individual fruits, showing the same initial coloration intensity, could ripen at almost any time. This meant that the level of the initial Hunter "a" value could only indicate a trend but could not provide a satisfactory single index for ripening time prediction.

REFERENCES

Akamine, E.K. and Goo, T. 1971. Relationship between surface color development and total soluble solids in papaya. Hort. Sci. 6(6): 567.
Brukner, P.F. and Kinch, D.M. 1968. Force-deformation ratio as index of papaya maturation. Trans. ASAE 11: 437.
Papayas in Hawaii. 1970. Circular No. 436. Cooperative Extension Service, University of Hawaii.
Peleg, M. and Gómez Brito, L. 1974. External color as a maturity index of papaya fruits. J. Food Sci. 39: 701.
Ms received 2/11/75; revised 2/24/75; accepted 4/28/75.

This study was financed by the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT) (National Venezuelan Council of Scientific & Technological Research).

The authors express their thanks to Mrs. Helga and Mr. Peter Bastiansen for the donation of fruits for the research.

G. S. STOEWSAND, J. L. ANDERSON and R. C. LAMB
Departments of Food Science and Technology, Pomology and Viticulture
New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456

A Research Note

CYANIDE CONTENT OF APRICOT KERNELS

INTRODUCTION

CYANOGENETIC GLYCOSIDES yielding hydrocyanic acid upon hydrolysis are widely distributed within approximately 150 plant species. Amygdalin, probably the first known of these complex glycosides, is present in the kernels of stone fruits and was first isolated from the seed of bitter almond (Liener, 1966).

Although accidental cyanide poisonings from consumption of stone fruit kernels are not frequently reported, actual poisoning, especially of children, is a worldwide occurrence (Sayre and Kaymakcalan, 1964). Apparently many people like the taste of raw kernels. In addition, apricot, peach and cherry kernels have been utilized in Germany and the United States for the manufacture of a fixed oil, bitter-almond oil, and macaroon paste (Cruess, 1958). The purpose of this study was to investigate the cyanide content of kernels from specific cultivars of apricots.

EXPERIMENTAL

KERNELS of five cultivars of apricot, *Prunus armenica* L., were analyzed: Moorpark, an old English cultivar of unknown parentage; Summerland 4E-55-9 (Perfection open pollinated), a selection from the breeding program of the Research Station, Canada Department of Agriculture, Summerland, British Columbia; Veecot (Reliable open pollinated) a cultivar introduced by the Horticultural Research Institute of Ontario, Vineland, Ontario in 1965; Viceroy (Geneva × Naramata) also introduced by the Horticultural Research Institute of Ontario in 1965; Vineland 51175 (Perfection × Geneva) a selection from the breeding program at the Horticultural Research Institute of Ontario. The pits were removed from the fruit, air dried for 1 month, and stored at 0°C for 3 months prior to cracking for kernel excising. The kernels were ground in a laboratory mill and analyzed for hydrocyanic acid, after steam generated distillation by the fluorometric procedure of Jeffrey and Wiebe (1971). The method was slightly modified in that each sample in 5 vol of water was placed overnight at 50°C, and 10% tartaric acid was added prior to distillation for more efficient amygdalin hydrolysis.

RESULTS & DISCUSSION

AS SEEN IN TABLE 1, the cyanide content of the five cultivar kernels ranged from about 12 to 177 mg/100g. Tasting the kernels showed that only those of Moorpark containing significantly lowered ($P < 0.05$) cyanide were sweet, while the other cultivar kernels had a strong bitter flavor. This bitterness has been shown to be due to the relatively large quantities of amygdalin (McCarty et al., 1952). Although the Summerland 4E-55-9 cultivar had the highest amount of cyanide ($P < 0.05$), and therefore amygdalin, the taste seemed to have about the same level of bitterness as the lower cyanide containing kernels of Vineland 51175 and Veecot.

The amount of cyanide in apricot kernels may vary with cultivation practices, i.e., increase with increased fertilization, irrigation and use of certain pesticides (Gunders et al., 1969), as well as cultivars. Ingested cyanide salts have a minimum lethal dose of about 2–4 mg/kg body weight, but even larger doses have occasionally been followed by spontaneous recovery (Rieders, 1965). The problem of children consuming apricot kernels in potentially toxic amounts may be of public health concern in Israel (Yatziv, 1969) and Turkey (Sayre and Kaymakcalan, 1964) as well as other apricot producing areas of the world. The industrial hazards of cyanide released in the processing of apricot kernels for the manufacture of oils and flavoring pastes has been investigated (Grabois, 1954).

Apricot pits yield about 24% of kernels which is higher than most other stone fruits. The utilization of waste fruit pits has become an important industry, primarily in California (Cruess, 1958). Thus, development and cultivation of low amygdalin, or sweet kernel apricot cultivars would yield a double benefit: Decreased public and occupational health hazards and increased marketability of a by-product. Consideration of the quality of usable by-products is important to the entire fruit and vegetable industry.

Table 1—Cyanide content of air-dried apricot kernels

Cultivar	CN^{-a} (mg/100g)
Moorpark	11.7 ± 0.5[1]
Summerland 4E-55-9	177.1 ± 11.1[3]
Veecot	143.5 ± 9.9[2]
Viceroy	164.0 ± 10.9[2,3]
Vineland 51175	132.5 ± 9.0[2]

[a] Mean values ± standard error of 4 replicates/cultivar. The content of cyanide exhibiting significant differences ($P < 0.05$) are indicated by having a different superscript number, e.g., Moorpark significantly lower than all other cultivars.

REFERENCES

Cruess, W.V. 1958. "Commercial Fruit and Vegetable Products." McGraw-Hill, New York.
Grabois, B. 1954. Exposure of hydrogen cyanide in processing of apricot kernels. Monthly Rev. N.Y. State Dept. of Labor 33: 33.
Gunders, A.E., Abrahamov, A., Weisenberg, E., Gertner, S. and Shafran, S. 1969. Cyanide poisoning following ingestion of apricot (Prunus armenica) kernels. J. Isr. Med. Ass. 76: 563.
Jeffrey, J.G. and Wiebe, L.I. 1971. Cyanide determination on the fruit of ornamental plants grown in Saskatchewan. Can. J. Phar. Sci. 6: 53.
Liener, I.E. 1966. Cyanogenetic glycosides. In "Toxicants Occurring Naturally in Foods," p. 58. NAS/NRC Publ. 1354, Washington, D.C.
McCarty, C.D., Lesley, J.W. and Frost, H.B. 1952. Bitterness (benzaldehyde content) of kernels of almond-peach F, hybrids and their parents. Proc. Am. Soc. Hort. Sci. 59: 254.
Rieders, F. 1965. Noxious gases and vapors. 1. Carbon monoxide, cyanides, methemoglobin and sulfhemoglobin. In "Drill's Pharmacology in Medicine," Ed. DiPalma, J.R., p. 939. McGraw-Hill, New York.
Sayre, J.W. and Kaymakcalan, S. 1964. Cyanide poisoning from apricot seeds among children in Turkey. New Eng. J. Med. 270: 1113.
Yatziv, A.S. 1969. Cyanide intoxication in childhood after ingesting apricot kernels. J. Isr. Med. Ass. 76: 535.
Ms received 3/13/75; revised 5/7/75; accepted 5/14/75.