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

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ABSTRACTS:
IN THIS ISSUE

FLAVOR AND CHEMICAL CHARACTERISTICS OF CONVENTIONALLY AND MICROWAVE REHEATED PORK. K.K. PENNER & J.A. BOWERS. *J. Food Sci.* 38, 553–555 (1973)–Sensory evaluations and chemical measurements were made on freshly cooked (conventionally heated), conventionally-reheated (after 5 wk frozen storage), and microwave-reheated pork loin muscle. Freshly cooked and microwave-reheated pork had sweeter aroma and less metallic flavor than conventionally-reheated pork. Microwave reheated pork was less juicy than pork heated by the other treatments. Freshly cooked pork had the lowest TBA values and the highest moisture content. Heating treatment did not affect ninhydrin-reactive compounds or nitrogen content in the various extracted protein fractions.

SOME ASPECTS OF RAW MEAT TENDERNESS. A Study of Some Factors Affecting Its Change with Cooking and a New Means of Measurement. R.W. PURCHAS. *J. Food Sci.* 38, 556–559 (1973)–A hand-operated biting instrument which is quick and simple to use was developed to investigate the possibility of assessing the tenderness of raw meat at the carcass or wholesale cut stage. Comparison with the Warner-Bratzler shear device revealed an overall close relationship for cooked meat ($r = 0.96$, $n = 66$), greater response in terms of proportional change in readings per unit change in tenderness ($P < 0.01$) but a greater variability in a series of readings on meat cores from the same steaks ($P < 0.001$). Relationships between readings on raw and cooked samples were low. It is suggested that this may be due to the fact that the effects of cold shortening and aging on cooked meat tenderness are not apparent on the tenderness of raw meat. The response to cold shortening in terms of tenderness appears to develop as meat is heated from 50°C to 60°C while the response to aging is already partially developed at a cooking temperature of 50°C. It is concluded that although the instrument may be better than existing methods under some situations due to its simplicity, it is unlikely to be useful on raw meat.

FREEZING OF RAW BEEF: INFLUENCE OF AGING, FREEZING RATE AND COOKING METHOD ON QUALITY AND YIELD. B. JAKOBSSON & N. BENGTSSON. *J. Food Sci.* 38, 560–565 (1973)–The combined effects of aging, freezing rate, frozen storage and cooking method on beef quality and yield were studied in a multifactorial experiment, using 1.5 cm slices of LD muscles from young steers. Aging for 2 wk resulted in significantly more tender, but slightly less juicy frozen meat than aging for 4 days. Prolonged frozen storage gave tougher meat and a larger tenderness difference between aging times. Freezing rates of 13, 2.0 and 0.04 cm/h differed but little in their effect on sensory quality, but slightly lower yield and redness value were noted for the lowest rate. Pan frying directly from the frozen state resulted in slightly higher juiciness and cooking yield, with larger differences in yield between freezing rates, than cooking after previous thawing. Significant interactions were noted for cooking method × freezing rate and for aging time × frozen storage time.

TISSUE DISTRIBUTION AND METABOLISM OF AFLATOXIN B_1-^{14}C IN LAYER CHICKENS. M.S. MABEE & J.R. CHIPLEY. *J. Food Sci.* 38, 566–570 (1973)–The effects of administering low levels of aflatoxin B_1-^{14}C by crop intubation daily for 14 days to layer chickens were determined. Studies on the distribution of ^{14}C in the blood, selected organs, tissues, eggs and excreta were conducted. No toxic effects were observed in layer chickens during the course of the experiment. The layer chickens excreted 92.15% of the ^{14}C administered. Of the ^{14}C retained, 19.5, 16.1, 3.9, 7.2, 26.4 and 26.9% were detected in the blood, liver, heart, gizzard, breast and leg, respectively. No radioactivity was detected in egg samples collected. Chemical assay of those samples demonstrating radioactivity revealed that 81.2% of the radioactivity in these substrates was soluble in aqueous extracts while approximately 10% was extractable by classical extraction procedures. Treatment of the aqueous extracts for conjugated steroids by treatment with Beta-glucuronidase revealed that 31.5% of the ^{14}C detected in the aqueous extract was a liberated glucuronide conjugate of aflatoxin M_1-^{14}C.

COMPOSITION AND PROPERTIES OF EXTRUDED, TEXTURIZED POULTRY MEAT. J.C. ACTON. *J. Food Sci.* 38, 571–574 (1973)–Hand deboned leg and thigh tissues from broilers and mechanically deboned broiler neck meats were extruded and texturized using a dry heat process. During heat processing of all meat tissues, there was a significant ($P < 0.05$) reduction of moisture content and significant increases in fat and protein levels as heating time increased. Meat strands from the texturization process became firm, possessing structural integrity as determined by the binding strength among meat tissue particles and strand resistance to shearing. Significant ($P < 0.05$) increases were observed in tissue water-holding capacity and emulsion stabilizing capacity although salt-soluble protein extractability was significantly ($P < 0.01$) reduced by the heating during texturization.

POSTMORTEM QUALITY CHANGES IN ICED PACIFIC SHRIMP (Pandalus jordani). S.C. FLORES & D.L. CRAWFORD. *J. Food Sci.* 38, 575–579 (1973)–The postmortem biochemical changes in intact raw shrimp during iced storage were investigated and the relationship between these changes and the quality of the cooked product was evaluated. Changes in pH, microbial numbers, total and nonprotein nitrogen, tyrosine and carotenoid pigment content, proteolytic and polyphenolase enzyme activities and levels of trimethylamine-oxide, trimethylamine, dimethylamine and formaldehyde were determined. Chemical changes were shown to be mediated by a combination of bacterial action and endogenous enzymatic activity. The washing action of melting ice could have played a major role. Organoleptic quality as shown by flavor panel evaluations progressively declined during an 8-day storage period. Dimethylamine and formaldehyde levels in intact shrimp progressively increased as flavor panel scores decreased and may offer additional sensitive indices of cooked meat quality.

BACTERIAL COUNTS AND RANCIDITY ESTIMATES OF STORED QUICK-SALTED FISH CAKES. F.R. DEL VALLE, J. HINOJOSA, D. BARRERA & R.A. DE LA MORA. *J. Food Sci.* 38, 580–582 (1973)–Total plate, halophilic and staphylococcal counts were determined in quick-salted fish cakes after 0, 1, 2 and 3 months' storage without packaging at an ambient temperature of 35–40°C. A rancidity (thiobarbituric acid or TBA) index was also determined in sun-dried and tunnel-dried cakes, before and after desalting, stored under the same conditions for the same lengths of time. Species studied were skipjack, mullet, Spanish mackerel and shark from the Gulf of California. It was found that total plate and halophilic counts behaved similarly, initially increasing with time, passing through a maximum, and then decreasing. Counts increased with decreasing salt and increasing moisture contents of the cakes. Maximum counts obtained were of the order of 10^6 per gram for cakes made from shark, while negative counts were obtained with cakes made from skipjack after 3 months. No growth of staphylococci was obtained at any time in any of the plated dilutions (10^{-1} to 10^{-6}).

Rancidity of the cakes increased with time, depending upon the following factors: oil content of the species; degree of unsaturation of the oil; drying time of the cakes; presence or absence of hematin pigments; presence or absence of sunlight when drying. Some of the rancid components in the cakes were removed by desalting in boiling water. In a limited taste panel evaluation of the cakes, order of preference was found to correlate well with decreasing rancidity.

MICROWAVE FINISH DRYING OF POTATO CHIPS. V.L. PORTER, A.I. NELSON, M.P. STEINBERG & L.S. WEI. *J. Food Sci.* 38, 583–585 (1973)–The effect of microwave finish drying of potato chips on their texture, color and oil content was studied. The intermediate moisture content (IMC) of the chips before microwave application and the reducing sugar content of the raw potatoes were the primary variables. The slices were individually submerged in oil at 320°F to various IMC and finish dried with 2500 watts microwave power for 1–3 min. Chip color was rated against PCII Color Standards. Texture was evaluated subjectively by a panel and objectively by the L.E.E. Kramer Shear Press. Oil determination was made by Soxhlet extraction. The results showed that potato chips removed from the oil at IMC above 13% were unacceptably tough after microwave drying. Potatoes containing more than 0.9% reducing sugar had to be removed from the oil at IMC above 13% in order to obtain acceptable color of the microwave-finished product. Therefore, microwave finishing raises the limiting reducing sugar content from about 0.4 to about 0.9%. Oil content of microwave-finished chips was 90% that of conventional controls.

FLAVOR QUALITY AND STABILITY OF POTATO FLAKES. Effects of Raw Material and Processing. G.M. SAPERS, O. PANASIUK, F.B. TALLEY & R.L. SHAW. *J. Food Sci.* 38, 586–589 (1973)–Potato flakes, produced experimentally to test the effects of raw material and processing variables on storage stability, were examined by a trained taste panel and by GLC analysis for evidence of oxidative and other flavor changes. Air-packed samples produced from unpeeled potatoes, defective raw material and potatoes cooked with excess water were more highly oxidized than conventional flakes after 12 months storage at 23°C. Raw material sugar content and piece size had no effect on stability. The flavor of nitrogen-packed samples was not affected by storage for 12 months at 23°C.

PILOT PLANT EVALUATION OF INDIVIDUAL QUICK BLANCHING (IQB) FOR VEGETABLES. J.L. BOMBEN, W.C. DIETRICH, D.F. FARKAS, J.S. HUDSON, E.S. DE MARCHENA & D.W. SANSHUCK. *J. Food Sci.* 38, 590–594 (1973)–A pilot plant IQB blancher was field tested at a California freezing plant. The system included: a warming and drying preconditioning unit, a steam chamber for single layer belt heating, a deep moving-bed holding chamber for temperature equilibration and enzyme inactivation and an air-water spray cooling unit. IQB with and without preconditioning were compared to conventional steam blanching at equivalent levels of enzyme inactivation in green beans, lima beans, Brussels sprouts and green peas. Effluent volume and total-solids concentration were measured. Product yields were determined by measuring residual peroxidase activity, chlorophyll conversion and ascorbic acid content as well as by sensory panel testing for color and flavor. Results showed that vegetables blanched with IQB and IQB with preconditioning were essentially the same in quality as those blanched at commercial conditions. Solids loss in the effluent was substantially less with IQB and preconditioning than with conventional steam blanching.

NUTRITIVE CONTENT OF CANNED TOMATO JUICE AND WHOLE KERNEL CORN. R.P. FARROW, F.C. LAMB, E.R. ELKINS JR., N. LOW, J. HUMPHREY & K. KEMPER. *J. Food Sci.* 38, 595–601 (1973)–Most of the information on the nutritive value of canned foods in the technical literature was obtained 25–30 years ago. A resurvey of the nutrient content of canned tomato juice and canned whole kernel corn has been carried out on a nation-wide basis with sampling methods directly comparable to the older survey procedures. Although there have been extensive changes in varietal types and processing methods during the past 30 yr the mean nutritional values for these products have changed very little. Comparison of the nutrient values for these products listed in USDA Handbook No. 8 reveals no differences that could not be attributed to seasonal variations. A possible exception is the ascorbic acid content of tomato juice, which for the 1969 season was found to be somewhat lower than the listed value. While sample-to-sample variation is small in terms of the RDA of most nutrients, it can be critical in terms of the regulatory requirements proposed for nutritional labeling. Under this proposal canners would have to grossly "under declare" some nutrients such as ascorbic acid in order to ensure compliance. The indication is that mean values derived from adequate sampling programs are relatively stable although the population may encompass wide ranges of variability. For this reason, nutrient labeling declarations based upon properly constituted average values provide the most practicable solution to the inherent technical problems in the proposed nutritional labeling regulation.

COMPOSITION OF THREE FOOD PRODUCTS CONTAINING DEFATTED CORN GERM FLOUR. C.W. BLESSIN, W.J. GARCIA, W.L. DEATHERAGE, J.F. CAVINS & G.E. INGLETT. *J. Food Sci.* 38, 602–606 (1973)–Incorporation of defatted corn germ flour in a standard cookie formula improved amino acid and mineral composition of the baked product. Similar effects, but of less magnitude, occurred in corn muffins. Addition of defatted germ flour to ground beef increased yield of the broiled meat product. Amino acid composition remained essentially constant, but mineral composition changed considerably.

STUDIES ON THE UTILIZATION OF COCONUT MEAL. A New Enzymic-Chemical Method for Fiber Free Protein Extraction of Defatted Coconut Flour. M.R. MOLINA & P.A. LACHANCE. *J. Food Sci.* 38, 607–610(1973)–A simple enzymic-chemical protein extraction process was developed. Proteases rather than carbohydrases were more efficient for the enzymatic treatment. Dilute NaOH was more effective than dilute HCl for the complementary chemical treatment. The combined treatments were more efficient than each of the two separately. The method devised was equally effective when applied to five coconut meals obtained through different oil extraction techniques. The partial hydrolysis of the protein during the proteolytic enzyme treatment appears to enhance the protein extractability by the complementary chemical treatment. Further, the resultant product is free of fiber.

ISOLATION AND CHARACTERIZATION OF MICROORGANISMS INVOLVED IN THE FERMENTATION OF TRINIDAD'S CACAO BEANS. K. OSTOVAR & P.G. KEENEY. *J. Food Sci.* 38, 611–617 (1973)–In order to isolate, identify and characterize the microflora of cacao beans before, during and after fermentation and locate possible sources contributing to microbial contamination, cacao beans from the Centeno and San Louis Estates in Trinidad were investigated. Prior to fermentation, the interior and exterior of the pods, hands of employees, utensils, dried pulp material of the sweatboxes and finally fruitflies (*Drosophila melanogaster*) were studied microbiologically. At Centeno Estate, beans were sampled at 5, 45 and 90 cm depths at 8-hr intervals for the first 72 hr and every 12 hr thereafter for 7 days. Sampling at San Louis Estate was carried out at 24 hr intervals for the same period. The changes in microbial population of the beans sampled at Centeno Estate ranged from 1.48×10^5/g at 0 hr to 4.1×10^5/g at the completion of the fermentation, whereas, at San Louis Estate they ranged from 6.8×10^4/g to 9.2×10^5/g during the same period. Taxonomical studies of isolates obtained during the fermentation period revealed the identification of 44 microorganisms at both Estates. Yeasts *Zymomonas mobilis* and several species of lactic acid organisms dominated the flora during the early stages of fermentation. As the fermentation progressed, these and other isolates were taken over by several species of genus *Bacillus*. Microbiological examination of dried and polished beans resulted in the identification of 22 organisms at Centeno Estate and 15 organisms at San Louis Estate.

ABSTRACTS:
IN THIS ISSUE

IRON-FORTIFIED SYRUP BLENDS: PREPARATION, CHARACTERISTICS, APPLICATION. G.N. BOOKWALTER, L.T. BLACK & K.A. WARNER. *J. Food Sci.* 38, 618–622 (1973)–Stable iron-containing syrups were developed to provide a liquid iron source that could be conveniently added to foods. Iron-fortified syrups were prepared by combining either corn syrup or sucrose, or both, with water, heating to boiling, cooling to 180–200°F and blending in an aqueous iron solution. Syrups were all-sucrose (67% solids); blends of 15% sugar (sucrose) with either regular or high-conversion corn syrups (75% solids). Iron sources were ferric ammonium citrate, ferrous sulfate, ferric choline citrate and ferrous gluconate at the available iron level of 0.015% (100 mg/pint). These iron-fortified syrups were stable during storage for 2 months at 120°F, 6 months at 100°F and 1 year at 77°F, except for combinations of ferrous forms with blends containing either 15% sugar and regular corn syrup or ferrous sulfate in all-sugar. Although flavor evaluations indicate that iron is readily detectable, iron-fortified syrups had satisfactory flavors before and after storage. These fortified syrups seem suitable for enrichment of infant formulas.

NEW COMPUTATIONAL PROCEDURE FOR DETERMINING THE APPARENT THERMAL DIFFUSIVITY OF A SOLID BODY APPROXIMATED WITH AN INFINITE SLAB. K-i. HAYAKAWA & A. BAKAL. *J. Food Sci.* 38, 623–629 (1973)–A computational procedure was developed for determining the thermal diffusivity of food. For this development, a theoretical formula for estimating heat conduction in an infinite slab was used. This formula was derived by assuming that temperatures on the surface of a slab change arbitrarily with time and also that its thermal diffusivity is temperature independent. A thermal diffusivity value of a solid sample is determined through estimation of the following specific diffusivity value: The specific value, which gives the least sum of squares of differences between temperatures calculated by the derived formula and those determined through heat transfer experimentation. A set of FORTRAN IV computer programs was prepared to determine diffusivity values of solids. The diffusivity values of ice, frozen grapefruit juice, frozen 50% sucrose solution and frozen ground beef were determined by using these computer programs. Experimental temperature data, which were used for this determination, were obtained by utilizing specially fabricated rectangular cells.

TEMPERATURE DISTRIBUTIONS DURING HEAT/HOLD PROCESSING OF FOOD. M.K. LENZ & D.B. LUND. *J. Food Sci.* 38, 630–632 (1973)–The unsteady-state differential equations for conduction heating were solved for the heat/hold process. The heat/hold process consists of heating the food to a certain mass average temperature and then adiabatically holding the piece until the desired reactions are complete. Charts for predicting the center and mass average temperatures for three basic geometries (infinite cylinders, infinite slabs and spheres) are presented along with methods to combine these solutions for other geometries. The use of these charts in blanching situations is shown.

IMPROVED REVERSE OSMOSIS PERMEATION BY HEATING. L.E. MONGE. B.J. McCOY & R.L. MERSON. *J. Food Sci.* 38, 633–636 (1973)–At higher temperatures reverse osmosis membrane coefficients increase and cause permeation rates to increase. Diffusion coefficients also increase and viscosity coefficients decrease causing concentration polarization to decrease and improve permeation. Experiments were performed with 15% (w/w) sucrose solutions in tubular cellulose acetate reverse osmosis membranes at 500 psig for low flow rates (Reynolds numbers between 10 and 1000). Depending upon the flow rate and mode of heating, raising the temperature from 25 to 35°C increased permeation rates up to 20%, and from 25 to 45°C up to 55%. Two methods of heating were tested: preheating the entire feed to the system, and heating the boundary layer at the membrane surface. Comparisons with theory are discussed.

COMPARISON OF THE PROTEIN NUTRITIONAL VALUE OF TVP, METHIONINE-ENRICHED TVP AND BEEF FOR ADOLESCENT BOYS. M. KORSLUND, C. KIES & H.M. FOX. *J. Food Sci.* 38, 637–638 (1973)–The protein nutritional value of an extruded soybean product resembling beef (TVP), a 1% DL-methionine-enriched TVP product and beef for adolescent boys was compared. The experimental diets supplied food energy to approximately maintain weight and were supplemented with vitamins and minerals. Mean nitrogen balances of subjects fed 4.0g nitrogen as TVP, methionine-enriched TVP, or beef in successive 6-day periods were −0.08, +0.48 and +0.32g per day, respectively. Nitrogen retention was significantly higher in response to methionine-enriched TVP or beef than to TVP alone.

INHIBITION OF RIPENING AND INDOLE-3-ACETIC ACID OXIDASE OF BANANA FRUIT BY p-2,4-CHLOROPHENOXY-ISOBUTYRIC ACID. N.F. HAARD. *J. Food Sci.* 38, 639–641 (1973)–p-2,4-dichlorophenoxy-isobutyric acid (p-CPIB) was observed to repress the ripening of green banana fruit after injection into the peel-pulp juncture at concentrations ranging from 10^{-5} to 10^{-3}M. Application of 10^{-2}M p-CPIB resulted in rapid necrosis at the site of injection and accelerated ripening at distal areas of the banana. Repression of ripening occurred regardless of whether fruit were gassed with exogenous ethylene. Indole-3-acetic acid oxidase isolated from the pulp of banana fruit was inhibited by concentrations of p-CPIB which repressed ripening. In addition, injection of indole 3-acetic acid and 2,4-dichlorophenoxy acetic acid into the peel-pulp juncture delayed ripening for up to 15 days at 10^{-3}M. It is suggested the p-CPIB affected ripening in banana fruit by blocking the oxidative metabolism of endogenous auxins.

CHILLING INJURY IN GREEN BANANA FRUIT: CHANGES IN PEROXIDASE ISOZYMES IN SOLUBLE AND PARTICULATE POOLS. N.F. HAARD & D. TIMBIE. *J. Food Sci.* 38, 642–645 (1973)–Green banana fruit manifested severe chilling injury after 5–7 days storage at 5°C and 80% relative humidity. (Severe chilling injury is described here as a failure of the fruit to ripen on removal to conditions conducive to normal ripening.) The recoverable "wall bound" peroxidase fraction exhibited a sharp decline during this initial period of stress. Subsequent storage of severely chilled fruit at 5°C resulted in a gradual increase in the "soluble" peroxidase pool and a rise in "wall bound" peroxidase to levels comparable to normal green fruit. After 30 days storage of fruit at 5°C, the "soluble" and "wall bound" fractions of peroxidase peaked and declined rapidly in the latter pool. The increase in peroxidase of cold stressed fruit involved qualitative changes in isozyme species that differed from those isozymes which emerge during normal fruit ripening. It is suggested that the initial decrease in "wall bound" peroxidase results in a loss in capability of the tissue to adapt to low temperature stress. The belated increase in peroxidase activity may be associated with a typical "cold hardening" response which develops only after an irreversible or "plastic strain" damage has occurred.

CHARACTERISTICS OF PECTINS ISOLATED FROM SOFT AND FIRM FLESHED PEACH VARIETIES. Y.S. CHANG & C.J.B. SMIT. *J. Food Sci.* 38, 646–648 (1973)–Pectins obtained from ripe and unripe samples of Elberta (melting fleshed variety) and Babygold 6 (firm fleshed variety) peaches had methoxyl levels ranging from 11.4–12.1% and anhydrogalacturonic acid levels between 84–90%. The apparent molecular weight of the pectin from ripe Elberta peaches was very low compared with that of the other samples, resulting in a low breaking pressure of jelly prepared with this sample. Jelly grades ranged between 102–123 and it is suggested that these low values were a result of the presence of acetyl groups present in peach pectins and their relatively low apparent molecular weights and high methoxyl levels.

ANTHOCYANIN PIGMENTS OF SOUR CHERRIES. A.J. SHRIKHANDE & F.J. FRANCIS. *J. Food Sci.* 38, 649–651 (1973)–Fresh fruits of tart cherries (Prunus cerasus L. var. Montmorency) were extracted with acidic methanol. The anthocyanin pigments were isolated and purified by conventional paper chromatography. They were identified by spectral and R_f data and by acid hydrolysis. The pigments were cyanidin-3-glucosylrutinoside, cyanidin-3-glucosylsambubioside, cyanidin-3-sophoroside, cyanidin-3-rutinoside, cyanidin-3-glucoside and peonidin-3-rutinoside. No free cyanidin or peonidin was found. Cyanidin-3-glucosylsambubioside, reported in cherries for the first time, was also found in the varieties of English Morello, Early Richmond and Meteor.

GINGER RHIZOME: A NEW SOURCE OF PROTEOLYTIC ENZYME. E.H. THOMPSON, I.D. WOLF & C.E. ALLEN. *J. Food Sci.* 38, 652–655 (1973)–The proteolytic activity of ginger rhizome was studied with bovine serum albumin (BSA), collagen and actomyosin as substrates. A semipurified, powdered enzyme preparation was prepared by buffer extraction of an acetone powder of ginger rhizome and subsequent acetone precipitation of the proteolytic principle from the buffer extract. With 3% BSA as substrate, a relatively high proteolytic activity occurred over a pH range of 4.5–6.0, with an optimum pH of 5.0. The optimum temperature for proteolysis of BSA was 60°C during a 10 min reaction time, with rapid denaturation of the enzyme occurring at 70°C. NaCl in conc up to 10% produced about a 20 and 50% reduction in proteolysis of collagen and BSA, respectively. The ginger protease was protected by dithiothreitol during extraction and reaction, indicating the involvement of -SH groups at the active site. The analyses of soluble peptide amino acids or terminal amino acids suggest that the proteolysis of collagen is many fold greater than that of actomyosin. The combined proteolysis of these two muscle protein fractions by the ginger protease resulted in significantly more tender meat. According to conventional nomenclature, "Zingibain" is the proper name for this proteolytically active principle in *Zingiber officinale roscoe* or ginger rhizome which is commonly referred to as gingerroot. For meat applications, a possible advantage of zingibain over papain and ficin is the greater proteolysis of collagen in comparison to actomyosin. When compared to reported values for bromelain, zingibain has a higher optimum activity temperature, which is desirable in some applications.

MEASUREMENT OF CHLOROGENIC ACID AND FLAVONOL GLYCOSIDES IN APPLE JUICE BY A CHROMATOGRAPHIC-FLUOROMETRIC METHOD. J. VAN BUREN, L. de VOS & W. PILNIK. *J. Food Sci.* 38, 656–658 (1973)–Methods are described that measure chlorogenic acid at concentrations as low as 1 mg/100 ml of juice and flavonol glycosides at levels as low as 0.2 mg/100 ml of juice. Results are given for several apple juices, indicating concentrations of chlorogenic acid ranging from 12–31 mg/100 ml juice. Flavonol glycosides in conventional juices were 0.4–0.7 mg/100 ml, while special juices had 6.5–8.5 mg/100 ml.

SPECTRAL CHARACTERISTICS OF THREE VARIETIES OF FLORIDA ORANGE JUICE. D.R. PETRUS & M.H. DOUGHERTY. *J. Food Sci.* 38, 659–662 (1973)–The combined ultraviolet and visible absorption spectral characteristics of the juices of Florida Hamlin, Pineapple and Valencia oranges, harvested at approximately 2-wk intervals, were all found to be similar in shape but different in peak absorbance. Absorption maxima, recorded on alcoholic solutions of the juices, were observed at 465, 443 and 425 nm of the visible spectrum and at 325, 280 and 245 nm of the ultraviolet spectrum. Processing variables, such as maturity and extractor pressure, appeared to influence absorbance intensities.

A STUDY ON SURVIVAL OF Staphylococcus aureus IN DARK AND MILK CHOCOLATE. K. OSTOVAR. *J. Food Sci.* 38, 663–664 (1973)–Dark and milk chocolate bars were inoculated with *Staphylococcus aureus* to establish an initial population of approximately 10^2, 10^4 and 10^6 cells per gram. Samples were stored at room temperature and examined for the survival of staphylococci at 2-day intervals for the first 6 days and every 8 days thereafter. When samples were inoculated with 10^2 cells per gram, absence of staphylococci was observed after 2 days in dark and 14 days of storage in milk chocolate. Bars with 10^4 cells per gram were free of cells after 38 days in dark and 86 days of storage in milk chocolate. Dark chocolate bars with 10^6 cells per gram, were staphylococci free after 86 days of storage; whereas the milk chocolate bars with same level of inoculation were shown to be staphylococci free after 110 days of storage.

STRUCTURAL FUNCTIONS OF TASTE IN THE SUGAR SERIES: EFFECTS OF AGLYCONES ON THE SENSORY PROPERTIES OF SIMPLE GLYCOSIDE STRUCTURES. G.G. BIRCH & M.G. LINDLEY. *J. Food Sci.* 38, 665–667 (1973)–Reports the sensory properties of a number of glycosides of increasing chain length and size of aglycones, all in the favored Cl conformation. Shows bitterness appears to increase in intensity as molecular weight of the aglycone increases. Hydroxy functions which contribute to the intense sweetness of β-D-fructopyranose are also discussed.

POLYSACCHARIDE 13140: A NEW THERMO-GELABLE POLYSACCHARIDE. H. KIMURA, S. MORITAKA & M. MISAKI. *J. Food Sci.* 38, 668–670 (1973)–Polysaccharide 13140, a curdlan-type polysaccharide produced from a culture filtrate of *Alcaligenes faecalis* var. myxogenes strain NTK-u, has the unique property of forming a gel on heating. This polysaccharide is composed of β-1,3 linked glucose residues. When heated, aqueous suspension of the polysaccharide forms an elastic gel which is thermally irreversible, heat stable and acid stable. The texture properties of the polysaccharide gel lie in between agar-agar gel and gelatin gel. Potential applications of the polysaccharide in food processing are quite diverse and promising because it can improve such properties as viscoelasticity, palatability, binding quality, water holding capacity, heat stability and compatibility.

STUDIES ON MECHANISMS OF RETENTION OF VOLATILE IN FREEZE-DRIED FOOD MODELS: THE SYSTEM PVP-n-PROPANOL. J. CHIRIFE, M. KAREL & J. FLINK. *J. Food Sci.* 38, 671–674 (1973)–Retention of C^{14}-labeled n-propanol was studied in a freeze-dried system containing polyvinylpyrrolidone (PVP). Variables affecting retention during freeze drying were: initial concentrations of PVP and n-propanol, rate of freezing and sample thickness. Rehumidification above the BET monolayer value resulted in losses of propanol which increased with increasing water content. Behavior of the model system based on PVP is consistent with the "microregion volatile entrapment theory." The PVP system (and perhaps polymeric carbohydrates) differ from low molecular weight carbohydrates: water sorption below the BET monolayer is reduced by entrapped volatile, and absolute level of retention is lower.

PINK DISCOLORATION IN CHEDDAR CHEESE. S. GOVINDARAJAN & H.A. MORRIS. *J. Food Sci.* 38, 675–678 (1973)–Pink material isolated from defective Cheddar cheeses consisted of norbixin associated

ABSTRACTS:
IN THIS ISSUE

with phospholipid and casein. UV spectra and polyacrylamide gel electrophoretic studies indicated that the associated casein fraction was mainly Beta casein with at least three additional unidentified peptide components. The presence of norbixin was confirmed by chromatographic and spectral studies. Evidence for the phospholipid part of the complex is circumstantial and needs further confirmation. Model system studies indicated that a localized production of hydrogen sulfide can cause a microfine precipitation of norbixin due to a decrease in pH. Phospholipid may act to prevent the resolubilization of norbixin which appears as pink zones in the cheese.

IDENTIFICATION AND CHARACTERIZATION OF THE MICROFLORA AND SPOILAGE BACTERIA IN FRESHWATER CRAYFISH, Procambarus clarkii (Girard). N.A. COX & R.T. LOVELL. *J. Food Sci.* 38, 679–681 (1973)–Crayfish tails were collected from two commercial peeling plants in South Louisiana and stored at 0° and 5°C. Initially and after various periods of storage, bacteria were isolated from the peeled tails, identified generically and subsequently classified on the basis of their ability to produce "spoilage" in sterile crayfish tail flesh. *Micrococcus, Staphylococcus, Alcaligenes* and *Achromobacter* were the predominant genera in the fresh samples of tails. *Achromobacter* predominated during early storage and *Pseudomonas* and *Achromobacter* were the dominating organisms in the spoiled tails. Of the 280 isolated bacteria, 22.1% were classified as "rapid spoilers," 16.4% were "slow spoilers" and 61.5% were "nonspoilers." Most of the spoilers were *Pseudomonas*, while an appreciable number belonged to *Achromobacter.*

THE AROMA OF CANNED BEEF: MODELS FOR CORRELATION OF INSTRUMENTAL AND SENSORY DATA. T. PERSSON, E. VON SYDOW & C. ÅKESSON. *J. Food Sci.* 38, 682–689 (1973)–Stevens' and Fechner's relations have been applied in a generalized form: they have been expressed as functions in several variables. These functions have been formulated in analogy with models used in other psychophysical contexts and are considered to handle simple interactions between chemical stimuli compounds. It is suggested that the different outcomes can be classified into three categories: ad hoc relations, predictive relations and causative relations. The meaning and interpretability of these are discussed. The models have been tested on data from samples of canned beef: different formulations and heating times. A large number of high correlations were obtained for the different odor qualities and the presence of possible causative relations among these are discussed.

EFFECT OF POSTMORTEM CONDITIONS ON CERTAIN CHEMICAL, MORPHOLOGICAL AND ORGANOLEPTIC PROPERTIES OF BOVINE MUSCLE. F.C. PARRISH JR., R.B. YOUNG, B.E. MINER & L.D. ANDERSEN. *J. Food Sci.* 38, 690–695 (1973)–Paired sides from U.S. Choice grade beef were aged immediately after slaughter at 2 and 16°C. Samples were removed from longissimus and semitendinosus at slaughter and at 1, 3 and 7 days postmortem for ATPase assay, phase microscopy, shear and organoleptic evaluation. Rib steaks from sides aged at 16°C for 1-day postmortem were as tender as steaks from sides aged at 2°C for 7 days postmortem. Flavor development of rib steaks also was more rapid at 16°C than at 2°C. Tenderness of semitendinosus steaks was improved by aging sides at 16°C; the difference in improvement of tenderness of semitendinosus, however, was not as great between 2° and 16° as it was for rib steaks. Ca^{++}-, Mg^{++}- and EGTA-modified ATPase activity of myofibrils from both muscles increased with postmortem time, with myofibrils from muscles held at 16°C having slightly higher ATPase activity than myofibrils from muscles held at 2°C. Increased EGTA-modified ATPase activity was indicative of loss of calcium sensitivity of the myofibril. Sarcomeres of myofibrils from longissimus were longer at 1-day postmortem than those from at-death longissimus and they remained essentially unchanged during the remainder of postmortem aging; however, tenderness improved at 16°C for 1 day and at 2°C for 3 days. Also greater fragmentation of myofibrils from longissimus postmortem aged at 16°C for 1 day and at 2°C for 3 days was observed, suggesting that the rate of myofibril fragmentation is an important factor in tenderization.

EFFECT OF POSTMORTEM AGING ON CHICKEN MUSCLE LIPIDS. J.D. HAY, R.W. CURRIE & F.H. WOLFE. *J. Food Sci.* 38, 696–699 (1973)–Neutral lipids of fresh chicken breast muscles are shown to be triglycerides, sterols and sterol esters with only traces of mono- and diglycerides and free fatty acids. Phospholipids include measurable quantities of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, sphingomyelin, diphosphatidyl glycerol, lysophosphatidyl choline and lysophosphatidyl ethanolamine. Fatty acid analyses of several of the lipid fractions are also included. Decreases in phosphatidyl choline and phosphatidyl ethanolamine coupled with increases in lysophosphatidyl choline, lysophosphatidyl ethanolamine and free fatty acids after 48 hr postmortem in the cold indicate phospholipase A activity concurrent with other postmortem changes. The significance of the results is discussed.

EFFECT OF POSTMORTEM AGING ON CHICKEN BREAST MUSCLE SARCOPLASMIC RETICULUM. J.D. HAY, R.W. CURRIE, F.H. WOLFE & E.J. SANDERS. *J. Food Sci.* 38, 700–704 (1973)–FSR (fragmented sarcoplasmic reticulum) isolated from chicken breast muscle (Pectoralis major) at 0 hr, 48 hr and 7 day postmortem was purified using linear density gradient centrifugation. The Ca^{++}-accumulating ability of the FSR was found to increase with postmortem aging. No loss in ATPase activity was noted nor was any significant change observed in the SDS-gel electrophoresis patterns of the proteins with postmortem aging. The FSR from the aged muscle contained a higher proportion of phospholipids These studies indicate that the Ca^{++} sequestering properties of sarcoplasmic reticulum from chicken breast muscle are not impaired during postmortem aging.

ON THE INTERACTION OF MYOGLOBIN AND HEMOGLOBIN WITH MOLECULAR OXYGEN AND ITS LOWER OXIDATION STATES AND WITH CYTOCHROME c. G.G. GIDDINGS & P. MARKAKIS. *J. Food Sci.* 38, 705–709 (1973)–Ferrimyoglobin and ferrihemoglobin are oxidized to the ferryl (+4) state by the xanthine-xanthine oxidase system under the same conditions under which the system causes rapid reduction of ferricytochrome c. Catalytic amounts of ferrimyoglobin exert an apparent inhibition of ferricytochrome c reduction by the system. Oxidation of ferrimyoglobin and ferrihemoglobin is most likely due to their reacting with products of xanthine oxidase reduction of oxygen, O_2^- ($HO_2^\cdot$) and/or H_2O_2. The apparent inhibition of ferricytochrome c reduction is direct (interception of O_2^- by ferrimyoglobin) and/or indirect (reoxidation of ferrocytochrome c by ferrylmyoglobin). The latter mechanism is supported by spectral evidence of one-equivalent redox reactions (intermolecular electron transfers) between ferrylmyoglobin and ferrocytochrome c and between ferromyoglobin and ferricytochrome c. Both electron transfers have favorable redox potentials and therefore would be expected to occur from that standpoint. During autoxidation of oxymyoglobin O_2^- dissociation was not detected. This is consistent with theoretical arguments which indicate that O_2^- cannot dissociate during autoxidation although the conjugate acid ($HO_2^\cdot$) can. If so, this would at least partially explain the known decrease in stability of the oxyheme complex as the pH of fresh red meat, meat extracts or oxy-

hemoprotein solutions is lowered. Mechanisms of autoxidation that are consistent with known pH and oxygen partial pressure effects and chemical reactions, as well as with available quantitative data and pertinent theoretical arguments are discussed.

POST-SLAUGHTER pH VARIATION IN BEEF. A.W. KHAN & W.W. BALLANTYNE. *J. Food Sci.* 38, 710–711 (1973)–Tests on over 1200 comparable carcasses in two major packing plants showed that a large variation occurred in the muscle pH values recorded 1 hr post slaughter. The percentage of carcasses having 1-hr post-slaughter pH values of 6.2 or lower, between 6.3 and 6.6, and 6.7 or higher varied by 2–23%, 38–63% and 15–62%, respectively, between plants and time of measurement. Variation between muscles from a single animal was 0.4 units or less.

EXAMINATION OF BONE CONTENT IN MECHANICALLY DEBONED POULTRY MEAT BY EDTA AND ATOMIC ABSORPTION SPECTROPHOTOMETRIC METHODS. L.P. GRUNDEN & J.H. MAC NEIL. *J. Food Sci.* 38, 712–713 (1973)–Mechanically deboned poultry meat from broiler parts, spent layer carcasses and turkey racks was evaluated for percent bone solids (PBS) by EDTA titration and atomic absorption spectrophotometry. The PBS for the deboned meat ranged from 0.33–1.44 with the higher values being from the more mature types of poultry. In comparing the EDTA and spectrophotometric methods, the PBS values correlated very well (r = 0.99).

EFFECT OF FRANKFURTER CURE INGREDIENTS ON N-NITROSODIMETHYLAMINE FORMATION IN A MODEL SYSTEM. W. FIDDLER, J.W. PENSABENE, I. KUSHNIR & E.G. PIOTROWSKI. *J. Food Sci.* 38, 714–715 (1973)–The effect of cure ingredients on the nitrosation of dimethylamine was investigated in a model system containing sodium nitrite. The ingredients used were: sodium chloride, sodium nitrate, glucono-Δ-lactone (GDL), sodium acid pyrophosphate (SAPP), sodium erythorbate (NaEry), sodium ascorbate (NaAsc), ascorbic acid (AscH) and sodium tripolyphosphate (STPP). Under conditions approximating those used in the processing of frankfurters, GDL by itself was found to increase the formation of dimethylnitrosamine (DMNA), whereas reductants reduced the amount formed. Of the reductants, NaAsc or NaEry, in combination with other cure ingredients have similar inhibitory activity. The use of NaCl, $NaNO_3$ and SAPP had little or no effect on DMNA formation.

SOME OBSERVATIONS ON THE COLOR MEASUREMENT OF CANNED TUNA. A. KHAYAT. *J. Food Sci.* 38, 716–717 (1973)–The effect of different sample holders and various sample preparation techniques on the color of albacore, yellowfin and skipjack canned tuna was examined. It was observed that sample holder A, an empty 8 cm diam stainless steel can with a well 1.2 cm deep, gave the highest reflectance values with the lowest standard deviations. In addition, it saved considerable time in the color measurement. Investigations on the effect of various sample preparation techniques on the color of canned tuna showed that a regular meat grinder with a plate with 1/20 in. holes gave more uniform-sized particles which, in turn, resulted in high reflectance values with low standard deviations and reduced the time of sample preparation greatly.

QUANTITATIVE CHANGES IN WHOLE MYOFIBRILS AND MYOFIBRILLAR PROTEINS DURING FROZEN STORAGE OF TRUE COD. E.A. CHILDS. *J. Food Sci.* 38, 718–719 (1973)–The extractability of whole myofibrils from true cod decreased more rapidly during frozen storage at −40°C than did the extractability of the component myofibrillar proteins. There was a 95% decrease in extractable myofibrils after 6 months in storage, but only a 23% decrease in extractable myofibrillar proteins.

USE OF THE PENETROMETER FOR DEFORMATION TESTING OF FOODS. M.C. BOURNE. *J. Food Sci.* 38, 720–721 (1973)–The cone of a penetrometer is replaced by a flat, light-weight plastic horizontal disc which is allowed to compress an article of food for 5 sec, after which a reading is taken from the dial of the instrument. Next a weight is placed on the upper end of the vertical shaft that carries the disc and the food is compressed again for 5 sec, after which a second reading is taken from the dial. The difference between the two dial readings gives the deformation to the nearest 0.1 mm. Examples of its use on tomatoes and marshmallows are given. The test works well on foods that are deformable, but is not recommended for rigid foods because of lack of precision.

A SIMPLE SHEAR PRESS FOR MEASURING TENDERNESS OF WHOLE SOYBEANS. J. SPATA, M.P. STEINBERG & L.S. WEI. *J. Food Sci.* 38, 722–723 (1973)–A simple shear press instrument for measuring the tenderness of whole soybeans was developed. A perforated plate was forced through a sample of beans by a hydraulic piston and the required pressure of the hydraulic fluid indicated the force required which was related to tenderness of the beans. Soybeans were processed to various degrees of tenderness. Samples were tested by both the simple shear press and a L.E.E. Kramer shear press. The correlation coefficient between these instruments was 0.9933. The average of the coefficients of variation of all samples tested with the simple shear press was 4.61%. This showed that the simple shear press could be used in place of the L.E.E. Kramer shear press for determining tenderness of whole soybeans.

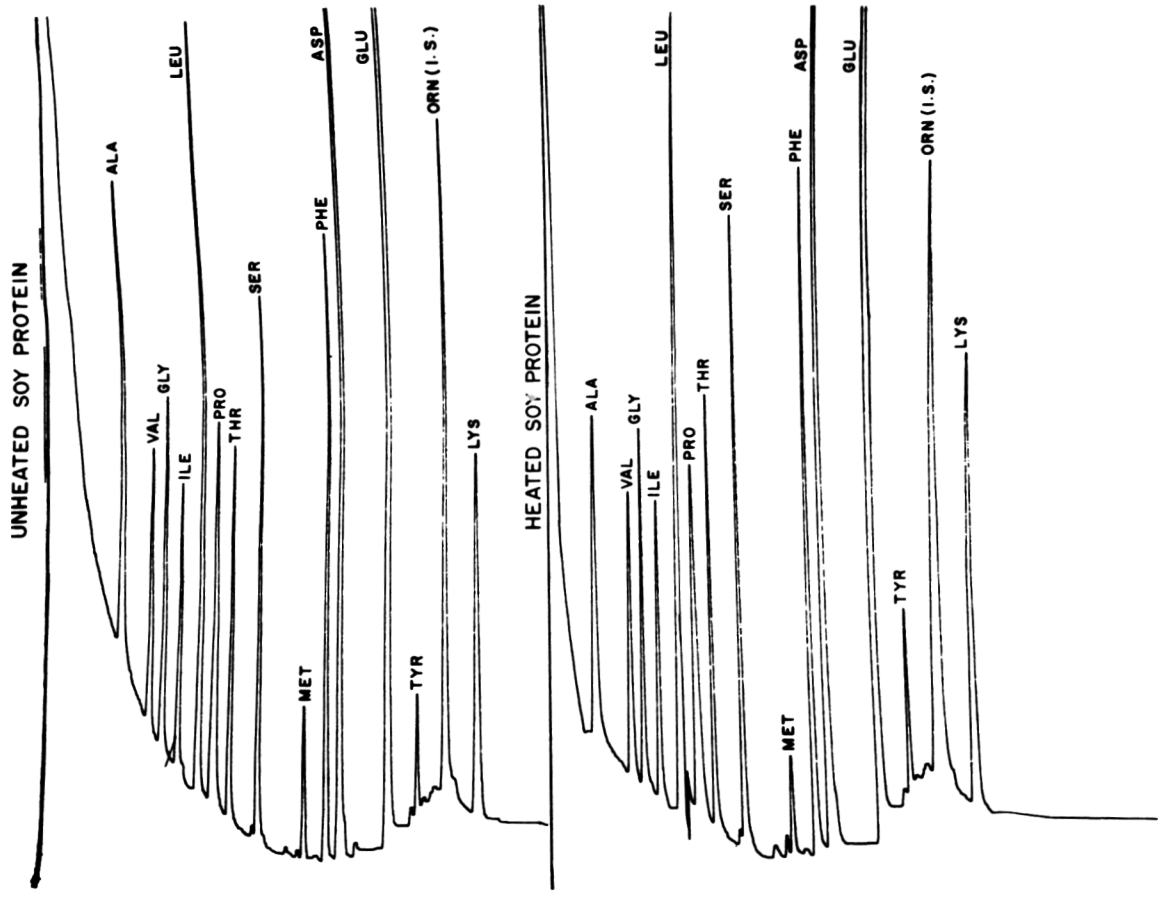
FLAVOR DETECTION THRESHOLD VALUES FOR ETHYL CAPRYLATE AND PHENYL ETHYL ALCOHOL AND ESTIMATES OF THE PERCENT POPULATION HAVING GREATER SENSITIVITY. J.J. POWERS & M.C. QUINLAN. *J. Food Sci.* 38, 724–725 (1973)–Threshold valuesfor ethyl caprylate in distilled water, 10% ethanol and corn oil were 0.15, 1.43 and 25.0 mg/l. For phenyl ethyl alcohol, the respective values were 0.175, 6.3 and 25.0. The threshold determinations for ethyl caprylate were made 1 yr apart with two different panels. Extreme-value analysis was applied to the first set of data to estimate the percent of individuals whose thresholds might be lower than the lowest threshold observed and this estimate was compared with the results of the second series of trials. There was moderately good agreement.

REFINEMENT AND EXTENSION OF f_h/U:g PARAMETERS FOR PROCESS CALCULATION. K.S. PUROHIT & C.R. STUMBO. *J. Food Sci.* 38, 726–728 (1973)–A digital computer program was written to refine and extend currently available f_h/U:g tables for thermal process evaluation. Some characteristics of the resulting parameters are discussed and an error-analysis is presented.

MERCURY IN FOOD. A Scientific Status Summary. IFT EXPERT PANEL ON FOOD SAFETY & NUTRITION. *J. Food Sci.* 38, 729–734 (1973)–Since mercury is ubiquitous, everyone consumes trace amounts. It can be found in all food and water, with higher concentrations in foods grown in areas having higher concentrations of mercury in soil. The short-chain alkyl mercury compounds, methyl, dimethyl and ethyl mercury, are more toxic than elemental mercury, inorganic salts of mercury and the aryl mercury compounds. Since mercury is excreted in feces, sweat and exhaled breath as well as in urine, a daily intake of 1.0 mg per day of elemental mercury or inorganic mercury salts appears safe. The USDL established an allowable level of 0.1 mg elemental mercury vapor or inorganic salts of mercury and 0.01 mg alkyl mercury per cubic meter of air for industrial exposures. The average U.S. diet contains less than 1/50 this amount. Epidemiological studies in several locations of elevated mercury content show no cases of methyl mercury poisoning resulting from fish consumption in the U.S. F&DA does not allow the sale of fish containing more than 0.5 ppm (0.5 mg/kg) of mercury, and the canned tuna industry now includes mercury content as one of their quality specifications. The naturally occurring element, mercury, does not appear to pose a toxic hazard in the food supply of the U.S. and there is no evidence that alkyl mercury formed by microorganisms in nature has led to methyl mercury poisoning; however, this does not imply there is no reason to control the release of elemental mercury and inorganic salts of mercury into the environment from industrial operations. It does indicate the problem has been recognized. Steps have been taken to reduce discharges of elemental mercury and inorganic salts of mercury and to reduce or eliminate use of mercurials in agriculture providing an increasing margin of safety against the possibility of an excessive amount of mercury in any form finding its way into any portion of the U.S. food supply.

Erratum Notice

J. Food Sci. 38(1): 112–115 (1973), M. Shemer, L.S. Wei and E.G. Perkins: "Nutritional and chemical studies of three processed soybean foods." Redproduction of Figure 4 (p. 113) was not clear; substitute new Figure 4 reprinted herewith.



JFS APPLIED SCIENCE and ENGINEERING

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FLAVOR AND CHEMICAL CHARACTERISTICS OF CONVENTIONALLY AND MICROWAVE REHEATED PORK

INTRODUCTION

USING PRECOOKED and frozen meats can save time and labor. However, eating quality of reheated meats generally is less desirable than that of freshly cooked (Bowers, 1972; Cash and Carlin, 1968; Korschgen and Baldwin, 1971). Certain chemical changes may occur in stored cooked muscle. Lipid oxidation can cause rancid odors and flavors. Using antioxidants (Chang et al., 1961) and irradiation (Chang et al., 1961; Greene and Watts, 1966) to inhibit deterioration of precooked meats has not been entirely effective.

Although studies of the effect of cooking raw meat with microwaves have been made, the reheating of precooked meats with microwaves has not been investigated thoroughly. Causey and Fenton (1951) reported acceptable palatability scores for several precooked and frozen meat combination dishes reheated with microwaves. Cipra and Bowers (1971) reported flavor was more intense and less stale for microwave reheated turkey than for conventionally reheated turkey. No information was found on the effect of reheating cooked pork with microwaves.

Flavor and aroma of microwave- and conventionally-reheated and stored pork were compared with that of fresh conventionally cooked pork and the relation of flavor and aroma to various chemical characteristics of the heat-treated meat was studied.

EXPERIMENTAL

TEN PAIRED, frozen, boneless loins (cut from the 10th thoracic vertebra to the 5th lumbar vertebra) from five 200 to 250-lb barrows were obtained from the Dept. of Animal Science and Industry, Kansas State University. Each loin was thawed and divided into thirds. The treatments–(1) freshly cooked; (2) conventionally precooked, frozen, conventionally-reheated; and (3) conventionally precooked, frozen, microwave-reheated–were assigned randomly to portions of each loin.

Two portions of each loin were wrapped in 3M Scotchpak Oven Service film, heated to 65°C in a rotary hearth gas oven at 163°C, cooked at room temperature for 15 min, packaged in polyethylene bags, and frozen at –17°C for 5 wk. The other portion was frozen raw. Before each evaluation, the three loin portions (one for each treatment) were removed from the freezer and thawed at 5°C for 24 hr. The raw portion then was cooked at 163°C in a rotary hearth gas oven to an internal temperature of 77°C. One precooked portion was reheated to 55°C in the gas oven; the other to 55°C in an Amana Radarange (model RR-2, MHz 2450) microwave oven. Spirit (instead of mercury) thermometers were used because they do not arc in microwave ovens. Cooked portions were cut immediately to minimize post-oven temperature rise. Total cooking times and losses were recorded. The cooked meat was trimmed of fat and browned surfaces. A center portion of the muscle was removed for sensory evaluation and the remainder was ground for chemical determinations.

From the center of each cooked portion, ¼-in. slices were cut from the longissimus muscle and placed in the upper part of 1-pt enamel double boiler over hot water and held (not longer than 15 min) until all samples were ready for evaluation. Samples then were transferred to warm coded snifters, covered with watch glasses, for sensory evaluation. Samples were evaluated by a six-member panel in individual booths. (Before the evaluation periods, panelists had been trained to identify the selected flavors and aromas.) Intensities of the flavor and aroma components were scored (0, absent; to 3, strong). Juiciness also was scored (1, very dry; to 6, very juicy).

Objective measurements

These duplicate measurements were made on ground meat samples:

Percentage total moisture. Percentage total moisture was determined in a 25-lb vacuum convection oven. 1–2-g samples were dried 4 hr at 110°C.

TBA values. The 2-thiobarbituric acid values were determined by the method of Tarladgis et al. (1960). Slurries were prepared from 7-g samples. Optical density readings (Beckman DU Spectrophotometer) were multiplied by a factor of 7.8 and adjusted for sample size to convert to mg of malonaldehyde per 1,000-g tissue (Tarladgis et al., 1960).

Total ninhydrin-reactive compound. A deproteinized filtrate, using 1% picric acid (Tallon et al., 1954), was prepared from 2-g samples. The quantity of ninhydrin-positive material in the filtrate was determined by a colorimetric method, based on the reaction with ninhydrin (Yemm and Cocking, 1955) and expressed as μmoles per g of meat based on a standard curve prepared from glycine.

Protein fractionation. To determine changes in the various protein and nonprotein nitrogen components of freshly cooked and reheated LD muscle, protein fractions were prepared from 5-g samples, following procedures of Randall (1969). They were: a low ionic strength fraction, a soluble and a denatured fibrillar protein nitrogen fraction and a sarcoplasmic and a nonprotein nitrogen fraction.

Nitrogen analysis. Protein nitrogen in each fraction was determined using micro-Kjeldahl methods. Nitrogen in each fraction was expressed as mg of nitrogen per g of muscle.

Analysis of data

A complete randomized block design with 10 replications of each treatment was used to analyze the data. The analysis removed animal and loin (cooking period) variation from the desired comparisons and provided ample degrees of freedom for estimating error variance. The data were subjected to analysis of variance; LSD's were calculated when F-values were significant. Correlation coefficients between variables were determined.

RESULTS & DISCUSSION

Sensory evaluation

Most aroma and flavor components of freshly cooked and reheated pork were similar (Table 1). No significant differences were found between freshly cooked and microwave-reheated pork, but some differences were noted between conven-

tionally reheated muscle and that of the other two treatments. Sweet aroma of freshly cooked and microwave-reheated pork was similar and significantly higher ($P < 0.05$) than that of conventionally reheated pork. However, the difference was small and probably of no practical significance. Metallic flavor of freshly cooked and microwave-reheated pork was similar and significantly lower ($P < 0.05$) than that of conventionally reheated pork. In similar work, comparing conventional and microwave-precooked and -reheated turkey, Cipra et al. (1971) found microwave heating intensified the meat, while lessening the stale flavor. Bowers (1972) reported that freshly cooked and microwave-reheated turkey had similar meaty-brothy flavors (higher than that of conventionally reheated turkey) and similar rancid aroma scores (lower than that of conventionally reheated turkey). Results of those studies and this study indicate that microwave-reheated muscle is more like freshly cooked muscle than is conventionally reheated muscle.

At several evaluation periods, panelists noted sulfurous and fleeting rancid-oily flavor components in the conventionally reheated pork, resulting perhaps from the longer exposure to heat (than in the other treatments). (That also could have been responsible for the decreased sweet flavor and increased metallic flavor found by the taste panel.) Correlation coefficients indicated that meaty-brothy flavor was related negatively to stale aroma ($r = -0.40$ $P < 0.05$) and flavor ($r = -0.58$, $P < 0.01$) and astringent flavor ($r = -0.42$ $P < 0.05$). Similar results for turkey were noted by Cipra (1969). In our study, significant correlations also were found for metallic flavor and bitter flavor ($r = 0.61$, $P < 0.01$) and for metallic aroma with astringent flavor ($r = 0.53$, $P < 0.01$).

Microwave-reheated pork was significantly ($P < 0.05$) less juicy than either freshly cooked or conventionally reheated pork (Table 1). Others have reported lower juiciness scores for microwave-cooked lamb roasts (Headley and Jacobson, 1960) and pork roasts (Apgar et al., 1959). Law et al. (1967) also reported a significant negative correlation (-0.55, $P < 0.01$) between total cooking loss and juiciness score of longissimus steaks prepared in a microwave oven.

Table 1—Means of sensory evaluation scores for pork

Factor	Freshly cooked	Conventionally reheated	Microwave reheated	Sign. of F-value	LSD*
Aroma components[a]					
Meaty-brothy	1.6	1.5	1.7	ns	
Acid	0.3	0.3	0.3	ns	
Sweet	0.2	0.1	0.2	*	0.1
Bitter	0.1	0.1	0.1	ns	
Stale	0.7	0.9	0.6	ns	
Metallic	0.3	0.5	0.4	ns	
Animal-like	0.4	0.4	0.3	ns	
Flavor components[a]					
Meaty-brothy	1.8	1.7	1.9	ns	
Acid	0.4	0.6	0.4	ns	
Sweet	0.4	0.3	0.4	ns	
Bitter	0.0	0.1	0.1	ns	
Stale	0.4	0.5	0.4	ns	
Astringent	0.2	0.3	0.2	ns	
Metallic	0.2	0.4	0.2	*	0.2
Animal-like	0.4	0.3	0.4	ns	
Juiciness[b]	4.7	4.8	4.1	*	0.5

[a] Based on intensity of component, 3-(strong); 0-(absent)
[b] 6-(very juicy); 1-(very dry)
* $P < 0.05$; ns = not significant

Table 2—Mean values of total moisture, total cooking losses, cooking time, TBA numbers, and ninhydrin-reactive compounds of heated pork

Factor	Freshly cooked	Conventionally reheated	Microwave reheated	Sign. of F-value	LSD*
Total moisture, %	63.59	60.67	57.79	***	1.61
Cooking losses, %	28.47	30.85	35.54	***	2.46
Cooking time, min[a]	69	50	9	***	5
TBA value[b]	3.31	4.97	4.01	***	0.36
Ninhydrin-reactive compounds[c]	11.379	10.567	10.039	ns	
Protein nitrogen					
Low ionic strength[d]	4.18	4.12	4.40	ns	
Sarcoplasmic[d]	3.31	3.31	4.15	ns	
Nonprotein nitrogen[d]	0.86	0.81	0.85	ns	
Soluble fibrillar[d]	0.56	0.54	0.51	ns	
Denatured fibrillar[d]	34.21	34.49	34.44	ns	

[a] Final cooking time immediately prior to evaluation
[b] mg malonaldehyde/1000g tissue
[c] μmoles glycine/g of tissue
[d] mg nitrogen/g of muscle on a wet-weight basis
* $P < 0.05$; *** $P < 0.001$; ns = not significant.

Objective measurements

Mean values for percentage total moisture, percentage total cooking losses, cooking time, TBA values, ninhydrin-reactive compounds and protein nitrogen in extracted tissue fractions are presented in Table 2. The moisture content of pork muscle differed significantly ($P < 0.001$) among the three treatments. Microwave-reheated pork contained the least moisture, freshly cooked pork had the highest percentage moisture, and conventionally reheated pork had intermediate moisture content.

Percentage total cooking losses were significantly greater ($P < 0.001$) for microwave-reheated pork than for freshly cooked or conventionally reheated pork, which had similar losses. A significant correlation ($r = -0.60$, $P < 0.01$) was found for percentage total moisture and percentage total cooking losses. Those results would account in part for differences in juiciness detected by the taste panel.

Differences among cooking times, at the time of final preparation, were all significant ($P < 0.001$). Both reheating methods took significantly less preparation time than did cooking from the raw state. Reheating by microwave was five times faster than reheating the conventional way.

TBA values, determined as a measure of fat oxidation, differed significantly ($P < 0.001$) among the three heating treatments. TBA values were highest for pork exposed to the longest total heating time (precooked and conventionally reheated, 106 min) and lowest for pork exposed to only one heating application (freshly cooked, 69 min). The microwave-

reheated pork, though exposed to heat during precooking (55 min), was subjected to reheating for only a short time (9 min) before evaluation. Heat has the catalytic effect of increasing the oxidative-reaction rate, so those portions of pork exposed to the longest total heat treatment would be expected to have the largest TBA values. Significant correlations ($P < 0.05$) were found for TBA values with stale aroma ($r = 0.42$, $P < 0.05$) and with astringent flavor ($r = 0.37$, $P < 0.05$). However, panel scores did not reflect differences in stale aroma or flavor among the heating treatments. Jacobson and Koehler (1970) reported a significant correlation ($r = -0.77$) between flavor scores and TBA values of precooked and stored turkey. In their study greater flavor differences were observed and the importance of storage temperature was noted–refrigerated cooked turkey had higher TBA values than did frozen cooked turkey.

Total ninhydrin-reactive compounds and amount of protein in various tissue extracts were not affected significantly by the heating treatments. During heating, amino acids may combine with other compounds to yield flavor products, or they may be carried out of the meat with other solubles and lost in the drip. Macy et al. (1964) found that heating meat increased some amino acids and decreased others. Changes in protein solubilities occur over various time-temperature ranges; heating treatments in this study evidently did not differ enough to alter the protein fractions significantly. Freshly cooked pork was heated to 77°C. Pork assigned to the other two treatments was precooked to 65°C and reheated to 55°C. Microwaves might have affected the proteins more if they had been used to precook the meat, because most protein changes would have occurred during the initial heating. Hamm and Deatherage (1960) found that most changes in muscle protein resulting from heat occurred between 40°C and 65°C.

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SOME ASPECTS OF RAW MEAT TENDERNESS. A Study of Some Factors Affecting Its Change with Cooking and a New Means of Measurement

INTRODUCTION

BECAUSE TENDERNESS is generally considered to be one of the most important palatability characteristics of meat (Brayshaw et al., 1967), many methods of measuring it have been developed (Pearson, 1963; Szczesniak and Torgeson, 1965). Some of these produce useful measures of tenderness and are widely used, although none is entirely satisfactory. However, in order to justify the introduction of any new method it must be superior to previous methods in some important characteristic(s) such as accuracy, sensitivity, simplicity or rapidity of use. This paper describes a simple, hand-operated biting instrument which was designed to assess meat tenderness on quartered beef carcasses or on wholesale cuts. This, of course, would entail the measurement of raw meat tenderness which has been shown in a number of studies to be poorly related to cooked meat tenderness (McBee and Naumann, 1959; Carpenter et al., 1965; Dikeman et al., 1972). One possible explanation for such poor relationships is that determinants of tenderness which exert their influence prior to cooking interact with the effect of cooking on tenderness. Therefore, experiments were conducted with the aim of measuring such interactions between immediate postmortem holding temperature or postmortem aging and cooking temperature with respect to their effects on beef tenderness.

EXPERIMENTAL

THE MEAT used in all experiments was obtained from Friesian steers ranging in age from 17–24 months and in carcass weight from 200–350 kg. Methods used for preparation, storage, cooking and tenderness assessment of steaks by Warner-Bratzler shear device were outlined in detail by Purchas (1972), with the exception that all steaks were dipped in a solution of 10 ppm chlortetracycline prior to being put in polyethylene bags. The procedure involved removing the section of M. longissimus from the 9-10-11 rib region 2 days postmortem, preparing 2.5 cm thick steaks, and storing them in polypropylene bags at 2–4°C until they were cooked for 1 hr by immersing the bags in a water bath preheated to the appropriate temperature. Tenderness measurements were made on 14 × 14 mm cores after cooling overnight at 2–4°C.

In experiments which involved cold shortening as a treatment, one or both Mm. semitendinosus were removed from the carcass and within 40 min of slaughter some parts were stored at 2–4°C while others were stored at 16–20°C. At this time pins were placed approximately 12 cm apart along the length of the muscle sample and the change in the distance between these pins after 24 hr postmortem was recorded. To minimize resistance to shortening the samples were placed on glass microscope slides which in turn were placed on two smooth metal rods (4 mm diam) at 1 cm intervals so that they were free to slide. At 24 hr postmortem the samples at 16–20°C were transferred to 2–4°C. The preparation, storage and tenderness assessment of steaks were as described for M. longissimus, with cooking being carried out at 7 days postmortem.

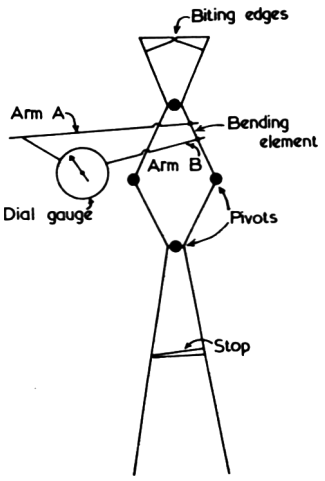
The roasts used in the comparison of biting instrument values on raw and cooked meat comprised primarily M. semimembranosus and M. adductor and were cooked to an internal temperature of 74°C in a domestic oven preheated to 163°C.

The biting instrument, shown in Figure 1, was built from a pair of bone forceps. The rounded biting edges (1 mm radius) are 27 mm long. As the biting edges meet with increasing resistance in a meat sample, the bending element bends to an increasing extent and the resulting movement of arm A away from arm B is recorded on the dial gauge. In the comparison of the biting instrument with the Warner-Bratzler device, measurements were made by each instrument on each core of meat. However, it was envisaged that in actual use, biting instrument measurements would not be made on prepared cores but on a meat surface produced by a cut at right angles to the muscle fibers. It was used in this way on the raw roasts where two parallel cuts 14 mm apart and approximately 5 mm deep were made with scalpel blades, so that the cross-sectional area of meat between the biting edges was the same each time.

RESULTS & DISCUSSION

Relationship between biting instrument and Warner-Bratzler readings

Regression equations and correlation coefficients for the relationship between biting instrument readings and Warner-



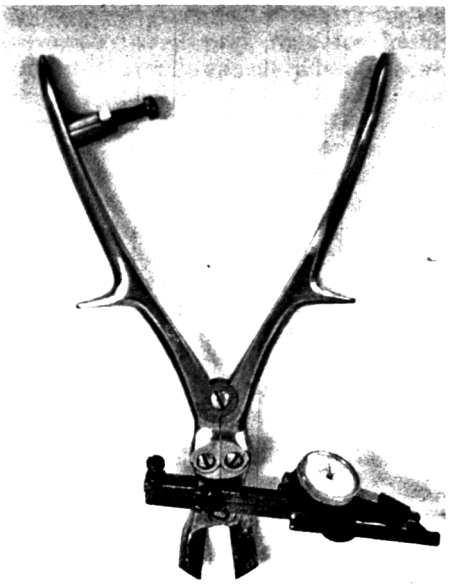


Fig. 1—Sketch and photograph of the biting instrument.

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Bratzler shear values are given in Table 1. The 66 pairs of values included those from cold shortened and noncold shortened samples from M. semitendinosus from 33 animals. The standard deviation of the Warner-Bratzler shear values within the cold shortened group was more than three times larger than that for the values in the noncold shortened group (4.40 and 1.27, respectively). It was probably mainly because of this that the relationship between the readings from the two methods was closer within the cold shortened group (r = 0.90) than within the noncold shortened group (r = 0.60).

The relationships outlined in Table 1 are close and indicate that the two techniques are probably measuring the same thing. The negative intercept in the first regression equation may indicate that the relationship is slightly curvilinear or it may reflect mechanical imperfections of the biting instrument which did not always return to zero.

If the response of any tenderness measuring device is defined as the proportional change in reading per unit change in "true" tenderness, then it can be shown that the regression coefficient of a log/log relationship between any two methods of measurement is equal to the ratio of the responses of those methods. From this point of view the biting instrument appears to be slightly superior to the Warner-Bratzler device as the regression coefficient in Table 1 is significantly greater than 1.0 ($P < 0.01$). However, it is possible that this is also a reflection of mechanical imperfections and, in fact, response in this sense is probably a minor problem in tenderness measurement compared with unexplained variation along a single core of meat, for example.

Each value used in the regression analysis was the mean of shear values from 16 cores, and when the standard errors of these means were expressed as a percent of the means, it was found that the mean of these percent standard errors for the Warner-Bratzler device was significantly lower than that for the biting instrument (4.13% vs. 5.38%; $P < 0.001$). This situation may be expected if the biting instrument does in fact show better response. In either case the mean standard errors are reasonably low for tenderness measurements.

Table 1—Relationship between Warner-Bratzler shear values (x) and biting instrument values (y) on 66 steaks from beef M. semitendinosus

Regression equation	n	r	$SE_{\bar{y}}$
$y = 2.3670x - 5.1310$	66	0.96	0.540
$\text{Log } y = 1.1614 \log x - 0.1049$	66	0.95	0.008

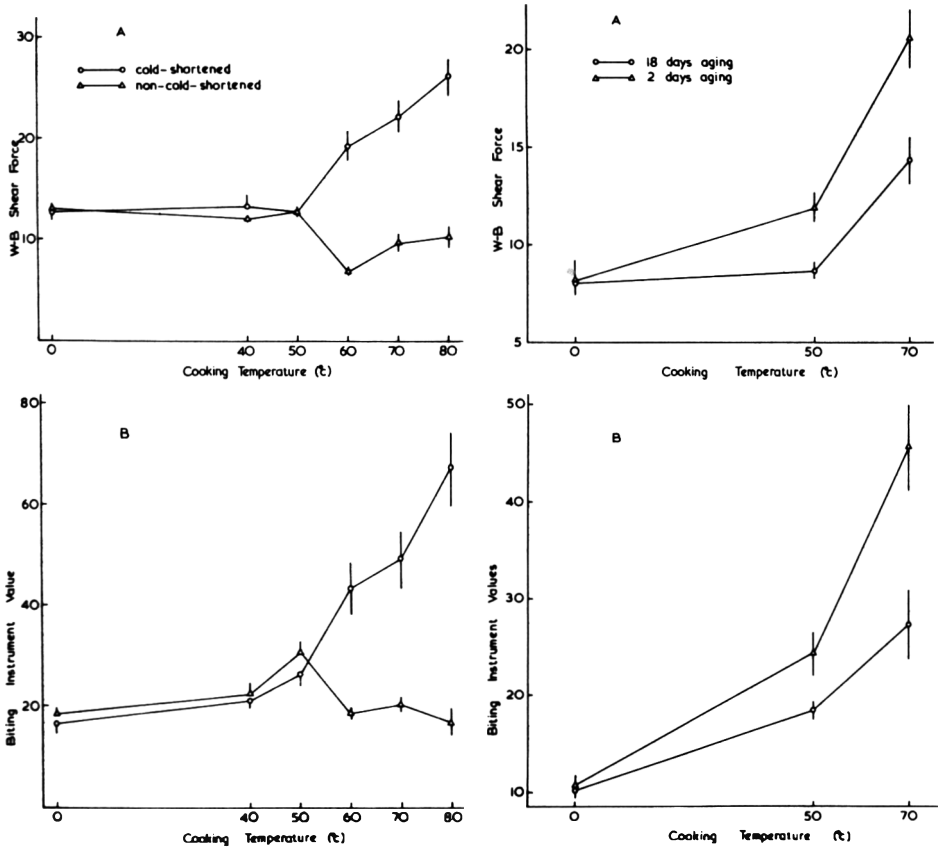

Fig. 2—Effect of cold shortening on changes in tenderness of beef M. semitendinosus as temperature of cooking is increased. (Means ± SE's, n = 6). A—Warner-Bratzler shear values; B—biting instrument values.

Fig. 3—Changes in tenderness of beef M. Longissimus aged for 2 or 18 days as temperature of cooking is increased. (Means ± SE's, n = 6). A—Warner-Bratzler values; B—biting instrument values.

Factors affecting the relationship between measures of tenderness on raw and cooked meat

It was expected that relationships between biting instrument values and Warner-Bratzler shear values on cooked meat would be closer than relationships between biting instrument values on raw and cooked meat samples. This proved to be very much the case and for the 24 roasts the simple correlation coefficient between raw and cooked values was 0.186. This could be partly attributed to the low variability in this group of samples, but the results are consistent with those of other workers using different methods (McBee and Naumann, 1959; Carpenter et al., 1965; Dikeman et al., 1972; Carpenter et al., 1972). Hinnergardt and Tuomy (1970), however, obtained quite close relationships between raw and cooked penetrometer values and between these values and taste panel assessment of pork tenderness.

Poor relationships between raw meat tenderness and cooked meat tenderness would arise if factors affecting tenderness of meat some time prior to cooking interacted with the effect of cooking temperature on tenderness. Two precooking factors which are known to have important effects on ultimate meat tenderness are cold shortening (Marsh and Leet, 1966) and aging at above freezing temperatures (Busch et al., 1967).

Figure 2 shows the changes in measures of tenderness with increasing cooking temperature for cold shortened and noncold shortened beef M. semitendinosus samples from six animals. Meat treated in these ways did not differ in tenderness when raw or when cooked at 40°C or 50°C. However, between 50°C and 60°C major changes took place so that at 60°C and to a greater extent at 70°C and 80°C cold shortened meat was

much tougher. The pattern was not greatly different between Warner-Bratzler shear values (Fig. 2a) and biting instrument values (Fig. 2b).

Similar results were obtained when the tenderness of aged and unaged M. longissimus samples from six animals were compared after cooking at different temperatures (Fig. 3). In this case, however, cooking for 1 hr at 50°C was sufficient to produce aging effects on tenderness. Again the patterns of change were similar for Warner-Bratzler shear values and for the biting instrument values.

These phenomena whereby the effects of cold shortening and of aging were very apparent on the tenderness of meat cooked at 70°C but undetectable in raw meat (Fig. 2 and 3), were assessed statistically by considering the interactions between pre-cooking treatment (cold shortening or aging) and cooking treatment (raw or cooked at 70°C). The results in Table 2 indicate that these interactions were highly significant, and therefore these would appear to be real effects, at least under the conditions used. It follows that there is little point in classifying beef according to its raw meat tenderness if it has been subjected to varying degrees of cold shortening or aging. According to Locker and Hagyard (1963), an important characteristic of cold shortening is its variability between individual animals, so that it seems possible that even in a group of carcasses exposed to the same postmortem conditions, raw meat tenderness values may be of little use due to the interactions outlined above.

Animal age is another determinant of meat tenderness which appears to interact strongly with the effect of cooking. This has been well demonstrated for several beef muscles by Bouton and Harris (1972). For example, they showed that whereas the mean Warner-Bratzler shear value for the deep pectoral muscle of calves (0–2 months) was greater than that for 5–7 year-old cows when the muscles were raw, after cooking to 75°C the mean shear value of the cows was more than twice that of the calves. They suggested that this age effect on the patterns of change during cooking was due to connective tissue changes but the results in Figure 2 suggest that they could be at least partially attributable to an increased response to cold shortening with increased age. The observation by Locker and Hagyard (1963) that meat from "boner" cows cold shortened to a greater extent than that from "prime" animals is consistent with such a suggestion.

Apart from the practical implications of the interactions already mentioned, it is also of interest to consider possible explanations for the effects in terms of known structural changes taking place during cold shortening, aging and cooking. Cold shortening and aging effects on tenderness are generally considered to involve the myofibrillar portion of muscle (Goll et al., 1970; Newbold and Harris, 1972) and according to Hamm (1966) most of the myofibrillar proteins denature as the temperature is raised from 50° to 65°C. This implies that it is when the myofibrillar proteins denature that previous shortening is manifested in tenderness differences, but the reason for this is not clear. It is possible that the temperature treatments which were applied with the aim of inducing differences in shortening may have brought about the different responses to cooking (Fig. 2) independently of their effect on physical shortening. The effect on shortening was not particularly large with the difference in mean percent shortening over 24 hr between samples stored at 2–4°C and those stored at 16–20°C being 18.1.

The pattern of changes in Figure 3 suggest that the muscle samples involved had cold shortened to some extent, and this was probably the case, as the carcasses were put in a cooler (4–6°C) within 2 hr of slaughter. According to Goll et al. (1970) the main structural changes taking place during postmortem aging are a weakening of the actomyosin interaction and the degradation of the Z line region. It may be that these changes had no measurable effect on raw meat tenderness because it was determined primarily by "background" connective tissue factors, rather than by myofibrillar characteristics (Newbold and Harris, 1972).

General discussion

The biting instrument described in this paper is smaller in size and is simpler and quicker to operate than most machines commonly used for meat tenderness assessment (Szczesniak, 1972). These advantages and the suggestion that it measures essentially the same characteristic(s) as the Warner-Bratzler shear device indicate that there may be practical situations where it would be useful.

However, it would not appear to be of much use for measuring the tenderness of raw meat. Also, more work needs to be done to correct mechanical imperfections and to ascertain the relationship between biting instrument values and taste panel assessments of cooked meat tenderness.

Table 2—Statistical significance (probabilities) of main and interaction effects on tenderness as measured by Warner-Bratzler shear and by the biting instrument

Source of variation	Method of measurement	
	W-B shear	Biting instrument
M. longissimus		
Cooking temperature[a]	< 0.001	< 0.001
Aging time[c]	< 0.025	< 0.005
Interaction	< 0.025	< 0.010
M. semitendinosus		
Cooking temperature[a]	< 0.025	< 0.001
Cold shortening[b]	< 0.001	< 0.001
Interaction	< 0.001	< 0.001

[a]Either uncooked or cooked at 70°C for 1 hr
[b]Stored at 2–4°C or 16–20°C for 24 hr postmortem
[c]Aged at 2–4°C for either 2 or 18 days

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FREEZING OF RAW BEEF: INFLUENCE OF AGING, FREEZING RATE AND COOKING METHOD ON QUALITY AND YIELD

INTRODUCTION

CURRENTLY there is considerable interest in Sweden in frozen, presliced raw beef for the consumer and institutional markets. This has caused a need for more knowledge on the combined effects of variables such as aging, freezing rate and frozen storage on sensory quality and yield. It is also of great practical interest to determine whether finely cut beef should preferably be cooked directly from the frozen state or only after previous thawing. Most of the pertinent literature concerns large cuts and, as a rule, only a few variables have been studied at a time. Since raw material and experimental conditions vary widely, and since they are sometimes not very well defined, published results are difficult to compare. The present study was undertaken to investigate the combined effects of the variables presently considered to be of main practical concern to the industry, using a multifactorial experimental design and as well defined conditions as possible.

It is apparent from the literature reviewed by Kondrup and Bolt (1960) and by Jakobsson and Bengtsson (1969) that the freezing rate, at least to some extent, influences sensory quality, thaw drip and yield of small cuts of beef. Overall, there is some evidence that tenderness and drip loss "improve" with increasing freezing rate, while juiciness may actually decrease. In freezing experiments with slices of LD muscles over a very wide range of freezing rates (from 0.03 to 100 cm/h) Jakobsson and Bengtsson (1969) found noteworthy quality differences only in frozen product color, which was very pale at the highest rates, and in thaw drip, which varied inversely with the freezing rate.

As to the influence of frozen storage, results reported by different investigators are contradictory with regard to tenderness, while there is reasonably good agreement that drip loss tends to increase and yield to decrease with increasing frozen storage time.

Combined effects of aging and freezing were studied by McCoy et al. (1949), who found no interaction between these variables, in spite of an observation that differences between aged and unaged meat decreased with frozen storage.

Vold (1968), noted an increase in tenderness with prolonged frozen storage at −20°C (of lamb steaks) above that resulting from aging alone, and Gac and Lafon (1969), found aging for 8 days before freezing to represent an optimum.

With regard to cooking beef from the frozen or thawed condition, Smith et al. (1969) and Slot et al. (1966) found no difference in yield or tenderness, while Brady et al. (1942) and Gac et al. (1966) reported a higher yield when they cooked beef slices directly from the frozen state.

EXPERIMENTAL

Raw material and preparation

The posterior end of LD muscles from eight steers of SLB-breed were used. The animals were of Swedish prime grade (1+) with a slaughtered weight of about 300 kg. The intramuscular fat content of the meat was low, varying between 1.5 and 3%. The muscles from the left half of the carcass were dissected after 3 days' cold storage at +3°C, and those from the right, after 13 days. The dissected muscles were trimmed on the following day and cut into transverse slices of 1.5 cm thickness (100g) and frozen. About 40 slices were obtained in this way from each animal and systematically assigned to the variables under study (Table 1).

Freezing, packaging and storage

The beef slices were immediately frozen at three widely different rates. Spray freezing with liquid nitrogen in a pilot freezer of our design gave a freezing rate of about 13 cm/h, while air blast freezing at −35°C resulted in a rate of 2 cm/h. The lowest freezing rate, 0.04 cm/h, was obtained by freezing in an insulated box in a freezer at −20°C, simulating commercial freezing of pallet loads of products packed in cartons.

For the two more rapid freezing methods the meat was frozen unpacked and then packed in aroma-tight pouches of saran-cellophane-polyethylene laminate, while slowly frozen samples were packed before freezing. Samples were not vacuumized prior to heat sealing, but headspace volume was reduced to a few cc. Samples were stored at −20°C for 1 month, and at −30°C for 8 months followed by 1 month at −20°C to simulate the range of storage conditions in the commercial frozen food chain.

Thawing and cooking

The meat was either cooked directly from the frozen state or after it had been thawed on a grid inside a plastic pouch at +20°C until it reached a central temperature of +10°C. Such prethawing was chosen as the more suitable procedure on the basis of a preliminary factorial experiment comparing two air temperatures (+5 and +20°C) and three different end temperatures (0°, +5° and +10°C) for frozen beef at the two levels of aging time mentioned. No interaction was found between thawing method and aging time. As found from previous experience, the method chosen was considered to sensitize yield comparisons between freezing rates.

In preliminary tests it was found that heating beef slices by pan frying directly from the frozen state requires lower temperature of the frying table (and longer heating time) than when heating from the thawed state, in order to reach a desired central temperature without overheating the surface layer. To secure the best results when frying in margarine to a central temperature of 75°C, frying from the frozen state was done at a pan temperature of 160°C for 12–13 min, and heating from the thawed state at 175°C for 10 minutes.

Analytical methods

Sensory evaluation was made using 9-grade intensity scales for off flavor, flavor, juiciness and tenderness (1 = no off flavor, or extremely poor, dry, tough; and 9 = extremely high off flavor or extremely good, juicy, tender). In all sessions a reference stored at −80°C was included, and its rating determined for each session as a means of "calibrating" the panel. The standard was cooked directly from the frozen state. It was of slightly higher quality than the samples and showed a slight directional decrease in sensory quality with frozen storage time.

Surface color was measured by a Hunter Color and Color Difference meter using a brown ceramic standard with L, a and b values in the range of the samples.

Tenderness-toughness. As a measure of tenderness-toughness we determined shearing force perpendicular to the fibres as a function of time for 1 × 1 × 5 cm pieces, cut from fried beef. An Instron TM-M, equipped with a CW-1 meat shear cell, and a shearing rate of 10 cm/min were used. Both maximum shearing force and the area under the force-time curve were measured.

Thawing and cooking losses (combined) as a measure of yield were determined and expressed in percent of frozen sample weight.

TBA-test as a measure of rancidity was performed on the raw meat according to Tarladgis et al. (1960), measuring extinction at 530 nm divided by sample weight of fat.

Centrifugation loss was used as a measure of water-holding ability for the thawed raw meat. Samples of 2g were centrifuged at 1200g according to a method by Aitken et al. (1962).

Table 1—Experimental plan and allocation of animals and LD slices from the hind part (numbered from the 11th vertebra towards posterior end)

Aging time	4 days												2 weeks											
Freezing method & rate	Liquid nitrogen spray, 13 cm/h				Air blast (−35°C) 2 cm/h				"Pallet freezing" 0.04 cm/h				Liquid nitorgen spray, 13 cm/h				Air blast (−35°C) 2 cm/h				"Pallet freezing" 0.04 cm/h			
Frozen storage[a]	A		B		A		B		A		B		A		B		A		B		A		B	
Pan frying[b]	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T
Allocation of samples	"Left side" LD muscle												"Right side" LD muscle											
Sensory evaluation:																								
Animal no.	1,5	1,5	2,6	2,6	1,5	1,5	2,6	2,6	1,5	1,5	2,6	2,6	1,5	1,5	2,6	2,6	1,5	1,5	2,6	2,6	1,5	1,5	2,6	2,6
Slices no.	1,7 13	4,10 16	1,7 13	4,10 16	2,8 14	5,11 17	2,8 14	5,11 17	3,9 15	6,12 18	3,9 15	6,12 18	1,7 13	4,10 16	1,7 13	4,10 16	2,8 14	5,11 17	2,8 14	5,11 17	3,9 15	6,12 18	3,9 15	6,12 18
Physical and chemical evaluations:																								
Animals no.	3,7	3,7	4,8	4,8	3,7	3,7	4,8	4,8	3,7	3,7	4,8	4,8	3,7	3,7	4,8	4,8	3,7	3,7	4,8	4,8	3,7	3,7	4,8	4,8
Slices no.	1,7 13	4,10 16	1,7 13	4,10 16	2,8 14	5,11 17	2,8 14	5,11 17	3,9 15	6,12 18	3,9 15	6,12 18	1,7 13	4,10 16	1,7 13	4,10 16	2,8 14	5,11 17	2,8 14	5,11 17	3,9 15	6,12 18	3,9 15	6,12 18

[a] A = frozen storage 1 month at −20°C; B = frozen storage 8 months at −30°C followed by 1 month at −20°C
[b] F = pan frying directly from frozen condition, T = pan frying after thawing

Experimental plan

A 2 × 3 × 2 × 2 multifactorial design with replicates was used to study the combined effects of aging time (4 and 14 days), freezing rate (13, 2 and 0.04 cm/h), frozen storage (1 month at −20°C and 8 months at −30°C followed by 1 month at −20°C) and sample temperature in cooking (−20° and +10°C). The experimental plan and allocation of raw material are given in Table 1. Muscles were systematically distributed between aging times (left-right), and slices were systematically distributed between treatments and methods, so that treatment comparisons for a given property would be made between equivalent slices from the same pair of muscles. However, because of the limited number of slices available from each muscle, different pairs of animals had to be chosen for the two storage times, in spite of thus reducing the validity of that comparison. For the same reason no initial control was included. The levels chosen for the different parameters are considered representative of the normal range of conditions in Swedish practice, with the exception of the two extremes of freezing rate used.

RESULTS & DISCUSSION

THE COMBINED influence of aging, freezing rate and frozen storage on raw beef fried after previous thawing or directly from the frozen state is shown in Tables 2 and 3. Since the available computer program could not handle the complete experiment, the analysis of variance was made separately for the two cooking variables. An additional reason was that preliminary data had indicated an interaction between cooking method and freezing rate.

Aging and frozen storage

As seen from Tables 2 and 3, a significant interaction was found between aging time and frozen storage time for sensory and/or instrumental tenderness irrespective of cooking method used. The decrease in tenderness by prolonged frozen storage appears to be considerably smaller for the well aged beef (Table 4), in disagreement with findings reported by McCoy et al. (1949). Overriding this interaction, a significant advantage in tenderness is seen for the longer aging time. For aging, significant differences were also noted for juiciness and centrifugation loss, the longer aging time showing slightly lower juiciness (compare also Table 5) and stronger water-holding ability. The favorable effect of aging is in partial agreement with Gac and Lafon (1969), who studied the influence of aging for 1 to 15 days in combination with frozen storage at −21°C for 150g beef samples, and claimed best overall quality for 8 days' aging.

For the longer frozen storage time, the scores for tenderness were significantly lower or the shear force values higher. However, as previously mentioned, the samples used for the two frozen storage times were not from the same animals,

Table 2—Influence of aging time, freezing rate and frozen storage time for raw beef cooked by pan frying after thawing

		Source of variation, degrees of freedom, mean squares and level of statistical significance of differences							
		Mean squares							
Source of variation	dF	Flavor	Juiciness	Tenderness	Instron max shear force	Instron peak area	Thawing + cooking losses	TBA-value	Centrifugation loss
Aging time (A)	1	0.0042	2.400*	47.700**	133.7***	259700***	0.0267	0.0015	111.30***
Freezing rate (F)	2	0.0875	1.3540	1.029	1.095	2599	7.029	0.0018	13.30
Storage time (S)	1	7.704**	0.0167	7.704	105.1**	367400***	20.17*	0.2427***	781.6***
A × F	2	0.4042	0.3875	0.054	1.12	3528	2.39	0.00048	7.563
A × S	1	0.3375	0.0167	4.538*	9.40*	20620*	0.015	0.0052	8.25
F × S	2	0.0792	0.3042	0.792	0.136	1325	9.545	0.0015	10.48
Error MS		0.8125	0.4917	0.700	1.396	4220	2.554	0.0043	8.246
Error dF		48	48	48	84	84	12	36	36

	Mean values, number of observations and S.E. of means							
Treatment	Flavor score	Juiciness score	Tenderness score	Instron max shear force, kp	Instron peak area	Thawing + cooking losses, %	TBA-value E530/g fat	Centrifugation loss, %
Aging time: 4 days	5.5	4.3	5.0	5.8	320	30.3	25.3	,0.20
14 days	5.5	3.9*	6.8**	3.4***	216***	30.4	22.3***	0.16
Number of observations	30	30	30	48	48	12	24	24
S.E.	0.16	0.13	0.15	0.14	7.6	0.65	0.83	0.013
Freezing rate: 13 cm/h	5.5	3.8	6.0	4.5	263	30.2	23.6	0.19
2 cm/h	5.6	4.3	6.0	4.5	263	29.5	23.0	0.18
0.04 cm/h	5.5	4.2	5.6	4.8	279	31.4	24.8	0.18
Number of observations	20	20	20	32	32	8	16	16
S.E.	0.20	0.16	0.19	0.17	9.4	0.8	1.01	0.0164
Frozen storage: 1 month	5.9	4.1	6.2	3.6	207	29.5	19.8	0.11
9 months	5.2**	4.1	5.5	5.6**	330***	31.3*	27.6***	0.25***
Number of observations	30	30	30	48	48	12	24	24
S.E.	0.16	0.13	0.15	0.14	7.6	0.65	0.83	0.0134

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

and no initial control was included. On the other hand, great care was taken to select animals of equivalent background and quality. The changes observed in tenderness with storage time for beef from the two pairs of animals were in good agreement with the corresponding change in Instron readings for the other two pairs (Table 4). Also, separate statistical analysis showed small variation between animals within the different pairs (which had been randomly grouped). We therefore considered it justified to draw certain conclusions from the storage data, even though the LD muscles used for the two storage times were not identical. If so, the texture data will indicate an overall decrease in tenderness with prolonged frozen storage, particularly for beef aged for only 4 days, and certainly do not support the wide belief in the trade in a tenderizing effect from frozen storage. The conclusion is in agreement with McCoy et al. (1949). Unfortunately, we made no pH measurements to ascertain that muscle shortening during thawing could not have affected results for the short aging time because of freezing the meat before reaching its ultimate pH. However, in an investigation of postmortem pH development during cold storage of beef under normal Swedish conditions, Nilsson (1965) found that pH usually reaches its minimum between the 2nd and 3rd day after slaughter. Therefore we do not consider it likely that the changes we observed in tenderness with aging and frozen storage were connected with muscle shortening. Neither were any such tendencies observed visually during thawing or during cooking from the frozen state.

Apart from tenderness, our data also indicate a general decrease in quality (flavor, TBA-value, yield and water-holding ability) from combining frozen storage for 1 month at $-20^{\circ}C$ with 8 months' storage at $-30^{\circ}C$. This would indicate somewhat shorter shelflife than what is commonly reported in the literature for raw beef, but is in agreement with an earlier investigation of ours (Jakobsson and Bengtsson, 1969). There was no evidence of freezer burn in the present study.

Freezing rate

In the frozen state, a clear visual color difference was seen between freezing rates, the highest rate resulting in a very light and pale appearance, in agreement with earlier observations (Guenther and Henrickson, 1962; Jakobsson and Bengtsson, 1969). After thawing, only the meat frozen at a very slow freezing rate differed in color from the other samples,

Table 3—Influence of aging time, freezing rate and frozen storage time for raw beef cooked directly from frozen state.

Source of variation	dF	Source of variation, degrees of freedom, mean squares and level of statistical significance — Mean squares: Flavor	Juiciness	Tenderness	Instron max shear force	Instron peak area	Thawing + cooking losses
Aging time (A)	1	0.0667	4.8170*	42.5000***	113.6600*	180100*	3.082
Freezing rate (F)	2	0.4625	1.5170	1.0230	7.6500*	4944	26.45***
Storage time (S)	1	2.0170	0.8167	6.3380**	73.1400	77630	41.61***
A × F	2	0.1792	0.6167	0.5417	0.4803	1826	1.0280
A × S	1	2.0170	0.0667	3.0380	24.460***	32120*	1.5000
F × S	2	0.2041	0.1167	0.9375	0.5970	9566	1.6300
Error MS		0.5938	0.7292	0.8188	1.913	6287	2.8280
Error dF		50	50	50	86	86	14

Treatment	Mean values, number of observations and S.E. of means[a]: Flavor score	Juiciness score	Tenderness score	Instron max. shear force, kp	Instron peak area	Thawing + cooking losses %
Aging time: 4 days	5.6	4.7	5.2	6.4	362	28.1
14 days	5.6	4.1*	6.8***	4.2*	276*	28.8
Number of observations	30	30	30	48	48	12
S.E.	0.14	0.16	0.17	0.16	9.3	0.68
Freezing rate: 13 cm/h	5.5	4.4	6.0	4.9 b	308	26.9 b
2 cm/h	5.8	4.7	6.2	5.1 b	317	28.4 b
0.04 cm/h	5.5	4.1	5.8	5.9 a*	333	30.5 a***
Number of observations	20	20	20	32	32	8
S.E.	0.17	0.19	0.20	0.20	11.4	0.84
Frozen storage: 1 month	5.8	4.5	6.3	4.4	291	27.1***
9 months	5.4	4.3	5.7**	6.2	348	29.8
Number of observations	30	30	30	48	48	12
S.E.	0.14	0.16	0.17	0.16	9.3	0.68

[a] Different index letter signifies that values are significantly different at least at $P < 0.05$.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 4—Interaction between aging and storage times for sensory and instrumental measurements of tenderness[a]

		Influence of frozen storage: Cooking from +10°C 1 month	Cooking from +10°C 9 months	Cooking from −20°C 1 month	Cooking from −20°C 9 months
Tenderness score	Aged 4 days	5.6	4.3	5.7	4.6
	Aged 14 days	6.8	6.7	6.9	6.7
Maximum shear force kp	Aged 4 days	4.4	7.1	5.0	7.7
	Aged 14 days	2.7	4.2	3.9	4.6
Instron peak area	Aged 4 days	244	397	316	409
	Aged 14 days	169	264	266	286

[a] Mean values for 15 determinations of sensory quality and 24 instrumental measurements.

showing a Hunter redness (a value) of 12.4 compared with 17 for the samples frozen at higher rates.

No significant differences between freezing rates were found for any parameter when the meat was cooked after previous thawing, but the quality of the very slowly frozen meat tended to be lower, in overall agreement with the conclusions of Kondrup and Bolt (1960) and with our earlier findings (Jakobsson and Bengtsson, 1969). When cooking directly from the frozen state, a significant favorable effect of the higher freezing rates on yield and shear force value was obtained. The observations strongly indicate an interaction between freezing rate and cooking method, particularly for yield, which was also confirmed in a separate analysis of variance (Table 5).

Cooking from frozen or thawed state

A preliminary analysis of pooled data

Table 5—Combined influence of cooking method, freezing rate and aging time on juiciness and yield. Results of separate analysis of variance for 1 and 9 months frozen storage.

	Juiciness score							Thawing and cooking losses, %						
	1 month storage				9 months storage			1 month storage				9 months storage		
Source of variation	dF	MS	P	Mean values	MS	P	Mean values	dF	MS	P	Mean values	MS	P	Mean values
Cooking method (C)	1	6.07	0.05		0.833	NS		1	32.43	(0.01)[a]		13.56	near 0.05	
from thawed				4.03			4.08				29.5			31.3
from frozen				4.48			4.25				27.1			29.8
Freezing rate (F)	2	2.708	NS		1.008	NS		2	21.08	(0.01)[a]		10.93	near 0.05	
13 cm/h				4.05			4.08				27.7			29.3
2 cm/h				4.55			4.35				27.0			30.5
0.04 cm/h				4.18			4.08				30.1			31.7
Aging time (A)	1	7.00	0.05		7.50	0.01		1	0.010	NS		2.22	NS	
4 days				4.50			4.41							
14 days				4.02			3.92							
Interactions			NS			NS		F x C 2	11.56	0.05		1.69	NS	
Error	108	1.412			1.165			12	2.43			2.93		

[a] Not significant when tested against interaction

for the two cooking methods showed significant differences between them for juiciness (P = 0.001) and yield (P = 0.001). To study the combined influence of cooking method, freezing rate and aging, separate analysis of variance were performed on the data from 1 and 9 months' of frozen storage (Table 5).

Juiciness proved significantly higher for cooking directly from the frozen state after 1 month's storage, but only a directional difference was found after 9 months.

In yield a significant interaction was found between cooking method and freezing rate after 1 month's storage, obscuring the otherwise significant advantages for cooking from the frozen state and for higher freezing rates. After 9 months' storage no interaction was found, and differences between cooking methods and freezing rates were very nearly significant at the 0.05 level.

The interaction for yield was mainly caused by higher loss for the highest freezing rate when cooking after previous thawing, a possible explanation being that cracks formed during very rapid freezing were more effectively "sealed in" when the meat was fried directly from the frozen state. A repeated analysis of the data (not shown in the report), after excluding those for the highest freezing rate, eliminates interaction and gives a significant advantage in yield for cooking from the frozen state.

The advantages found for pan frying meat slices directly from the frozen state are in agreement with results reported for beef by Brady et al. (1942) and Gac et al. (1966), while Smith et al. (1969) found no such difference, between sliced LD muscles broiled at 175°C in an oven. No significant differences were found for tenderness or other qualities studied. Cooking the meat from the frozen state tended to improve (lower) the Instron readings but not sensory tenderness.

Correlation between sensory and instrumental methods

Fair general agreement was found between flavor scores and TBA-values, on one hand, and tenderness scores and shear cell Instron measurements on the other, in spite of the fact that sensory and instrumental determinations were not made on identical meat samples. The correlation was somewhat better for maximum shear force (r = 0.88) than for area under the shear force value-time curve.

CONCLUSIONS

FOR RAW BEEF significant interaction was found between aging time and frozen storage and between cooking method and freezing rate. Significant differences in one or more quality aspects were found between aging times, freezing rates, cooking methods and frozen storage times, the last comparison being less well defined because of a possible raw material difference.

The results indicate that, under Swedish conditions at least, beef should preferably be well aged prior to slicing and freezing, frozen individually at rates corresponding to good commercial practice, kept in the freezer chain less than a year (when not vacuum packed or nitrogen packed) and cooked directly from the frozen state when pan frying.

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TISSUE DISTRIBUTION AND METABOLISM OF AFLATOXIN B_1-^{14}C IN LAYER CHICKENS

INTRODUCTION

AFLATOXINS are a group of secondary metabolites produced by certain strains of the genus *Aspergillus*. These metabolites are recognized as food and feed contaminants of great economic importance throughout the world. The widespread distribution of the Aspergilli in nature suggests the potential hazard of human and animal intoxication.

The possibility of aflatoxicosis in man resulting from the ingestion of previously contaminated animal products, such as poultry meat and eggs, remains uncertain. Results of earlier research (Abrams, 1965; Allcroft and Carnaghan, 1962, 1963; Brown and Abrams, 1965; Sims et al., 1970) indicated that there was no transfer of aflatoxin to edible tissues of chickens fed diets containing crude aflatoxins. More recently, a report of possible aflatoxin dissemination into chicken muscle has been published (Van Zytveld et al., 1970).

Aflatoxins labeled with ^{14}C have been used to study toxin metabolism, distribution and excretion in laboratory rats (Dann, 1970; Wogan et al., 1967; Wogan, 1969) and monkeys (Dalezios and Wogan, 1971). Unlike conventional chemical assay procedures, this method is a specific, absolute method for determining the fate of ingested aflatoxins within a biological system.

Since discrepancies have been reported regarding the dissemination of aflatoxin in edible chicken tissues using conventional chemical techniques, the present study was designed to determine metabolism and tissue distribution of subclinical levels of aflatoxin in layer chickens using radioactive aflatoxin B_1-^{14}C as a tracer compound.

MATERIALS & METHODS

Aflatoxigenic organism

Aspergillus flavus NRRL-3145 was used for production of aflatoxin B_1 in this study. Stock subcultures were inoculated onto Potato Dextrose Agar (Difco), overlayed with sterile mineral oil, and stored at 22°C until used.

Preparation of aflatoxin B_1

Potato Dextrose Agar (PDA) slants were inoculated with the stock subculture and incubated for 7 days at 30°C. After incubation, the fungal spores were washed from the slant cultures with 5 ml of sterile distilled water per culture. The washings were pooled and the spore density was adjusted to 10^6 spores per ml with a Petroff-Hausser and Helber counting chamber (C.A. Hausser & Son, Philadelphia, Pa.). 1 ml of the spore suspension was added to each of 15 sterile, cotton-stoppered, 250-ml Erlenmeyer flasks containing 25g of wheat adjusted to 50% moisture content. The inoculated flasks were shaken to distribute the inoculum and incubated at 30°C and 93% relative humidity (RH) in a chamber within a forced air incubator. The RH was maintained in the chamber with the use of a concentrated ammonium sulfate solution (*Handbook of Chemistry & Physics*, 43rd ed, 1962) contained in shallow glass trays. The flasks were removed daily and the contents shaken in order to maximize spore dispersal and surface area within the flask.

After 5 days incubation, the wheat cultures were removed for toxin extraction. 90 ml of acetonitrile were added to each of the wheat cultures and the aflatoxins were extracted according to the procedure described by Stoloff et al. (1971). Since their procedure described the extraction of 50-g samples, the reagent volumes were halved for the present experiment. A second extraction was made to remove any remaining aflatoxins.

The crude extracts were pooled, taken to dryness and redissolved in chloroform. The redissolved crude extract was applied to silica gel G-HR (250μ thickness, Brinkman Instruments, Westbury, N.Y.) thin-layer chromatography (TLC) plates as a series of spots along a line 1 cm from the bottom edge of each plate. The spotted plates were developed in benzene–methanol-acetic acid (18:1:1) in unlined developing tanks according to Stoloff et al. (1971). Blue fluorescing spots corresponding to aflatoxin B_1 standards were scribed and scraped from the plates with a vacuum zone collection apparatus (Brinkman Instruments, Westbury, N.Y.). Aflatoxin B_1 was eluted from the silica gel with chloroform-methanol (9:1, v:v). The eluates were pooled and taken to dryness. Subsequent thin-layer chromatograms developed in chloroform-acetone (9:1) yielded pure aflatoxin B_1. The purified aflatoxin B_1 was dissolved in 2 ml of benzene-acetonitrile (98:2) and the absorbance was measured at 350 nm against standards of known concentration according to the methods of Rodricks and Stoloff (1970). A total of 45.575 mg of aflatoxin B_1 was produced from the wheat cultures.

Preparation of aflatoxin B_1-^{14}C

1 ml of spore suspension, prepared as described above, was added to each of five, 500-ml baffled Erlenmeyer flasks containing 100 ml of a sterile glucose-ammonium salts-trace minerals medium (basal medium) described by Hsieh and Mateles (1971). The cotton-stoppered flasks were incubated at 30°C in a forced air rotary incubator at 100 rpm for 24 hr, and then at 200 rpm for an additional 24 hr to maximize aflatoxin production. After incubation, the mycelia were collected by filtration on sterile cheesecloth and washed with sterile distilled water.

Resting medium, for the preparation of ^{14}C labeled aflatoxin B_1, was prepared according to procedures described by Hsieh and Mateles (1971). The resting medium, containing 30g of glucose per liter, was inoculated with 1.5g (wet weight) of washed mycelia per 10 ml of medium contained in rubber-stoppered 50-ml baffled Erlenmeyer flasks. The rubber stoppers were equipped with tubing connected from a positive pressure pump (Metaframe Aquarium Products, Maywood, N.J.) to a barium hydroxide trap system to collect the expired $^{14}CO_2$ produced by the resting culture. The air flow was adjusted to 1 ml per 4.5 sec.

Sodium acetate-1,2-^{14}C (specific activity 55.2 μCi/μM, New England Nuclear Corp., Boston, Mass.) was combined with 955.5μmoles of unlabeled sodium acetate to yield a total of 960 μmoles in 2.4 ml of sterile distilled water. The 2.4 ml of acetate solution was added to the resting culture in six aliquots of 0.4 ml per addition at 3-hr and 20-min intervals. After 20 hr incubation (3 hr and 20 min after the final addition of acetate) at 30°C and 200 rpm, the resting medium was filtered through sterile cheesecloth. The medium was extracted three times with equal volumes of chloroform, and the extracts were pooled and concentrated. Aflatoxin B_1-^{14}C was then isolated and purified as described above. The radioactivity of 1 μg in dioxane-based scintillation fluid was then measured with a Packard TriCarb model 2425 liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.). 60 μg of aflatoxin B_1-^{14}C with a specific activity of 455 μCi/μM were produced by the five resting cell cultures.

Preparation of aflatoxin B_1 inoculum

The labeled and unlabeled aflatoxin B_1 concentrates were pooled and taken to dryness in a sterile 500-ml rubber-stoppered Erlenmeyer flask. The dried pool was dissolved in 265 ml of propylene glycol to yield a total of 0.172 mg of aflatoxin B_1, including 0.22 μg of aflatoxin B_1-^{14}C, per ml of glycol. The specific activity was calculated to be 12,000 counts per minute (cpm) per ml. The ratio of aflatoxin B_1-^{14}C to aflatoxin B_1 was found to be 0.0013. This mixture was subsequently administered to layers as described below.

Experimental feeding trial

White Leghorn pullets, 21 wk of age, were used in this experiment. Nine pullets, housed individually in metabolism cages, were selected to receive the propylene glycol-containing aflatoxin. Three pullets were given propylene glycol containing no aflatoxin, and three additional birds received no inoculations. These two groups of control birds were housed in battery-type cages. All birds were fed pre-mixed standard rations for layer chickens. The birds were weighed individually, and feed consumption was determined every other day.

Propylene glycol was administered by intubation into the crop of each dosed bird with polyethylene tubing (0.067 in. OD) attached to a tuberculin syringe. Aflatoxin B_1-^{14}C was given to each test bird daily at a dose level of 0.1 mg/kg of body weight. Control birds receiving propylene glycol alone were dosed in the same manner as the test birds by calculating the weight-dose ratio. The birds were dosed daily for 14 days.

Collection of samples

Feces. Trays lined with aluminum foil were placed beneath each cage and the excreta was removed every 48 hr during the course of the feeding trial. At the time of collection, the feces were wrapped in foil liners and frozen.

Eggs. Eggs were collected daily, appropriately labeled and refrigerated at 4°C.

Blood. 5 hr after the final dose of aflatoxin and/or propylene glycol was administered, the birds in all groups were killed by exsanguination. Approximately 50 ml of blood from each bird was collected and frozen in a 150-ml beaker.

Organs and tissues. A postmortem examination was performed on each bird and the breast and leg muscles were removed. In addition, the heart, liver and gizzard from each carcass were removed and examined. Each of the organs and muscle tissues were placed in a separate plastic bag and frozen.

Preparation of samples for analyses

Feces. Frozen fecal samples were thawed at room temperature and 35g (wet weight) were taken from each of the thawed samples collected from each bird. Each sample was homogenized in 100 ml of distilled water in a Virtis model 45 homogenizer (Virtis Inc., Gardiner, N.Y.). Each homogenate was transferred to a shallow aluminum plate and lyophilized in a Virtis model 10-MRTR lyophilizer (Virtis Inc., Gardiner, N.Y.). The lyophilized samples were placed in plastic bags and stored at room temperature for further radioactive and chemical analyses.

Eggs. Individual eggs were broken, separated and placed in 100-ml beakers. The component yolks and whites were lyophilized separately, placed in individual plastic bags and stored at room temperature for radiological and chemical analyses.

Blood. The frozen blood samples were lyophilized, placed in individual plastic bags and stored at room temperature for radioactive and chemical analyses.

Organs and tissues. Each organ and tissue was thawed at room temperature, weighed and homogenized in a volume of distilled water equal to three times the weight of the tissue. The homogenates were lyophilized, placed in separate plastic bags and stored at room temperature for radioactive and chemical analyses.

Radioactive assay. Triplicate 30 mg aliquots of each lyophilized sample were placed in low-potassium glass scintillation counting vials (Packard Instrument Co., Downers Grove, Ill.). 1 ml of Soluene tissue dissolver (Packard Instrument Co., Downers Grove, Ill.) was added to each vial, and the vials were incubated in a water bath at 60°C until dissolution was complete (approximately 2 hr). After incubation, 0.01 ml of 30% hydrogen peroxide was added to each vial to decolorize the dissolved tissue. The samples then were shaken and 0.01 ml of concentrated hydrochloric acid was added to each vial to reduce the pH of the solution. The vials were again shaken and permitted to stand 15 min at room temperature. 10 ml of chilled (4°C) dioxane-based liquid scintillation counting solution were added to each vial. The vials were capped, shaken and placed in a Packard TriCarb liquid scintillation spectrometer (model 2425). Two sets of duplicate 2-min counts were recorded for each aliquot and the obtained cpm values corrected for quenching.

Chemical assay. A chemical assay for aflatoxin B_1 or its metabolites was performed on pooled samples representing each substrate where ^{14}C was observed by liquid scintillation spectrometry. The procedure, described below in general form, is a combination of methods designed to extract unbound aflatoxin or aflatoxin in conjugated form (glucuronide) from the representative substrates. Unbound aflatoxin is easily extracted from most substrates with the use of nonpolar solvent systems. Conjugated aflatoxins, however, are known to be soluble in polar or aqueous solvents.

The following quantities of lyophilized blood, tissues and excreta were pooled and chemically assayed for aflatoxin B_1 or its metabolites: excreta, 42g; liver, 45g; heart, 20g; gizzard, 20g; and blood, 40g. Triplicate samples of breast muscle (45.1, 59.2 and 64.9g) and leg muscle (55.3, 49.4 and 50.5g) were also chemically analyzed.

A pooled sample of lyophilized material was extracted with 400 ml of sodium acetate buffer (pH 4.5) in a Virtis model 45 homogenizer. The suspension was centrifuged for 10 min at 7970 × G in a Sorvall model RC2-B refrigerated centrifuge (Ivan Sorvall Inc., Newton, Conn.) adjusted to 10°C. The supernate was decanted into a 500-ml Erlenmeyer flask and stored at 4°C for further testing. The precipitate was extracted with 270 ml of acetonitrile. The suspension was centrifuged at 7970 × G and the acetonitrile was decanted into a 1-liter separatory funnel. Aliquots of the precipitate were dried and stored in a desiccator at room temperature for subsequent liquid scintillation spectrometry. 50 ml of sodium acetate buffer were added to the acetonitrile, and the mixture was extracted three times with 60 ml of chloroform. The chloroform extracts were combined (chloroform extract A), reduced to dryness and stored at 4°C for subsequent TLC and liquid scintillation spectrometry. The sodium acetate fraction was added to the initial sodium acetate extract and placed in a 1-liter separatory funnel. The combined sodium acetate extracts were treated with sodium chloride (3.5g/10 ml of extract) and an equal volume of 1N dipyridinium sulfate and shaken. The solution was extracted three times with an equal volume of ethyl acetate according to the method of Okerholm et al. (1970). The sodium acetate layer (sodium acetate buffer extract A) was transferred to an Erlenmeyer flask and stored at 4°C for further treatment. The ethyl acetate extracts were combined and reduced to dryness. The dried ethyl acetate extract was redissolved in 30 ml of sodium acetate buffer and transferred to a 250-ml beaker. 200 mg of β-glucuronidase (Worthington Biochemical Corp., Freehold, N.J.), with a specific activity of 41 units per g, were dissolved in 10 ml of sodium acetate buffer. One unit of β-glucuronidase is that amount which will liberate 1 μmole of glucuronic acid from a glucuronide conjugate per minute at 37°C. 10 ml of the enzyme were added to the redissolved ethyl acetate extract and incubated for 40 hr in a water bath at 37°C. After incubation, the enzyme-substrate mixture was extracted three times with 45 ml of chloroform. The sodium acetate layer (sodium acetate buffer B) from the enzyme reaction was stored at 4°C for subsequent TLC and liquid scintillation spectrometry. The chloroform extracts were combined (chloroform extract B), reduced to dryness and stored at 4°C for subsequent TLC and liquid scintillation spectrometry.

Chloroform extracts A and B were each redissolved in 1.0 ml of chloroform. Sodium acetate buffer extract A was reduced to dryness in a forced-air oven at 75°C and redissolved in 50 ml of sodium acetate buffer. 5 μl of chloroform extracts A and B, sodium acetate buffers A and B, and aflatoxin B_1 standards were spotted on silica gel G-HR thin-layer plates and developed in benzene-methanol-acetic acid (18:1:1).

Blue fluorescing spots with different R_f values from aflatoxin B_1 were scribed, scraped from the plate with a vacuum zone collection apparatus, and eluted from the gel with chloroform-methanol (9:1). The eluates were reduced to dryness and redissolved in absolute ethanol. Absorbance maxima for the eluates were determined on a Beckman DU-2 spectrophotometer. The eluates were reduced to dryness in scintillation counting vials and 10 ml of dioxane-based counting solution was added to each vial. Duplicate counts were recorded for each vial in a liquid scintillation spectrometer.

Duplicate 30 mg aliquots of extracted, lyophilized samples were prepared for liquid scintillation spectrometry according to the procedure outlined above. Duplicate 0.01 ml aliquots of chloroform extracts A and B, and sodium acetate buffers A and B were added to scintillation counting vials and radioactivity determined as described above.

RESULTS & DISCUSSION

Production of aflatoxin B_1-^{14}C from sodium acetate-1,2-^{14}C

Biollaz et al. (1968, 1970) performed chemical degradation studies on aflatoxin B_1-^{14}C produced by cultures of *A. flavus* from either acetate-1-^{14}C or acetate-2-^{14}C precursors. Based upon their presumptive evidence that acetate-1-^{14}C contributed to nine of the 16 ring carbon positions in aflatoxin B_1-^{14}C, and acetate-2-^{14}C contributed to the remaining seven ring positions, aflatoxin B_1-^{14}C was successfully produced in the present experiment by *A. flavus* from acetate-1,2-^{14}C. The specific activity obtained (455 μCi/μM) was calculated to be approximately twice that of aflatoxin B_1-^{14}C produced from acetate-1-^{14}C under the condition described by Hsieh and Mateles (1971).

Effects of low levels of aflatoxin B_1-^{14}C on the performance of layer chickens

The daily administration of 0.1 mg of aflatoxin B_1-^{14}C per kg of body weight (0.1 ppm) to layer chickens for 14 consecutive days did not significantly ($P < 0.05$) affect weight gain, feed consumption and egg production. Sims et al. (1970) observed weight loss in laying hens resulting from daily intubation of 8.0 ppm of crude aflatoxin to each hen. There are other reports that describe the effects of various levels of aflatoxins on chickens (Platonow, 1965; Cottier et al., 1968, 1969; Muller et al., 1970); however, these workers administered aflatoxin by incorporating it into feed at various levels. Their results are therefore based upon the amount of feed the test birds consumed.

A postmortem examination of the aflatoxin-treated layers revealed no gross evidence of pathology. All organs and tissues from aflatoxin-treated birds were comparable in weight, size and color to those of the control birds.

Detection of administered radioactivity (^{14}C) in selected organs and tissues

In order to account for the total amount of aflatoxin B_1-^{14}C retained by the test chickens, it was necessary to determine the amount of aflatoxin B_1-^{14}C excreted. Since fecal samples were collected at regular 48-hr intervals during the course of the trials, patterns in the excretion of aflatoxin as measured by radioactivity could be determined. The patterns of ^{14}C excretion by the test layers are presented in Figure 1. The data presented are average levels of net radioactivity (^{14}C) detected per g (dry weight) of the fecal material collected at each 48-hr interval from all aflatoxin B_1-^{14}C treated layers. This figure graphically illustrates that there was no significant change in the rate of excretion of ^{14}C in the feces of layers after the second and subsequent daily administrations of aflatoxin B_1-^{14}C. This observation is verified by the overlapping standard deviations of means representing radioactivity in feces collected at 48-hr intervals. The configuration of the curve indicates a trend toward a constant rate of excretion of ^{14}C by the layers.

The initial rapid increase in the rate of ^{14}C excretion by layers during the 24 hr immediately following the initial administration of aflatoxin B_1-^{14}C is consistent with data obtained by Wogan (1969). He observed maximum levels of radioactivity (^{14}C) in feces within 15–24 hr after the administration of ^{14}C labeled aflatoxin B_1 to Fischer rats. However, these data are not directly comparable to the data obtained in the present experiment since Wogan et al. (1967) and Wogan (1969) administered a single dose of aflatoxin B_1-^{14}C by intraperitoneal injection into rats and studied the distribution and excretion of ^{14}C for only a 24-hr period.

A histogram representing the distribution of ^{14}C in various organs and tissues of layers is presented in Figure 2. The amounts of ^{14}C detected in the excreta, blood and each tissue of layers was calculated by multiplying the net cpm/g of sample times the total grams of sample obtained. This calculation was performed for each sample collected from each layer and the average value for each sample was calculated for the nine layers.

The average level of radioactivity (^{14}C) detected in the blood, liver, heart, gizzard, breast muscle and leg muscle was 19.5, 16.1, 3.9, 7.2, 26.4 and 26.9%, respectively, of the total amount retained by the layers. Wogan (1969) administered a single intraperitoneal injection of aflatoxin B_1-^{14}C into Fischer rats and recovered 2.3 and 7.7% of the ^{14}C dose in the blood and liver, respectively. He also reported less than 0.1% of the dose was recovered from the hearts and skeletal muscles of the experimental rats.

In the present study the total radioactivity (^{14}C) detected in liver, heart, gizzard, breast meat and leg meat samples accounted for 7.85% of the total ^{14}C administered in 14 doses.

The average net cpm of the blood, organs and tissues of layers is presented in Table 1. The total amount of ^{14}C in the blood, organs and tissues was approximately equal to that amount in the final dose of aflatoxin B_1-^{14}C administered 5 hr before the birds were sacrificed. These data indicate that most of the aflatoxin B_1-^{14}C administered in the previous 13 doses was excreted before the final dose was given. This observation is compatible with that of Wogan (1969) who found that approximately 80% of a single intraperitoneal dose of aflatoxin B_1-^{14}C in

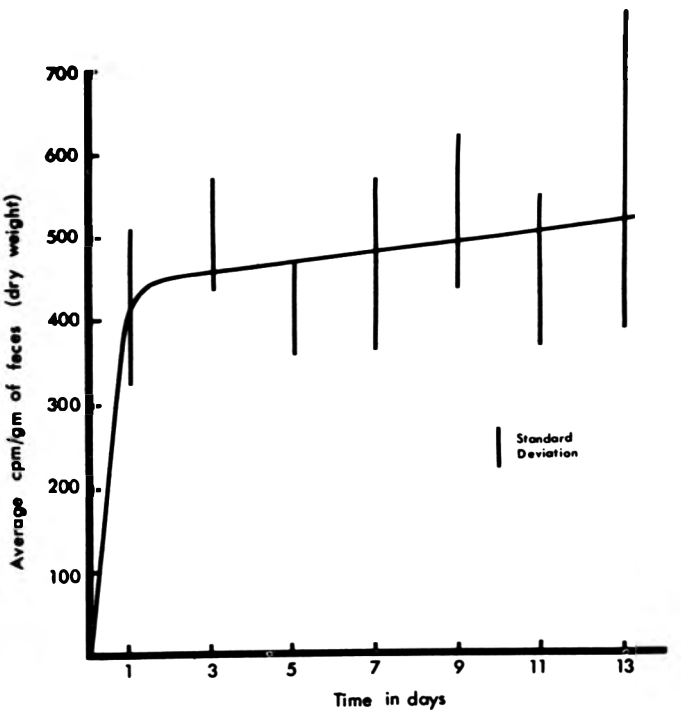

Fig. 1—Excretion of ^{14}C by layers administered aflatoxin B_1-^{14}C daily for 14 days.

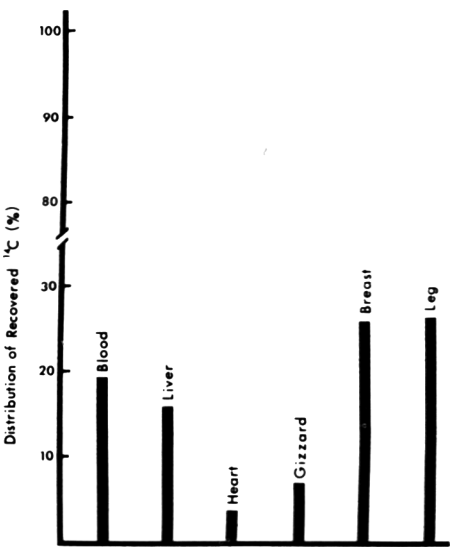

Fig. 2—Average net radioactivity (^{14}C) distributed in blood, organs and tissues. Variance of ± 10% within each set of samples.

rats was excreted within 24 hr post-injection.

Characterization of radioactive aflatoxins recovered from excreta and selected organs and tissues

The results obtained in the present experiment from the chemical assay of pooled lyophilized radioactive excreta, blood, organs and tissues of layers indicate that 81.2% of the radioactivity observed in these substrates (Table 1) was confined to the sodium acetate buffer (A) extract. Dalezios and Wogan (1971) observed that 85% of the administered radioactivity (aflatoxin B_1-^{14}C) present in the urine of rhesus monkeys was in the aqueous extract in conjugated form. Dann (1970) observed conjugated aflatoxin M_1 and B_{2a} in the bile of rats administered aflatoxin B_1-^{14}C.

Treatment of sodium acetate buffer extract (A) for the presence of conjugated aflatoxins, followed by treatment with β-glucuronidase and subsequent chloroform extraction, revealed that 31.5% of the total radioactivity observed in the aqueous extract (sodium acetate buffer A) was located in the chloroform (B) extract. Thin-layer chromatography of chloroform extract (B) from each lyophilized sample yielded a bluish spot with an R_f value of 0.34. Absorbance maxima observed were the same as those described by Holzapfel et al. (1966) for aflatoxin M_1. Subsequent liquid scintillation spectrometry of the eluted fluorescing spots revealed that approximately 90% of the radioactivity observed in chloroform extract (B) was confined to the isolated fluorescing material.

Sodium acetate buffer (B) contained 49.7% of the total radioactivity observed in the aqueous extract (sodium acetate buffer extract A). Subsequent thin-layer chromatography yielded no discernible fluorescing spots under long-wave ultraviolet light.

The radioactivity (^{14}C) observed in chloroform extract (A) from each lyophilized sample was about 10% of that observed for each sample tested. The limitations of the chromatographic procedure precluded the possibility of visual observation because of high concentration of extracts used.

No radioactivity (^{14}C) was observed in sodium acetate buffer extract (A) after treatment for conjugates, and no ^{14}C was detected in the tissues after extraction.

The results indicate that layer chickens can metabolize the majority of aflatoxin B_1 when administered at relatively low levels. Aflatoxin conjugates are the predominating form of metabolite produced. Spectrophotometric maxima and radioactive fluorescing spots on TLC are suggestive evidence that aflatoxin B_1-^{14}C is metabolized to 4-hydroxy-aflatoxin B_1-^{14}C (aflatoxin M_1-^{14}C) and that this metabolite is soluble in aqueous extracts and is present in the form of a conjugated glucuronide. Aflatoxin M_1 glucuronides constituted 38.9% of the total conjugates extracted by ethyl acetate. Dalezios and Wogan (1971) reported sulfate conjugates of metabolized aflatoxin B_1 in addition to glucuronides from monkey urine.

Table 1—Average total radioactivity (^{14}C) of blood, organs and tissues

Sample	Counts per min[a]
Blood	2374.5
Liver	1961.7
Heart	481.6
Gizzard	871.8
Breast	3214.9
Leg	3292.4
Total	12,196.9
Final dose	11,611.1

[a] Birds were sacrificed and samples collected 5 hr after the final dose of aflatoxin was administered.

The radioactivity (^{14}C) observed in sodium acetate buffer (B) is attributable to metabolite(s) of aflatoxin B_1, possibly aflatoxin M_1, that is (are) conjugated in some form other than a glucuronide, possibly a sulfate conjugate.

Implications

The average net cpm detected in blood, organs and tissues from layers sacrificed 5 hr after the final dose was administered corresponded to approximately 0.1 mg aflatoxin B_1-^{14}C/kg body weight, or approximately equal to the final dose administered. In relation to the total amount of aflatoxin B_1-^{14}C administered during the 14-day feeding trial, 7.85% was detected in the layer blood, organs and tissues. It is likely that most of the remaining ^{14}C in the blood, organs and tissues would be excreted after a period of time if the administration of aflatoxin B_1-^{14}C was discontinued.

Metabolism of aflatoxin by animals resulting in the formation of conjugates is a possible explanation for the conflicting reports dealing with the isolation of aflatoxins from biological tissues or the potential toxicity of tissues taken from animals receiving aflatoxins. The fact that conjugated aflatoxins are not extractable from biological tissues by classical methods (Abrams, 1965; Brown and Abrams, 1965; Sims et al., 1970; Smith and Hamilton, 1970) indicates the possibility that aflatoxin might have been present in conjugated form and not observed by these and other researchers. Van Zytveld et al. (1970) observed, but did not quantify, fluorescing spots on the thin-layer chromatograms representing chemical extracts of livers and skeletal muscles of chickens administered aflatoxins by crop intubation. They reported that the fluorescing spots were, in all probability, metabolites of aflatoxins B_1 and G_1 (M_1 and M_2). However, aflatoxin M_2 is a metabolite of B_2 rather than G_1 as reported by Van Zytveld et al. (1970). It should be emphasized that aflatoxins or their metabolites were only detected in the livers and skeletal muscles from chickens that were severely affected as a result of aflatoxin ingestion.

The toxicity of aflatoxins in conjugated form has not been described and is at this time unknown. Allcroft and Carnaghan (1962, 1963) and Platonow (1965) fed ducklings and ferrets, respectively, organs and tissues from chickens previously fed aflatoxins. They observed no evidence of toxicity as a result of feeding organs and tissues from aflatoxin-fed chickens and concluded that there was no toxicity associated with those parts.

Conjugated aflatoxins can be liberated by animal systems in the presence of the appropriate enzyme. Since aflatoxin M_1-^{14}C was successfully liberated from a glucuronide conjugate with β-glucuronidase in an in vitro reaction in the present experiment, a similar reaction could take place in the liver and other tissues of animals administered aflatoxin conjugates. The liberated or unconjugated aflatoxin would probably then undergo re-conjugation as a part of the "detoxication" process in an animal's system, resulting in possible deposition in the animal's tissues.

Dann (1970) and Dalezios and Wogan (1971) have underscored the hazard involved in relying upon classical nonpolar extraction procedures and TLC R_f's for identifying aflatoxin in animal by-products. This conclusion was based upon the fact that aflatoxin B_1 metabolized by rats or rhesus monkeys was predominately in conjugated form not extractable by classical methods and not discernible by ultraviolet examination of TLC plates. The results of the present experiment are in agreement with the results reported by Dann (1970) and Dalezios and Wogan (1971) and indicate that classical nonpolar extraction procedures cannot be relied upon for the isolation and identification of aflatoxins in animal tissues.

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COMPOSITION AND PROPERTIES OF EXTRUDED, TEXTURIZED POULTRY MEAT

INTRODUCTION

MEAT from the major poultry carcass parts (thigh, drum, breast) and mechanically deboned meat from carcass necks, backs, wings and/or frames have been evaluated for use in meat emulsion-type products (Blackshear et al., 1966; Maurer et al., 1969; Baker et al., 1970; Froning, 1970; Froning and Janky, 1971; and Froning et al., 1971). The emulsifying capacity and emulsion stability performance of mechanically deboned poultry meat has been related to compositional differences in protein and fat content (Froning, 1970; Froning and Janky, 1971). Composition of muscle tissue from the major carcass parts (Maurer et al., 1969; Hudspeth and May, 1967), giblets and skin (Hudspeth and May, 1969), and mechanically deboned meats (Froning, 1970; Vadehra and Baker, 1970a) has been reported. There are considerable variations in the chemical and physical characteristics among deboned meats produced from different classes of poultry (Grunden et al., 1972), deboned with various machines (Froning, 1970), and processed with different levels of skin (Satterlee et al., 1971).

The USDA regulations for boneless poultry products specify natural proportions of meat, skin and fat as they occur on the whole carcass or carcass part (Federal Register, May 16, 1972). Deviations in proportion quantities must be labeled otherwise. Convenience items such as poultry rolls, loaves and roasts are normally prepared with hand deboned meat tissues which are easily excised from the carcass. Product development using mechanically deboned meat has been hindered by the puree form and comminuted consistency of this meat. Histologically, no muscle fibers have been observed in several samples of commercially deboned neck and back meats (Vadehra and Baker, 1970a).

The purpose of this study was to explore the possibility of texturizing machine deboned meat for use in further processing. Texturization by the particulate binding of tissue was considered in terms of meat firming and resistance to shearing. Structural units of meat were formed by an extrusion method for composition and functional property analyses.

EXPERIMENTAL

Sources and preparation of meats

Thigh and leg tissues were hand deboned from carcasses of 8-wk old broilers. Visible fat, skin and connective tissues were separated from the lean and discarded. Cryovac packages containing approximately 0.8 kg of meat were sealed and frozen at −20°C for 4–5 months. Thawing was conducted at room temperature (22°C) for 10–12 hr followed by 4–6 hr storage at 4°C. The thawed meat was finely ground twice through a 4 mm plate, mixed with 0.5% NaCl (w/w) and reground two additional times through the same plate. The meat was held at 4°C prior to further processing.

Two batches of machine deboned neck meat from 9-wk old broilers were obtained from a regional poultry processing plant. A Beehive Deboner, operated at a setting of 9-B, was used to prepare both meat batches. One batch of necks had been deboned with the normal complement of neck skin left intact. The second batch of necks was deboned after complete removal of adhering skin tissue. The machine deboned meats were frozen in approximately 2.7 kg units, transported frozen to the laboratory and stored at −20°C for 2–3 months. After thawing as previously described, the meats were each thoroughly blended with 0.5% NaCl (w/w). The two batches of neck meat were held at 4°C until extruded and heat-processed.

Meat extrusion and texturization

The ground thigh and leg meat was extruded using an Osterizer Model 480 Food Grinder. The meat was forced along the grinder path (cutting blade omitted) through a plate with a single 4 mm orifice. Meat strands of 4 mm diameter were collected on 22 × 30 cm sheets of aluminum foil. Approximately 60g of meat yielding 10–12 meat strands 28 cm in length and spaced 10–12 mm apart were collected per sheet.

Texturization of the raw meat strands was accomplished by placing the foil sheets in a mechanical convection oven (Blue M, Model OV-490A-2). The air temperature was maintained at 100° ± 1°C. Sheets of meat strands were removed after 1, 3, 5, 7.5 and 10 min of heating. The strands were cut to lengths of 35–45 mm. Meat samples at each time interval were combined and stored in glass jars at 4°C for further analysis.

A second extrusion system was utilized for the machine deboned neck meats. A sausage stuffer equipped with a horn having a 4 mm orifice was used to extrude strands of these meats. Under applied pressure, meat was easily extruded and collected on foil sheets as previously described. Texturization of the extruded strands, division into smaller strand segments and storage for analysis was conducted as outlined above. Due to the consistency or rheological characteristics of the neck meats, heating times at 100°C were altered to 3, 5, 7.5, 10 and 12 min. Three replicate preparations of each of the texturized meats were used for chemical, physical and physiochemcial analyses.

Chemical analyses

Initial meat samples and texturized strands were analyzed for moisture, protein and lipid contents. Moisture was determined by the AOAC (1970) method. The Kjeldahl nitrogen method following AOAC (1970) was used for protein determinations. Lipid content was calculated from ether extractables (Soxhlet). All analyses were conducted in duplicate.

The pH of meat samples was measured using homogenates. Duplicate 10-g portions of the meats were blended for 1 min with 100 ml quantities of distilled water in an Osterizer and the pH of the homogenate recorded.

The extractability of salt-soluble proteins

Table 1—Composition, binding strength and shear force values of extruded thigh and drum meat strands from dry heat texturization[a]

Min at 100°C	Moisture %	Fat %	Protein %	Binding Strength[b]	Shear force, kg/g
0	74.07[a]	3.52[a]	19.05[a]	–	–
1	72.33[a]	3.65[a]	21.60[b]	1.27[a]	1.95[a]
3	69.90[b]	5.02[b]	22.50[b]	3.13[b]	3.05[ab]
5	68.23[b]	5.82[b]	23.15[b]	4.83[c]	4.22[ab]
7.5	65.93[c]	6.02[b]	26.90[c]	7.47[d]	5.03[b]
10	62.27[c]	7.20[c]	28.40[c]	8.63[d]	7.83[c]

[a]Any two means within a column having one of the same letters are not significantly different at $P < 0.05$.
[b]Binding strength range: 1 = extremely poor bind; 9 = extremely good bind.

from the initial neck meat samples and texturized strands was evaluated using a modification of the method reported by Acton (1972). 6g of meat (finely ground) were placed in 120 ml of 0.6M NaCl solution in a 250 ml beaker. The meat slurry was blended for 1 hr on a Corning stirrer, Model PC-353 at 465 rpm. The extraction was accomplished at 4°C using salt solution of the same temperature. Following extraction, the slurry was centrifuged at 12,000 × G for 15 min. The supernatant was decanted, filtered through a small pad of glass wool to remove any fat particles, and stored at 4°C. Extracts were analyzed for protein by the Kjeldahl nitrogen method (AOAC, 1970). Total extract nitrogen was corrected for nonprotein nitrogen using 5% trichloroacetic acid (TCA) filtrates. Salt-soluble protein was expressed as a percent of the total meat protein.

Physical analyses

Texturized samples were analyzed for binding strength (Acton, 1972) and shear force values. A trained panel of eight members evaluated the binding strength of the meat strands using a nine-point hedonic scale (1 = extremely poor bind; 9 = extremely good bind). Panel members were trained as previously reported (Acton, 1972). The meat strands were at room temperature (22°C) and panelists were furnished two to three strands per sample. Panelists were instructed to disregard appearance and texture (softness, hardness) in rating the binding since some samples of the meat were raw in appearance and texture.

Triplicate samples of strands were tested for shear force values using a Kramer press with a 250 lb ring and 30 sec downstroke. Samples of the texturized strands averaging 3g were placed at 90° angles to the open grid slots in the cell chamber to accomplish the shearing action across the strand length. Shear forces were calculated as kg force/g sample.

Physiochemical analyses

Water holding capacity and emulsion stabilizing capacity tests were conducted. The water holding capacity method was adopted from a centrifugation technique reported by Hamm (1960). Samples of the meats were ground twice through a 5 mm plate. Duplicate quantities of 17.5g of meat were placed in 2.8 × 11 cm centrifuge tubes, 21 ml of 0.6M NaCl added and the slurry stirred for 1 min with a glass rod. After holding for 15 min at 4°C, the meat slurry was stirred again for 1 min and centrifuged at 12,000 × G for 15 min. The supernatant layer was decanted and the volume recorded. The amount of added solution retained by the meat is reported as the water holding capacity in ml/100g.

Emulsions of 60g of texturized meat and 40g of rendered pork fat were prepared for stability evaluation. The meat was ground twice through a 5 mm plate and blended with 0.3% NaCl (w/w) for 1 min. Pork fat in 10–15g quantities (to 40g total) was blended with the meat over a 5 min period for emulsion formation. Duplicate 30g emulsion samples were stuffed in 2.8 × 11 cm test tubes, covered with aluminum foil and placed in an 80°C water bath for 20 min. After heating, aliquots of 80°C water were added to the tubes to separate liquid fat released by the emulsion. The volume of liquid fat obtained from 30g of emulsion was used for comparisons of emulsion stability.

Statistical analyses

Regression analysis was used to estimate the linear relationship between the observed variables of the chemical and physical analyses and the length of the texturizing intervals. In addition, all results were subjected to analysis of variance and the significance of means tested by Duncan's method (Steel and Torrie, 1960). Linear correlation analysis for the relationships among variables of the two batches of chicken neck meat was included in the statistical analysis.

Table 2—Regression equation, standard error of the coefficient (s_b) and R^2 value for the relationship between observed variables and the time (minutes) of heating during texturization of extruded broiler neck meat

Variable observed y	Regression (y = a + bt) intercept, a	coefficient, b	s_b	R^2
Neck, with skin				
Percent moisture	70.638	−1.408**	0.068	0.977
Percent protein	14.210	0.760**	0.046	0.964
Percent fat	14.385	0.574**	0.118	0.702
Bind strength	0.770	0.544**	0.035	0.968
Shear force, kg/g	2.624	0.301**	0.041	0.870
Neck, without skin				
Percent moisture	75.400	−1.193	0.046	0.985
Percent protein	16.377	0.666**	0.056	0.934
Percent fat	7.327	0.368**	0.064	0.766
Bind strength	2.515	0.546**	0.089	0.825
Shear force, kg/g	1.261	0.429**	0.027	0.968

**Highly significant ($P < 0.01$)

Table 3—Shearing force, binding strength, water holding capacity and emulsion stabilizing capacity of texturized broiler neck meat strands[a]

Minutes at 100°C	Shear force, kg/g		Binding strength[b]		Water holding capacity[c]		Emulsion stability[d]	
	with skin	without skin	with skin	without skin	with skin	without skin	with skin	without skin
0	–	–	–	–	17.7[a]	16.0[a]	12.4[a]	11.0[a]
3	3.7[a]	2.6[a]	2.2[a]	3.6[a]	11.4[b]	10.3[a]	12.2[a]	13.5[b]
5	4.1[a]	3.2[a]	3.8[b]	5.2[a]	16.6[a]	15.4[a]	7.0[b]	10.5[a]
7.5	4.6[ab]	4.7[b]	4.7[b]	7.6[b]	41.1[c]	36.0[b]	6.2[c]	4.5[c]
10	5.4[b]	5.6[c]	6.2[c]	8.2[b]	53.4[d]	53.1[c]	7.8[d]	3.2[d]
12	6.5[c]	6.3[c]	7.4[d]	8.4[b]	65.1[e]	63.7[d]	4.0[e]	3.5[cd]

[a]Any two means within a column having one of the same letters are not significantly different at $P < 0.05$.

[b]Binding strength scale: 1 = extremely poor bind; 9 = extremely good bind.

[c]ml 0.6M NaCl bound/100g meat tissue

[d]ml pork fat released/30g emulsion

RESULTS & DISCUSSION

THE COMPOSITIONAL change occurring in ground thigh and drum meats during the heat induced texturization is shown in Table 1. The unit of product material, referred to as "meat strand," undergoes a significant ($P < 0.05$) loss of moisture with an increase of heating time. The moisture loss in turn significantly increases the protein and fat contents of the meat strands. There is greater firming of the tissue with longer process times as shown by an increase of the binding strength and shearing force.

Thigh, drum and breast muscle tissue from poultry carcasses are relatively easy to hand debone and are the more desirable raw material for preparation of filets or whole bound products such as rolls and roasts. Machine deboned meat from the carcass neck, back, wing or frame does not possess the same textural characteristics due to the communition involved in processing. Texturization of machine deboned meats may be advantageous in providing extended usage for further processed products. Thus, a more comprehensive study of machine deboned meat was undertaken using the same process as previously described.

Chemical analysis of broiler neck meat with and without accompanying skin tissue is shown in Figure 1. The neck meat containing skin initially had a higher fat

content and lower moisture and protein content than the meat without skin. It is possible to mechanically debone meats (neck, back and/or wing) to differing moisture, fat and protein levels. Satterlee et al. (1971) reported variations in chemical composition of back meat deboned at various equipment settings. These workers also reported that there was a significant relationship between percent skin included with the meat and the resulting product composition.

During the heating process there was a significant ($P < 0.01$) linear relationship between percent moisture, percent protein, percent fat and length of process time (Table 2). Protein and fat content increased inversely with respect to the decrease in moisture content. A significant ($P < 0.01$) loss of salt-soluble protein extractability due to heat denaturation occurred within the first 3 min of heating. Extractability had decreased approximately 67% for meat with or without skin. Further heating resulted in either no significant ($P < 0.05$) loss of protein extractability (meat with skin) or losses up to 38% (meat without skin) between 3 min and 10 min of heating. The quantity of extractable salt-soluble protein available for oil emulsification (Maurer et al., 1969; Hudspeth and May, 1967) and for binding of meat particles to each other (Vadehra and Baker, 1970b; Acton, 1972) is of importance in processing of sausage-type products or bound poultry meat products. The pH of the meat strands remained constant at pH 7.3 and was not affected by the amount of heating or level of skin.

The extent of texturization observed by changes in the physical properties of the meat strands is given in Table 3. As heating time increased, there was a significant ($P < 0.01$) increase in shear resistance. The degree of firming or tissue resistance to shear can be controlled by varying the length of exposure to heat. For neck meat with skin, there was an increase of approximately 77% in shear force when heating times of 3 min and 12 min are compared. Comparison of the same heating times for neck meat without skin shows a 144% increase in shear resistance. At each interval of heating after 5 min, the shear forces for the two types of meat strands are close in value and generally show the same rate of increase in resistance with time of heating. Significant relationships were observed between shear force and heating time (Table 2) and chemical components of the meat (Table 4).

Binding strength evaluations for the neck meat strands showed a significant ($P < 0.01$) increase in tensile strength with the increase of process time (Table 3). At 3 min, the strands had moderately poor binding characteristics (ratings of 2–4), whereas at 10–12 min exposure, the meat strands were more firmly bound and possessed good to very good binding quality (ratings of 7–8). The loss of protein extractability and the increase of tissue binding strength were significantly correlated (Table 4) for strands from meat without skin. No significant correlation occurred between these two variables for strands from meat with skin. Acton (1972) previously found that binding strength development during heat processing of meat loaves was significantly correlated with the denaturation and loss of extractability of salt-soluble protein. Binding strength was related to changes in other chemical components (Table 4).

The water holding capacity of the initial deboned meats and texturized strands is presented in Table 3. Both types of meat (with and without skin) showed a significant ($P < 0.01$) increase in the volume of 0.6M NaCl solution absorbed and bound as texturization time increased. Although heating resulted in a significant loss of extractable protein, the meat tissue's ability to hydrate solution increased. Normally the hydration capacity decreases as internal temperature of the meat increases, due to protein denaturation (Hamm and Deatherage, 1960; Hamm 1966). It is possible that the tis-

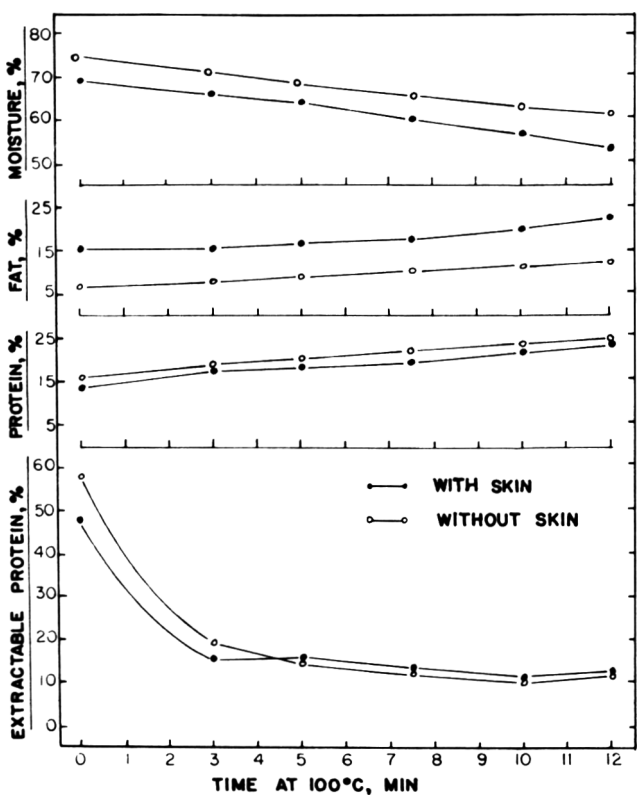

Fig. 1—Chemical composition of mechanically deboned broiler neck meats as a function of heating time.

Table 4—Correlation coefficients between chemical components and physical and physiochemical properties of extruded, texturized broiler neck meat

Variable	Shear force	Binding strength	Water holding capacity	Emulsion stability
Neck, with skin				
% Moisture	−0.96**	−0.97**	−0.94**	0.86**
% Fat	0.75**	0.87**	0.86**	−0.68*
% Protein	0.95**	0.97**	0.87**	−0.82**
% Salt-soluble protein	−0.58ns	−0.59ns	−0.45ns	0.64*
Shear force		0.93**	0.92**	−0.76**
Bind strength			0.95**	−0.81**
Water holding capacity				−0.78**
Neck, without skin				
% Moisture	−0.96**	−0.90**	−0.90**	0.85**
% Fat	0.75**	0.80**	0.82**	−0.79**
% Protein	0.93**	0.91**	0.85**	−0.83**
% Salt-soluble protein	−0.79**	−0.76**	−0.48ns	0.49ns
Shear force		0.88**	0.98**	−0.95**
Bind strength			0.90**	−0.95**
Water holding capacity				−0.93**

**Highly significant ($P < 0.01$)
*Significant ($P < 0.05$)
nsNonsignificant

sue's water holding capacity was enhanced on a comparative weight basis due to the decrease of moisture content and increase of protein content which occurred with progressive heating. Significant correlations were observed between the change of each chemical component, except extractable protein, and the water holding capacity (Table 4).

Emulsions prepared with the texturized meat and pork fat showed an increase of stability against fat separation as treatment heating time increased (Table 3). The fat stabilizing characteristic appeared to be enhanced by the heat treatments although the quantity of soluble protein responsible for emulsification (Hudspeth and May, 1967) had significantly decreased. Emulsions made from ground meat heat processed for 5 min and longer exhibited a grainy, coarse consistency in comparison to the normal fine batter consistency which occurred with the initial meat samples. Microscopic examination would be necessary to determine whether the fat was actually emulsified to dispersed globules (Borchert et al., 1967) or only physically adsorbed to the meat particle surface.

The many significant relationships (Table 4) occurring between chemical components of the meat and the functional properties of the meat strands indicate the complex nature of meat tissue. Simultaneous changes in meat characteristics during heat induced texturization and their effects on product quality will require further study.

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POSTMORTEM QUALITY CHANGES IN ICED PACIFIC SHRIMP (Pandalus jordani)

INTRODUCTION

PACIFIC SHRIMP represent a major seafood resource in the coastal waters of northern California, Oregon, Washington and Alaska. Landings in Oregon in 1969 amounted to over 10.5 million pounds round weight (Fish Commission of Oregon, 1971). Shrimp in this fishery are caught with a towed trawl net, separated from trash fish and washed with sea water. The whole shrimp are then packed in alternate layers of ice in boxes or bins in the hold of the trawler. The age of landed shrimp varies from 1 to 4 days post-catch.

Quality changes that occur in iced shrimp during storage are generally considered to result from the combined action of tissue enzymes and microbial contamination (Fieger et al., 1958; Nair and Bose, 1964; Sundsvold et al., 1969; Luna, 1971; Cobb and Vanderzant, 1971; Flick and Lovell, 1972). Changes in pH (Bethea and Ambrose, 1962; Bailey et al., 1956; Luna, 1971), microbial numbers (Campbell and Williams, 1952), trimethylamine (Iyengar et al., 1960; Fieger and Friloux, 1954), total nitrogen, nonprotein nitrogen, free amino acid (Velankar and Govindan, 1957, 1958; Gagnon and Fellers, 1958), volatile acid (Fieger and Friloux, 1954), indole (Duggan and Strasburger, 1946) and carotenoid levels (Collins and Kelley, 1960; Kelley and Harmon, 1972) have been used and/or proposed as indices of iced shrimp freshness.

Many of the previous investigations of the postmortem changes in iced shrimp have dealt with species from the Gulf of Mexico *(Penaeus aztecus* and *setiferus).* This investigation was undertaken to develop information on the postmortem biochemical changes that take place in iced Pacific shrimp and to evaluate the relationship between these changes and the quality of the cooked product.

EXPERIMENTAL

Materials and handling procedures

Analyses were carried out on samples from a 400-lb lot of shrimp taken from a single trawl about 40 miles off the North coast of Oregon. A portion of the intact shrimp, after washing with sea water, was immediately frozen on dry ice. The remaining larger portion of the sample was well iced. Upon landing, the iced intact shrimp samples were reiced and placed under refrigeration at 1–2°C.

Shrimp frozen on dry ice immediately after removal from the sea were analyzed after storage for 1 day at the temperature of dry ice and were considered as zero-time samples. Iced intact shrimp were analyzed daily for a period of 8 days. Enzymatic and microbiological evaluations were made on intact shrimp only. Chemical evaluations were carried out on both intact shrimp and cooked separated meat. Shrimp were cooked for 2 min in boiling water, cooled in air and the meat was removed by hand peeling. Prior to testing, samples for flavor panel evaluation were vacuum sealed in moisture vapor proof film, frozen and stored at −35°C.

Analysis procedure

Intact shrimp and cooked meat (200g) were blended with 200g of distilled water at 4°C for 2 min using a Waring Blendor. Sterilized distilled water and blender containers were used in the preparation of intact shrimp homogenates.

The total nitrogen content of the intact shrimp and cooked meat-water (1:1) homogenates was determined by a semi-micro Kjeldahl procedure (AOAC, 1970).

Shrimp-water (1:1) homogenates were analyzed for their total carotenoid content according to the procedure of Kelley and Harmon (1972). The carotenoid (astaxanthin and astacin) content was expressed as $A^{474nm}_{1\ cm}$ of the carotenoids from 100g of wet sample in 100 ml of cyclohexane.

A 50-g sample of the shrimp-water (1:1) blend was homogenized at 4°C for 10 min in 200g of 5% NaCl solution which contained $NaHCO_3$ (0.02M). This homogenate was divided into two equal portions. One portion was centrifuged at 1000 × G for 10 min at 4°C, the supernatant was removed and used for determining proteolytic activity. The second portion was centrifuged at 10,000 × G for 20 min at 4°C and the supernatant was used for assaying polyphenolase activity.

Proteolytic activity was determined according to methods outlined by Kakade et al. (1970) using a 1% solution of casein (Hammersten quality, Nutritional Biochemical Corp., Cleveland, Ohio) in 0.1M borate buffer (pH 7.6) at 37°C as a substrate. Proteolytic activity was arbitrarily defined as the average change in absorbancy of a trichloroacetic acid (TCA) filtrate at 275 nm over a 20 min time period. Activity was arbitrarily expressed as ΔA^{275nm}_{1cm} per min per 16 mg total nitrogen × 10^3.

Polyphenolase activity was estimated spectrophotometrically at room temperature. The change in absorbancy at 480 nm was determined for a reaction mixture consisting of 0.5 ml of 0.1M phosphate buffer (pH 6.4), 2.0 ml of 1% pyrocatechol, 0.3 ml of crude enzyme preparation, and 0.2 ml of distilled water. The absorbance change against a distilled water blank over a 1.0 min time period starting at 0.5 min after addition of the crude enzyme preparation to the reaction mixture was used as an estimate of activity. Activity was defined as $\Delta A^{480nm}_{1\ cm}$ per min per 16 mg total nitrogen.

A 40-g sample of the intact shrimp-water (1:1) homogenate was blended for 4 min with 160 ml of sterile distilled water (4°C) in a presterilized blender container. Portions of this homogenate were used to determine pH and aerobic plate count. Microbial growth was determined after 48 hr of incubation at 32°C on standard plate count agar. All plate counts were made in duplicate from appropriate dilutions.

A 50-g sample of blended shrimp-water (1:1) was blended for 3 min with 200g of 5.5% TCA. The TCA homogenate was filtered through S & S ribbon No. 589 filter paper and stored at −35°C prior to analysis for trimethylamine oxide (TMAO), trimethylamine (TMA), dimethylamine (DMA), nonprotein nitrogen (NPN), formaldehyde (FA) and tyrosine. The method of Yamagata et al. (1969) for reducing TMAO to TMA was used to estimate TMAO. The picric acid procedure of Dyer (1945) was used to measure TMA. The difference between total TMA (TMAO + TMA) and TMA values represented the amount of TMAO. DMA was measured by the copper-dithiocarbamate method of Dyer and Mounsey (1945). TMAO and TMA values were corrected for the interference of DMA. Dyer (1945) found that DMA will react with picric acid giving 21% of the absorbancy as an equivalent concentration of TMA. The interference of DMA was found to be constant over a wide concentration range (0–20 μg/ml) in the TMA-picrate test (Babbitt et al., 1972).

FA was estimated in the TCA filtrate using a modification (Babbitt et al., 1972) of the procedure of Sawicki et al. (1961). NPN was determined in the TCA filtrate by a semi-micro Kjeldahl method (AOAC, 1970). Free tyrosine was estimated by using the method of Ceriotti and Spandrio (1957).

Flavor panel evaluations were carried out approximately 2 wk post-processing. Samples were thawed over-night at 1–2°C and served in coded cups to judges seated in individual booths. Panelists were asked to judge the shrimp for texture, juiciness, degree of off-flavor and desirability on an intensity scale ranging from 9, "highest affirmative value," to 1, "lowest value." Because the experimental design involved nine treatments, the various treatments were randomly allocated to two different evaluations, each including a zero-time sample for reference and evaluated by 15

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judges. This evaluation was carried out in duplicate. Analysis of variance of the scores received by all treatments for each organoleptic factor showed that mean scores for the replicate evaluations did not vary significantly ($P < 0.05$) from each other. The scores for the two replicate evaluations were then combined by organoleptic factor and treatment and evaluated by analysis of variance procedures. Scores for zero-time samples from the second evaluation for each panel replication were arbitrarily deleted from the analysis so that the number of judgments per treatment would be equal. The significance of individual treatment means was tested by Duncan's multiple range test.

RESULTS & DISCUSSION

A PROGRESSIVE INCREASE in pH from 7.6 to 8.8 was observed for intact shrimp during 8 days of iced storage (Fig. 1). The pH of the cooked meat paralleled these changes closely. The pH of the zero-time samples of 7.6 was relatively higher than other values reported for fresh shrimp. A pH of 7.2 after 1 day of ice storage of Gulf shrimp *(Penaeus setiferus)* (Fieger et al., 1956), 7.4 for zero-day old shrimp *(Penaeus aztecus)* (Flick and Lovell, 1972) and 6.75 for fresh shrimp *(Penaeus braseliensis)* (Luna, 1971) have been reported. In a preliminary investigation, the pH at zero time of a sample from a 200-lb lot of Pacific shrimp taken from a single trawl was found to be 7.0. After iced storage of that same lot for 8 days, the pH of the sub-sample increased to 8.4. This suggests that seasonal variations and catch procedures may greatly affect the pH of shrimp immediately after removal from the water.

Microbial numbers in this storage test were shown to increase from 1×10^4 to 68×10^4 per g (Fig. 1). The apparent decrease in microbial load observed for the seventh day of storage was probably related to the washing effect of the melting ice and/or a sampling error. Green (1949) showed that melting ice washes bacteria down into a sample lot and results in an accumulation at lower levels. Although microbial numbers at the end of the storage period (68×10^4 per g) were fairly high, they fall within the range of 3.0×10^5 to 1.3×10^6 organisms per g found in commercial samples of fresh Pacific shrimp by Harrison and Lee (1969).

The total nitrogen and NPN content of intact shrimp was shown to decrease with iced storage (Table 1). A similar decrease, although variable, was shown for samples of the cooked meat. The largest loss in total nitrogen occurred during the first day of iced storage in both the intact shrimp and cooked meat samples. NPN levels in the intact shrimp, although somewhat variable, showed a considerable decrease after the fourth day of storage. Levels of NPN in the cooked meat showed a variable increase up to the fourth day of storage, followed by a considerable decrease on the fifth day. Levels remained relatively constant during the remainder of the test period.

Table 1—Changes in total nitrogen, NPN and carotenoid index during the iced storage of Pacific shrimp

Time (days)	Total N (μg/gm shrimp)		NPN (μg/16 mg N)		Carotenoid index	
	Intact	Cooked	Intact	Cooked	Intact	Cooked
0	26.4	25.5	5.1	2.3	10.8	2.4
1	20.4	32.3	4.7	2.1	9.2	2.3
2	18.6	32.7	4.7	2.5	8.6	2.2
3	17.0	29.4	4.1	2.5	8.0	2.1
4	19.9	28.9	5.0	3.4	8.8	2.0
5	17.0	30.5	3.9	1.9	8.6	2.0
6	16.3	28.5	3.6	1.7	8.2	1.9
7	17.2	28.8	3.3	1.7	8.7	2.1
8	17.1	29.5	3.5	1.5	8.6	2.1

A decrease of total carotenoid (astaxanthin and astacin) content was observed in both the intact shrimp and cooked meat during the first 2 days of storage (Table 1). After the second day of storage, carotenoid levels in both the intact and cooked meat remained relatively constant. The large loss in total carotenoid content observed in intact shrimp samples during the first 2 days of storage parallels losses in total nitrogen. The autolytic breakdown of the highly pigmented hepatopancreas in the stomach area of the shrimp which could be washed away by melting ice may explain this large initial loss. Losses observed in the cooked meat during this same period of time would not account for this initial large pigment loss from the intact shrimp.

The carotenoid levels of shrimp and other shellfish has been proposed as an index of product freshness (Kelly and Harmon, 1972; Collins and Kelly, 1969). The results of this investigation did not show a change in the pigment content of the cooked meat during the storage period of a magnitude or for a duration which would provide the basis for a good index of product quality with respect to storage time.

Tyrosine is liberated from proteins and peptides during the process of enzymatic proteolysis in muscle. It has been used as a measure of such activity (Hagihara et al., 1958; Kakade et al., 1970). Several investigations of the use of tyrosine levels as an index of quality in fin fish have been reported (Tarr and Bailey, 1939; Bradley and Bailey, 1940; Vaisey, 1956). Results of these investigations showed that tyrosine levels increased during cold storage and became most evident when advanced spoilage had taken place.

Tyrosine levels in intact shrimp were shown to increase rapidly during the first 2 days of iced storage (Fig. 2). Increased, or at least equal, proteolytic activity during the first 2 days of iced storage could explain this rapid increase (Fig. 2). After the second day of storage, tyrosine levels decreased in samples of intact shrimp. Although polyphenolase activity was shown to generally decrease during storage (Fig. 2), the oxidation of tyrosine to dihydroxyphenyl alanine (DOPA), to other more highly oxidized products and ultimately to melanin pigment would only partially explain this loss.

Conversely, the tyrosine level in the cooked meat generally increased during the storage period (Fig. 2). The meat or shrimp muscle would not be subject to the same degree of washing action by melting ice, as the remainder of the shrimp body after degradation had disrupted its physical integrity. Although a considerable amount of tyrosine was probably lost during the cooking process, levels would generally reflect proteolytic activity in the shrimp meat during ice storage.

TMAO has been found in most kinds of fin and shellfish and has been investi-

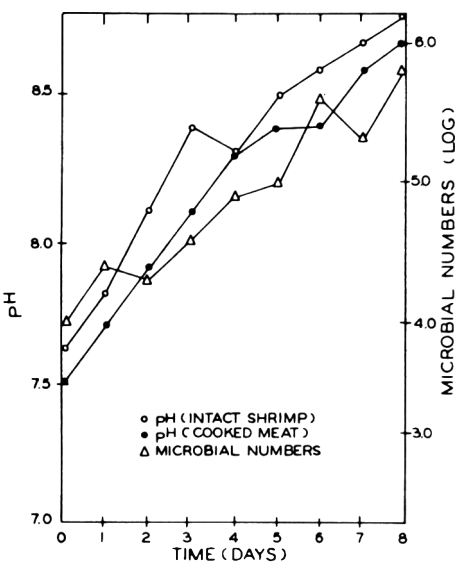

Fig. 1—Changes in the pH and microbial numbers of intact shrimp and the pH of cooked meat during iced storage.

Table 2—Mean[a] flavor panel scores[b,c] for cooked meat from Pacific shrimp stored on ice

Time (days)	Texture	Juiciness	Off-flavor	Desirability
0	6.87^{ab}	6.87^{ab}	7.63^{ab}	6.67^{a}
1	8.03^{c}	7.18^{ab}	7.87^{a}	6.92^{a}
2	7.47^{ac}	7.65^{c}	7.65^{ab}	6.68^{a}
3	7.15^{ab}	8.00^{cd}	7.58^{ab}	6.42^{ab}
4	7.38^{ac}	8.18^{d}	6.85^{cd}	5.88^{bc}
5	6.95^{ab}	7.63^{bc}	7.13^{bc}	5.82^{bc}
6	6.52^{b}	7.97^{cd}	7.13^{bc}	5.87^{bc}
7	6.90^{ab}	7.53^{bc}	6.58^{cd}	5.38^{c}
8	7.12^{ab}	7.18^{ab}	6.28^{d}	5.42^{c}

[a] n = 30
[b] Range of scores: 9, "highest affirmative value," to 1, "lowest value"
[c] Mean scores in a column with same exponent letter did not vary significantly ($P < 0.05$) from each other

gated because of its bacterial reduction to TMA. TMA has been proposed and is used as an index of seafood quality (Ronold and Jakobsen, 1947; Beatty, 1938; Collins, 1941; Tarr, 1938; Watson, 1939; Sigurdsson, 1947; Laycock and Regier, 1971). Amano and Yamada (1964) have suggested from their investigation of gadoid fish the possibility of two completely different systems for the reductive degradation of TMAO. One system proposed the bacterial or the exogenous enzymatic reduction of TMAO to TMA. The second system involves the endogenous enzymatic reduction of TMAO to DMA and FA. Whether TMA can be the substrate for, or an intermediate in the latter system is not presently clear.

TMAO-N levels increased from a zero-time level of 279 to 487 μg/16 mg N in intact shrimp after 1 day of iced storage (Fig. 3). Levels decreased progressively during the remaining 8 days. Conversely, the maximum level of TMAO in the cooked meat was achieved after 4 days of iced storage (Fig. 4). Levels in the cooked meat then declined. Although a portion of the decline of TMAO levels during iced storage reflects its degradation to DMA and FA and possibly to TMA, a large portion of the observed loss was probably related to the washing action of melting ice.

TMA-N levels in intact shrimp were shown to increase from 1.4 at zero-time to 15.3 μg/16 mg N (0.24 to 1.6 mg/100g shrimp) at the end of 8 days of iced storage (Fig. 3). These results are in agreement with the increase in levels reported by Collins et al. (1960) for Pacific shrimp (*Pandalus* species) of 0.24 at zero-time to 1.72 mg/100g shrimp after 8 days of iced storage. TMA levels in the cooked meat (Fig. 4) were shown to be considerably lower than those found in intact shrimp samples, but appeared to follow the same general pattern of development.

Both FA and DMA levels increased rapidly in a parallel manner in intact shrimp (Fig. 3) and cooked meat (Fig. 4) samples during iced storage. These results are not in agreement with the findings of Castell et al. (1970) who reported that TMAO was not reduced to DMA and FA in shrimp muscle. The variance in these results may be related to the fact that samples analysed by these investigators did not include the head and intestinal tract.

Enzyme systems that reduce TMAO to DMA and FA may be concentrated in the gastrointestinal tract. Amano and Yamada (1964) found a similar increase in FA which was parallel to the production of DMA and accompanied by a decrease in TMAO in the muscle of three different species of gadoid fish held at 1.0 to 4.0°C. Their investigations and those of Amano et al. (1963) and Yamada and Amano (1965) showed that the pyloric caeca of cod and Alaska pollock contained high levels of TMAO which was rapidly reduced to DMA and FA under sterile conditions. FA and DMA found in cooked meat could have originated either wholly or partially in the gastrointestinal tract of the shrimp.

Although levels of 3.0 and 7.9 μg/16 mg N of DMA-N and FA, respectively, were found at zero-time, the existence of

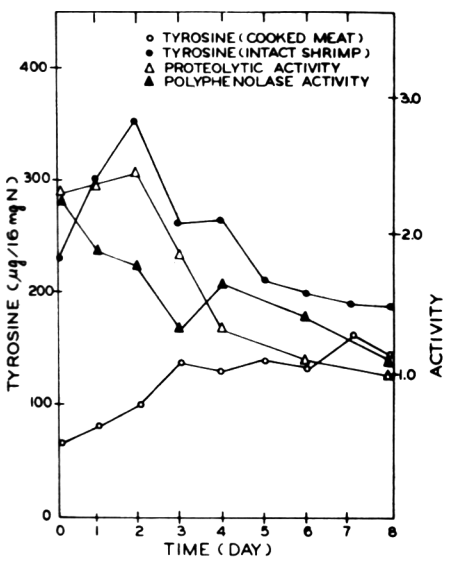

Fig. 2—Changes in the proteolytic and polyphenolase activity of intact shrimp and tyrosine levels in intact shrimp and cooked meat during iced storage.

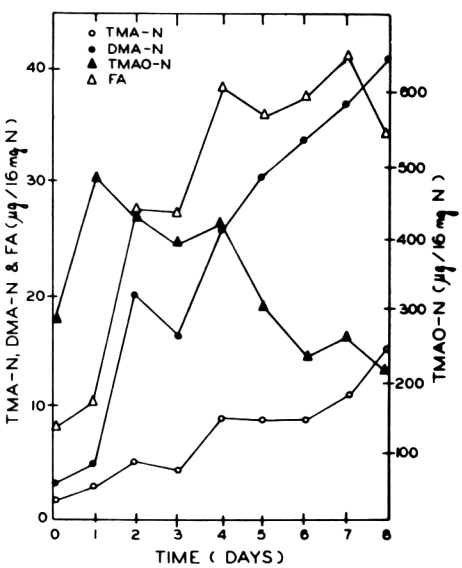

Fig. 3—Changes in the trimethylaine oxide (TMAO), trimethylamine (TMA), dimethylamine (DMA) and formaldehyde (FA) levels in intact shrimp during iced storage.

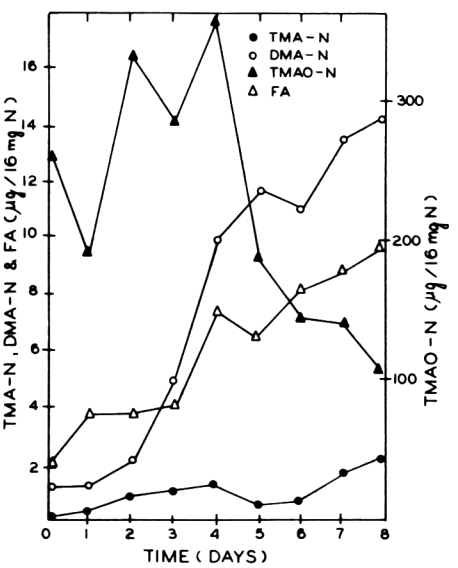

Fig. 4—Changes in the trimethylamine oxide (TMAO), trimethylamine (TMA), dimethylamine (DMA) and formaldehyde (FA) levels in cooked meat during iced storage.

FA and DMA in live shrimp is not conclusive. Samples were frozen on dry ice immediately after washing with sea water, about 1 hr post-catch, and held at dry ice temperature for about 24 hr prior to analysis. This may suggest a very rapid reduction of TMAO post-catch and prior to freezing or its presence in the gastrointestinal tract in vivo. Alternately, some reduction of TMAO could have occurred during the brief period the sample was held frozen. Castell et al. (1971) showed that DMA was produced in frozen cod, haddock, pollock, cusk and hake fillets held at $-5^{\circ}C$. TMAO levels in frozen hake fillets held in storage at $-20^{\circ}F$ have been shown to be reduced yielding a parallel production of both DMA and FA (Babbitt et al., 1972).

Flavor panel scores for the texture of shrimp meat were shown to be significantly lower for zero-time samples than for samples stored 1 day on ice (Table 2). Although not significant, scores for juiciness, off-flavor and desirability were also somewhat lower. Freezing the intact shrimp immediately post-catch and thawing during cooking reduced the over-all quality of the meat, particularly with regard to texture.

From the first day of iced storage, mean panel scores for texture generally showed only a slight decline. Mean scores at the end of 2 days of iced storage did not vary significantly from those observed at the end of 8 days.

Flavor panel scores for juiciness progressively improved during the first 4 days of iced storage. Mean panel scores at the end of 4 days were significantly higher than those observed after 1 day of iced storage. After the fourth day, scores generally declined to a level which was significantly lower than those found for shrimp stored 4 days. Scores at the end of 8 days of storage, however, did not vary significantly from those for shrimp stored only 1 day.

Panel scores for off-flavor and desirability generally declined during the 8 days of iced storage. The greatest loss in shrimp quality with regard to off-flavor did not occur until after the sixth day of storage. Scores for desirability did not vary significantly during the first 3 days of storage. Scores at time periods after 3 days progressively declined, but did not vary significantly.

Although it is very difficult to correlate biochemical changes with specific organoleptic factors, the formation of DMA and FA showed the widest measurable range of change that follow a general loss of organoleptic quality. The levels of these two products increased even though the washing action of melting ice may have greatly reduced the actual levels produced. TMA levels and pH were shown to increase, but the magnitude of change was observed to be much less and would not yield as precise an index of quality. Tyrosine levels in the intact shrimp changed in an irregular manner during storage, but levels in the cooked meat progressively increased and appeared to follow the observed loss of organoleptic quality. Changes in total nitrogen, NPN, carotenoid and TMAO levels and proteolytic and polyphenolase activities clearly reflect changes in quality, but they appear to be of little use in reflecting the organoleptic quality of the final cooked meat.

Microbial numbers, pH and TMA levels have been used in the past as indices of shrimp quality. The magnitude and duration of DMA and FA production during iced storage of intact shrimp observed in this investigation offer a sensitive test that potentially could better reflect the organoleptic quality of the cooked separated meat. Verification of the utility of these indices would require their statistical correlation with flavor panel scores for samples of shrimp representing a wide variation in quality resulting from iced storage time, season and catch and handling procedures.

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BACTERIAL COUNTS AND RANCIDITY ESTIMATES OF STORED QUICK-SALTED FISH CAKES

INTRODUCTION

PREVIOUS PAPERS have dealt with a quick-salting process for fish. Del Valle and Nickerson (1968) and Del Valle (1972) described the process; Del Valle and González-Iñigo [1](1968) studied its applicability to different species of fish; finally, Del Valle et al. (1973) produced quick-salted fish cakes in a pilot plant and conducted extensive acceptance trials with the cakes.

Because of their ease of preparation and low cost, quick-salted fish cakes have been suggested as a means for combatting protein malnutrition in developing countries. Another advantage of the cakes is that they have been found, by organoleptic testing, to require no refrigeration, even at tropical temperatures. This apparent stability is due to their high salt and low moisture contents, since the dry cakes contain approximately 50% salt and 10% water.

It was desirable, however, to obtain a quantitative measure of the stability of the dry cakes. This led to a study of bacterial counts and rancidity estimates of the product.

EXPERIMENTAL

BACTERIAL COUNT studies were directed to determination of microorganisms which could decompose the dried, salted cakes as well as of possible pathogens. With this in mind, plate counts were determined in freshly-made cakes as well as in cakes stored in the laboratory without packaging during 1, 2, and 3 months at an ambient tropical temperature (35–40°C) as follows: (1) total plate count, to obtain a measure of the general quality of the product as well as detect the presence of microorganisms which could decompose the cakes; (2) halophilic count, to detect the presence of salt-tolerant microorganisms which could decompose the cakes; and (3) staphylococci, which are potential pathogens capable of growing in fairly high salt concentrations. All counts were carried out after plating in the following media and incubating for 24 hr at the indicated temperature: total plate count in nutrient agar, incubating at 25°C; halophilic count in nutrient agar with 10% sodium chloride, incubating at 37°C; staphylococci in glycine tellurite agar, incubating at 37°C.

Dilutions from 10^{-1} to 10^{-6} were plated out in each case in the usual manner. The 10^{-1} dilution was prepared by mincing 10g of sample in 90 ml of sterile distilled water in a Waring Blendor. Stated incubation temperatures were selected for the following reasons: (1) Total plate count: 25°C was considered to be a good temperature for growth of contaminants of marine origin present in the fish; (2) halophilic count: 37°C is the normal incubation temperature for halophiles present in the salt; (3) staphylococci: 37°C is the normal incubation temperature for these organisms.

Cakes used in bacterial counts were purposely not dried at time zero, but were rather left to air dry during the duration of the runs. This was done in order to obtain a measure of the stability of the product under the worst possible conditions, i.e., if the cakes were sold directly without drying. It should be noted that this is not the usual case, since the cakes are normally sun-dried.

A rancidity index was determined in sun-dried and tunnel-dried cakes by the thiobarbituric acid (TBA) method (Sinhuber and Yu, 1958). Again, freshly-made cakes and cakes stored without packaging in the laboratory during 1, 2 and 3 months at an ambient temperature of 35–40°C were studied. 1g of material on a dry basis was used in each determination, employing the method described. Cakes used in these determinations were dried at time zero as follows: sun-dried cakes, 6 days at an ambient temperature of 33–35°C and 65–70% relative humidity; tunnel-dried cakes, 25 hr at 40°C and 60% relative humidity, with an air velocity of 600 ft/min. Final moisture contents of all dry cakes were of the order of 6–8%, and showed little variation during the duration of the runs.

Rancidity indices were obtained in order to determine if the product could become unacceptable after a prolonged storage time. Since fish oils are unsaturated, there were reasons to believe that this could become a serious problem, especially with cakes made from fatty species. On the other hand, it was thought that some of the rancid-flavored components, being of low molecular weight and/or soluble in water, might be eliminated by leaching or steam distillation when desalting the cakes in boiling water. This hypothesis was verified by making additional rancidity determinations in cakes which had been boiled for 5 min, using a water: cake ratio of 12.5:1.

Table 1—Common and scientific names of species studied and amounts of salt added to fresh fish muscle in order to form cakes

Spanish name	English name	Scientific name	Amt. salt added g/100g fresh muscle
Barrilete	Skipjack	*Katsuwonus pelamis*	45
Sierra	Spanish Mackerel	*Scomberomorus sierra*	45
Lisa	Mullet	*Mugil cephalus*	40
Tiburón	Shark	*Mustelus mustelus*	25

Table 2—Moisture contents of cakes for bacterial counts after different storage times

Species	Initial (%)	First month (%)	Second month (%)	Third month (%)
Skipjack	40.88	15.23	6.40	6.11
Sp. mackerel	45.74	16.63	8.31	8.00
Mullet	44.80	16.19	8.10	8.05
Shark	53.88	23.84	14.10	12.00

[1] Present address: Mexicana de Jugos y Sabores, S.A., Monterrey, Mexico
[2] Present address: Cervecería Cuauhtémoc, S.A., Fábrica de Malta, Monterrey, Mexico
[3] Present address: Troqueles y Esmaltes, S.A., Monterrey, Mexico
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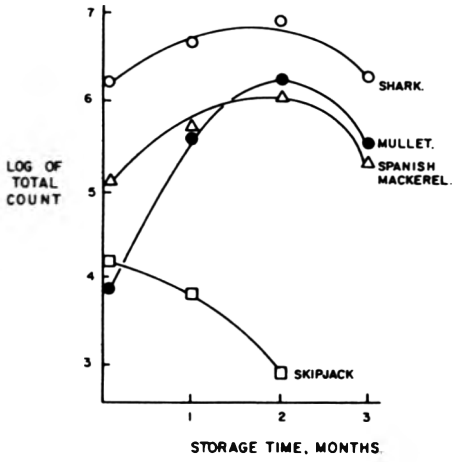

Fig. 1–Variation of total plate count with storage time for cakes made from different species. (Note: Skipjack count negative at 3 months)

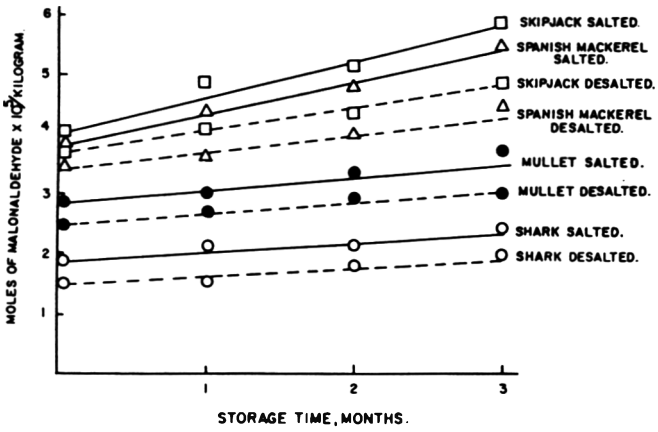

Fig. 3–Rancidity index versus storage time for sun-dried cakes.

In summary, rancidity determinations were made on all cakes, both before and after desalting.

Species studied in this work were skipjack, mullet, Spanish mackerel and shark from the Gulf of California. Table 1 shows scientific names and amounts of salt added in each case in order to form the cakes.

RESULTS & DISCUSSION

FIGURE 1 shows variation with time of total plate count for the different cakes. It may be seen that the general tendency was for the total count to increase, pass through a maximum and then decrease. The increase was probably due to the fact that the cakes were not dried at time zero, as noted previously, and thus permitted growth of halotolerant species. With the passage of time the cakes became drier; this produced an increase in salt concentration with a resulting suppression of bacterial growth. Table 2, which shows variation with time of the moisture contents of cakes used in bacterial counts, verifies this hypothesis. Cakes made from shark muscle, which was treated with the minimum amount of salt, retained a maximum amount of water and thus experienced more microbial growth than those made from other species.

The order of increasing total plate count was generally skipjack < mullet < Spanish mackerel < shark. By reference to Table 2 it may be seen that this order correlated well with decreasing salt and increasing moisture contents. That is, cakes with high salt contents tended to have low moisture contents and low bacterial counts, and vice-versa. These results show that it would be possible to obtain low bacterial counts by using high amounts of salt. It should be mentioned at this point that an incubation period of 24 hr was perhaps short for the employed incubation temperature of 25°C. If a longer period or higher temperature had been employed, total plate counts would have perhaps been higher, although the general trend would have probably been the same as shown in Figure 1.

No attempt was made to identify the organisms obtained in the total plate counts. On the other hand, it might be interesting to speculate on what types of bacteria would have survived such high salt concentrations. One possibility is *Bacillus* spores, which germinated when the plate counts were made; another possibility is halotolerant species, as distinct from obligate halophiles.

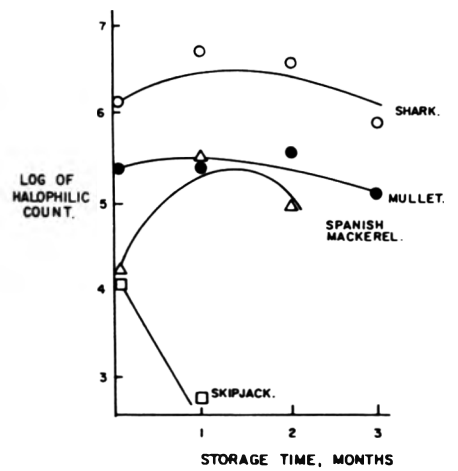

Fig. 2–Variation of halophilic count with storage time for cakes made from different species. (Note: Skipjack count negative at 2 and 3 months; Spanish mackerel count negative at 3 months).

Figure 2 shows variation with time of halophilic counts. It may be seen that the same general tendencies as those observed with total plate counts were exhibited by these counts. Finally, with respect to staphylococci, no growth was obtained in any of the plated dilutions at any time.

Figures 3 and 4 show results of rancidity determinations in cakes dried in the sun and in a tunnel, before and after desalting. Rancidity indices are reported per kilogram of cake on a dry basis. It may be seen that all cakes tended to become more rancid with time, although there were differences between the species. The order of increasing rancidity was shark < mullet < Spanish mackerel < skipjack. This order probably depended upon various factors, including oil content of the species and iodine number of the oil. Table 3 compares literature values for oil contents and iodine numbers of the different species. It may be seen that the order of increasing oil content was shark < Spanish mackerel < skipjack < mullet, while that of increasing iodine number was mullet < Spanish mackerel < shark < skipjack. The presence of hematin pigments in skipjack muscle was undoubtedly another factor in increasing the susceptibility of its oil to rancidity, besides the high degree of unsaturation of the oil.

Figures 3 and 4 also show another important point. Solid lines in the figures refer to cakes before desalting, while dotted lines refer to the same cakes after desalting by leaching in boiling water. Lower rancidity indices for the desalted cakes show that rancid components were indeed partially removed by the desalting operation, whether this was due to leaching, steam distillation, or both.

Concerning differences in rancidity between sun-dried and tunnel-dried cakes,

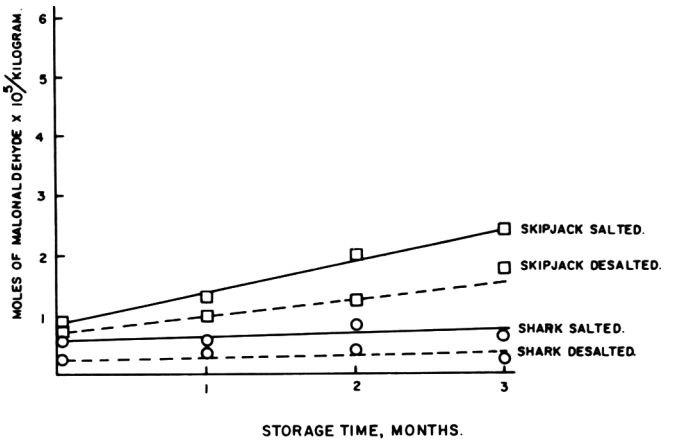

Fig. 4—Rancidity index versus storage time for tunnel-dried cakes.

Table 3—Oil contents and iodine numbers of oils of the different species[a]

Species	Oil content, wet basis	Iodine number of oil
Skipjack	6.09%	440
Spanish mackerel	2.06%	145
Mullet	9.36%	126
Shark	–	270

[a]Jacquot, R. 1962. Organic constituents of fish and other aquatic animal foods. Vol 1, p. 145. In "Fish as Food," ed. Borgstrom, G. Academic Press, New York.

Figures 3 and 4 show that the former were more rancid than the latter. This difference was probably due to the lower drying times required in a tunnel than in the sun, as well as to the presence or absence of sunlight, which is known to catalyze rancidity reactions.

Finally, a limited attempt was made to correlate rancidity indices with a taste panel evaluation. Cakes were desalted by leaching twice in boiling water, cooked in a chili-tomato-onion sauce and served to a panel of 15 tasters, who were asked to rank the different cakes in order of preference. Results obtained were as follows, in order of decreasing preference: shark > mullet > Spanish mackerel > skipjack. If this order is compared with that obtained for increasing rancidity (shark < mullet < Spanish mackerel < skipjack), a very good inverse correlation is seen to exist between preference and rancidity. A statistical analysis of the taste panel results was not performed due to the limited nature of the corresponding tests.

CONCLUSIONS

THE FOLLOWING conclusions may be made with respect to storage stability of quick-salted fish cakes:

(1) Bacterial contamination of the cakes depends upon their salt and moisture contents. Contamination is not high and tends to decrease with time after approximately 2 months.

(2) Staphylococci are apparently not present in the cakes.

(3) Points (1) and (2) verify previous organoleptic observations regarding stability of cakes without refrigeration.

(4) Cakes become rancid with time to different degrees. The amount of rancidity depends upon the following factors: oil content of the species used to make the cakes; degree of unsaturation of the oil; drying time; presence or absence of hematin pigments; presence or absence of sunlight in drying.

(5) Some of the rancid components in the cakes are removed by desalting in boiling water.

(6) Taste panel preference of the cakes apparently correlates well with decreasing rancidity.

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MICROWAVE FINISH DRYING OF POTATO CHIPS

INTRODUCTION

POTATO CHIP processing systems using microwave energy for finish drying have been reported (Davis et al., 1965; Smith, 1966; Blau, 1965; Jeppson, 1965; and O'Meara, 1966). The studies included both 915 and 2450 MHz microwave frequency with production capacities ranging from 62–1500 lb per hour. Their results were similar and indicated that microwave finish drying of potato chips with as high as 0.5% glucose content was economical and produced chips of acceptable quality. Some found that microwave-dried chips had more uniform moisture content from chip-to-chip. In contrast, conventionally fried chips produced from tubers with high reducing sugar levels were unacceptable in color.

Each of these researchers prefried the chips on a continuous belt and thus had to contend with moisture variability due to frying temperature, time in the fryer and slices sticking together. Since they used a microwave unit at maximum capacity in a commercial operation, they could not adjust power-to-product ratio. Thus, processing variables were not investigated under ideal conditions and some variables could not be studied. Texture was always evaluated by subjective methods and thus small differences could not be positively identified.

The purpose of this research was to study microwave finish drying of potato chips with the primary variables being reducing sugar content of the potato tubers and moisture level of the pre-fried chips passed to the microwave unit. An objective method for evaluating texture in potato chips made it possible to record small changes in texture. Oil content of the samples was determined and samples were organoleptically evaluated for both color and texture.

MATERIALS & METHODS

Microwave oven

The microwave oven (Model 1-2Lc Microwave research system, 2450 MHz, Cryodry Corp., San Ramon, Calif.), allowed continuous control of power between 0-2500 watts and of belt speed to within a few seconds. Temperature of circulating air in the chamber was controlled at 200°F for all experiments. Power input to all samples was 2500 watts but application time was varied from 1 to 3 min.

Processing

Russet Burbank potatoes from Michigan were stored at several temperatures. The potatoes were peeled by hand and 1/16-in. slices obtained with a Qualheim-Electrocut machine (Model 101, Qualheim Inc., Racine, Wis.). Slices were rinsed three times in cold tap water, blotted with a paper towel and deep-fat fried (Hotpoint Model HKO3B) in corn oil at 320°F (160°C). To accomplish individual frying, slices were impaled on a 1/8 in. diam stainless steel rod with about 1/2 in. spacing between slices. 25g of potato slices were held on three rods below the oil surface. Under these conditions, temperature reduction upon insertion of slices into the oil was negligible. Time in the oil was varied to obtain chips with different IMC. These unfinished chips were removed from the rods, quickly placed on pulp paper trays and immediately set in the microwave oven for finish drying. The desired final moisture content was 2%. To achieve this, time in the microwave oven was varied according to estimated IMC. This estimate was based on time of oil frying as well as oiliness and texture of the pre-fried chips.

Objective tests

Moisture content was determined by drying 5–8g of potato chips at 28–29 in. Hg vacuum and 65°C for 12 hr. The dry sample was used for oil determination by Soxhlet extraction (Joslyn, 1950) using 8-hr reflux with pet. ether (b.p. 30–60°C). Reducing sugar content was determined by the revised Somogyi (1952) method.

An objective texture evaluation method was developed using the L.E.E. Kramer Shear Press (L.E.E. Inc., Washington, D.C.); the maximum point of the output curve was recorded as the Shear Press reading. A sample size of 13g and descent time of 14.6 sec were established as standard. All readings fell on the 1000 scale; these were divided by 10 for reporting here. Seven to 10 readings were taken of each lot; these were statistically not different at the 1% significance level.

Organoleptic technique

Evaluations for color and texture were done by seven to 10 experienced judges. A hedonic rating scale was used; a score of 9 was considered as excellent and a score of 5 was considered barely acceptable. An unmarked commercial sample was always included as a control with the experimental samples. Another portion from the same commercial sample was scored and marked as a reference. If a judge rated the control different from the reference sample by more than 2 units his scores were

Table 1—Subjective and objective texture of potato chips pre-fried in oil to different intermediate moisture contents and finish dried by microwave oven

Sample no.	IMC %	Shear press readings[a]	Avg Organoleptic texture score (9 = perfect)
1	Control	25.7	8.9
2	Control	30.9	8.7
3	6.92	32.3	8.0
4	12.68	38.9	6.7
5	18.63	42.4	5.1
6	21.92	47.0	4.7
7	33.28	54.6	3.0

New Multiple Range Test[b]

Sample no.	1	2	3	4	5	6	7
Organoleptic texture means	8.9	8.7	8.0	6.7	5.1	4.7	3.0
Shear press means	25.7	30.9	32.3	38.9	42.4	47.0	54.6

[a] Mean of seven replications
[b] Values not connected by an underline are significantly different at the 1% level.

eliminated from the evaluation. Both tenderness and crispness were considered in scoring texture. The Potato Chip Institute Color Standard was used as a guide in color evaluation.

RESULTS & DISCUSSION

REDUCING SUGAR content of the raw potatoes was a prime variable in these experiments because it is so important to the color of the finished chips. To obtain the desired reducing sugar level, batches were removed from bulk storage at 34°F and conditioned at 45°, 50°, or 70°F for the necessary time. In general, potato slices were prefried in oil to different intermediate moisture contents (IMC) by controlling residence time and then finish fried in the microwave oven to 2.0 ± 0.3% moisture. Finished potato chips were evaluated for texture, color and oil content.

Texture

Reducing sugar content of the raw potato was below 0.3% for all experiments on texture. In a preliminary experiment, samples were removed from the oil bath at 17 different IMC ranging from 3.68 to 32.3%, microwave finished and objectively evaluated for texture. Statistical analysis gave a positive correlation of 0.98 between IMC and shear press readings, showing that the chips became tougher as the IMC was increased.

This was further investigated by microwave finish drying five lots of pre-fried chips from IMC of 6.9–33%; two controls were conventionally oil fried to final moisture contents of 2.1 and 2.6%. Texture was determined both by shear press and a panel of judges. The data are in Table 1. Most of the means were significantly different within each determination.

A correlation analysis between organoleptic texture scores and shear press readings resulted in a negative correlation, −0.8312, showing that a low shear press reading was closely related to a high organoleptic score. From these and other data not shown, it was determined that an organoleptic score of 5.0 which was considered as barely acceptable was related to a reading of 40 on the shear press. Therefore, a shear press reading above 40 was considered unacceptable in texture. Since a shear press reading of 40 was related to an IMC of 13.0%, it was concluded that the IMC should be below this level to obtain acceptable texture of the finished chip.

Color

Batches of Russet Burbank potatoes with different reducing sugar contents were pre-fried until they developed a color that matched 3–4 on the PCII Color Standard. After sampling, half of each batch was removed and finish dried by microwave; the other half remained in the oil until frying was completed.

The moisture data from tubers with reducing sugar levels of 0.45–2.0% are shown in Table 2. As the reducing sugar content of the potatoes increased, the pre-fried slices had to be removed from the oil earlier and at higher moisture levels to prevent the potato chips from being too dark in color. Without exception, high reducing sugar tubers resulted in high moisture pre-fried slices. All control samples finish fried in oil were scored 8 to 10 on the PCII Color Standard and thus were too dark for commercial use. All microwave finish-dried batches scored 3–4 on the PCII Color Standard, indicating that the color was good and had not changed in the microwave oven.

However, when these samples were tested for texture with the Shear Press, some samples indicated a tough texture. These results corroborated the above finding that the IMC must be below 13% to obtain satisfactory texture. Thus, in order to prevent excessive browning, the samples with reducing sugar contents of 1.0 and 2.0% had to be removed from the oil at the high moistures of 14.9 and 26.0%, respectively. Although the color of these samples after microwave finishing was still good, the texture was not.

It was therefore concluded that the application of microwave finish drying did not solve the reducing sugar problem. When the reducing sugar was above 0.9%, the potato chip was too dark if fried to completion in oil and too tough if finished by microwave. Previous reports indicated that the reducing sugar must be below 0.35–0.45% (Hawkins et al., 1958; Denny and Thornton, 1941; 1942) to obtain satisfactory color in oil-fried chips. Thus, microwave finishing does alleviate the reducing sugar problem and allows up to about 0.9% reducing sugar content in the potato slices without adversely affecting texture.

Flavor

Olsen and Drake (1966) reported that microwave finish drying appreciably extended shelf life of potato chips. Davis et al. (1965) found that microwave finished potato chips required 3 days longer to develop rancidity at 150°F than did the control. Therefore, no flavor evaluations were made here.

Oil content

Oil contents of microwave finish-dried potato chips were determined and compared to the oil contents of the conventionally fried controls. The average oil content of the microwave finish-dried potato chips from five lots was 35.1% as compared to 38.5% for the controls; the difference was significant at the 1% level. This is in good agreement with Davis et al. (1965) and Smith (1966) who found that the oil content of microwave-treated chips was 5.72% less than conventional chips in one study and 6.06% lower in another study.

In another experiment, the oil content was determined before and after microwave finish drying of the seven samples shown in Table 2. The data in Table 3 show that the oil content on a moisture-free basis averaged 37.2% before and 33.3% after microwave finish drying; this difference was significant at the 1% level. It was observed that this lost oil physically drained onto the paper plates holding the chips during microwave drying.

Table 2—Effect of reducing sugar content of the tubers on moisture content of the pre-fried chips removed when the color matched 3–4 on the PCII Color Standard

Reducing sugar, %	Moisture, %, of pre-fried chips at color of 3–4 on PCII Standard
0.45	8.04
0.65	9.60
0.75	10.70
0.90	12.10
1.00	14.90
2.00	26.00

Table 3—Oil content on a potato solids basis before and after microwave finish drying

Before Microwave % oil		After Microwave % oil
36.34		28.56
38.23		34.23
40.64		37.66
32.36		31.80
36.20		34.95
34.97		27.22
41.65		38.92
37.20	Average	33.33

T test showed a significant difference at 1% level.

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FLAVOR QUALITY AND STABILITY OF POTATO FLAKES
Effects of Raw Material and Processing

INTRODUCTION

PREVIOUS STUDIES of potato flake stability (Sapers et al., 1972) have indicated that the shelflife of this product is limited by the development of hay-like off-flavors associated with oxidative reactions. Under mild storage conditions, nonenzymatic browning appears to play a minimal role; however, large differences in levels of volatile products of the browning reaction may occur in fresh flakes prior to storage.

Large variations in the flavor quality of freshly produced potato flakes and in product shelflife have been observed by the potato processing industry (Sapers, 1970). While this variability is undoubtedly due to differences in raw material, processing conditions and packaging, there is no concensus in industry as to the relative importance or specific effects of these factors. One can speculate that deficiencies in potato flake flavor and stability might arise from sub-optimal commercial practices which, if optimized, might result in significant product improvement.

The present study is part of a research program undertaken at the Eastern Regional Research Laboratory to improve potato flake flavor quality and shelflife. The effects of certain raw material and processing variables are described herein.

EXPERIMENTAL

Materials and standard process

All potato flake samples were processed from Norchip variety tubers, harvested at the Red River Valley Potato Growers Association Research Farm (Grand Forks, N.D.) in September, 1970 and stored at 13°C (except where specified otherwise) until April, 1971. Tubers had a specific gravity of 1.094 and contained 0.14% fructose, 0.36% glucose and 4.24% sucrose (moisture-free basis).

Raw material and processing variables

Potato flake samples were prepared at the Red River Valley Potato Research Center pilot plant to test the effects of the following raw material and process variables on flavor quality and stability:

Conventional flakes (Product 1). Tubers were abrasion peeled, subdivided to 9.5 mm cross-cuts, precooked in water at 71–77°C for 20 min, cooled in water at 10–13°C, steam blanched for 20 min, and mashed in a Hobart Mixer (Model L-800). Sodium bisulfite was added via the cooking water. An aqueous suspension containing 20g mono- and diglycerides (Durkee EM400E), 15g sodium acid pyrophosphate, and 1.5 ml Tenox 4 (Eastman) was added to 22.7 kg of mashed potatoes in the Hobart Mixer. The mash was dried on a single drum drier, heated with steam at 100 psi, and the sheets of dried mashed potato were broken into flakes and packaged in polyethylene bags.

Cull raw material (Product 2). The raw material was inspected for defects, and cull tubers (subject to sprouting, rot and atypical shapes) were set aside. Flakes were produced from the cull raw material by the same process used for conventional flakes.

High sugar raw material (Product 3). Raw tubers were transferred from storage at 13°C to storage at 4°C 3 wk prior to processing. The sugar content increased to 0.66% fructose, 0.98% glucose and 7.13% sucrose (moisture-free basis). Flakes were prepared by the conventional flake process.

Unpeeled tubers (Product 4). The abrasion peeling step was omitted from the conventional flake process. Peel was in intimate contact with the product through the drying step where it was removed on the drum drier rolls.

Small raw potato pieces (Product 5). Peeled tubers were subdivided to 11.1 × 3.2 mm random length pieces rather than 9.5 mm cross-cuts.

Large raw potato pieces (Product 6). Peeled tubers were subdivided to 14.3 × 14.3 mm random length pieces.

Potato pieces held before cooking (Product 7). Peeled potatoes were subdivided to 11.1 × 11.1 mm random length pieces and held in water containing 1% sodium bisulfite for 18 hr prior to further processing.

Minimum process water (Product 8). The solids contents of the cooking and cooling waters were permitted to build up by the passage of 136 kg of potato pieces through the processing line with no water turnover. This material was discarded and fresh potato pieces were processed into product using the high solids process waters for cooking and cooling. The solids contents of the cooking and cooling waters were 1.76 and 0.32%, respectively, as compared to 0.85% and 0.02%, respectively, for the conventional flake process with which the process water was continuously replaced.

Maximum process water (Product 9). The solids build-up in the process water was reduced to negligible levels by using the maximum rate of water turnover possible in the cooker and cooler.

Preparation for storage

All products were shipped to ERRL for initial evaluation, canning and storage. Samples were analyzed for moisture (16 hr in 70°C vacuum oven), equilibrium relative humidity (Hygrodynamics Electric Hygrometer Model 15-3001, Hygrodynamics, Inc., Silver Spring, Md.) and sulfur dioxide (Nury et al., 1959; West and Ordoveza, 1962). Analytical data are summarized in Table 1.

Products were canned in No. 303 and No. 10 cans in air and under nitrogen (less than 2% oxygen) and were placed in storage at 23° and −18°C.

Table 1—Moisture content, equilibrium relative humidity and sulfur dioxide content of potato flake products

Flake product	Variable tested	Moisture (%)	ERH[a] (%)	SO_2 (ppm)
1	Conventional process (control)	6.76	31.9	423
2	Cull raw material	6.34	32.1	298
3	High sugar raw material	6.34	36.8	505
4	Unpeeled tubers	5.66	18.5	338
5	Small raw potato pieces	10.10	57.0	1544
6	Large raw potato pieces	6.92	31.0	494
7	Potato pieces held before cooking	7.78	35.6	752
8	Minimum process water	6.31	27.6	689
9	Maximum process water	5.84	22.9	160

[a] Equilibrium relative humidity

[1] A center cooperatively operated by the USDA North Central Region, ARS; The Minnesota Agricultural Experiment Station; the North Dakota Agricultural Experiment Station; and the Red River Valley Potato Growers Association.

Sensory evaluation

Products were evaluated for flavor quality initially and at intervals during storage using a trained panel as described by Sapers et al. (1972). Panelists were asked to rate flake samples on an eight-point scale ranging from "much better than standard" (8) to "extreme off-flavor" (1).

The conventional product (Product 1) stored at −18°C under nitrogen was used as a standard and hidden standard. The significance of differences reported by the panel was determined using Duncan's multiple range test (LeClerg, 1957).

Gas chromatographic analyses

Flake samples were analyzed by GLC for volatile components associated with nonenzymatic browning and oxidative rancidity (Table 2). Lower boiling compounds were determined in the headspace vapor; higher boiling components were determined in volatile concentrates prepared by steam distillation using procedures described previously (Sapers et al., 1972). Component concentrations were expressed as peak area ratios (component peak area/internal standard peak area). Duplicate analyses were performed initially and at intervals during storage.

RESULTS & DISCUSSION

Initial product flavor quality and volatile composition

The results of sensory evaluations and GLC analyses performed at the beginning of the storage study are summarized in Table 2. Differences between products in flavor score were small and not significant at the 0.05 level. Flakes processed from high sugar raw material (Product 3) were scored slightly lower than the conventional flakes (Product 1), probably because of their atypical sweet flavor. Likewise, flakes processed from unpeeled tubers (Product 4) were downgraded, probably due to the presence of an atypical peel-like flavor in the product. No other off-flavors were observed.

Major GLC headspace vapor (HV) and volatile concentrate (VC) peaks (Table 3) previously associated with typical storage off-flavors (Sapers et al., 1972) were examined for differences between fresh products. Levels of HV component 14 (2- and 3-methylbutanal), Strecker degradation aldehydes associated with nonenzymatic browning, were slightly elevated in flakes produced from cull raw material (Product 2), high sugar raw material (Product 3), unpeeled tubers (Product 4), potato pieces held before cooking (Product 7) and potato pieces cooked with maximum rate of process water turnover (Product 9). Similar results were obtained with VC components 40 (furfural) and 51 (phenylacetaldehyde) which are also associated with non-enzymatic browning. Presumably, these differences resulted from variations in heat damage to the flakes during processing and/or from different levels of browning reaction precursors.

The extent of oxidation in fresh potato flake samples was determined from levels of the major volatile oxidation products listed in Table 3. The sums of mean peak area ratios were computed for the four HV components and for the eight VC components used as indices of oxidation and are reported in Table 2. Flakes, analyzed within 3 wk of their arrival at our laboratory contained relatively low levels of oxidation products. Samples stored in their original shipping containers (polyethylene bags within closed fiber drums) for more than 3 wk at room temperature before analysis contained elevated levels of hexanal and benzaldehyde in volatile concentrates, as is shown in Table 4. This may have been due to the oxidation of lipids (including potato lipids, added emulsifiers and corn oil, the solvent in Tenox 4) on flake surfaces in contact with polyethylene. The very low levels of other volatile components characteristic of flake oxidation and the absence of hay-like off-flavors in these samples suggests that the reactions which occurred in bulk storage were not typical of potato flake oxidation. Fortunately, this phenomenon did not interfere with the storage study since all products intended for storage were canned within one week after their arrival. Subsequent storage stability was not correlated with the behavior of the products in bulk storage. Volatile concentrate analyses compromised by bulk storage oxidation were later repeated using nitrogen packed cans stored at −18°C. These data indicate that flakes prepared with maximum process water (Product 9), and flakes processed

Table 2—Summary of sensory and gas chromatographic data for potato flake products stored in air and nitrogen at 23°C

Flake product	Variable	Storage time (months)	Flavor score[a]	Sum of major volatile oxidation products[a]		2- and 3-Methylbutanal[d]
				Headspace vapor[b]	Volatile Conc.[c]	
1	Conventional process (control)	0	4.91	0.037	0.69	0.072
		6	–	0.040	2.03	0.052
		12	3.93	0.045	2.87	0.065
2	Cull raw material	0	4.56	0.045	0.73	0.094
		6	3.31[e]	0.063	3.91	0.082
		12	3.64[e]	0.098	6.88	0.086
3	High sugar raw material	0	4.44	0.037	0.88	0.089
		6	–	0.045	–	0.082
		12	3.71[e]	0.042	2.30	0.083
4	Unpeeled tubers	0	4.33	0.038	0.73	0.117
		6	3.54[e]	0.093	5.66	0.138
		12	3.14[e]	0.103	10.29	0.118
5	Small raw potato pieces	0	5.06	0.034	1.20[g]	0.055
		6	–	0.040	–	0.061
		12	4.36[f]	0.053	4.06	0.047
6	Large raw potato pieces	0	4.75	0.030	1.10[g]	0.059
		6	–	0.063	1.68	0.062
		12	4.21[f]	0.064	4.05	0.067
7	Potato pieces held before cooking	0	5.13	0.027	0.67	0.122
		6	4.15[e]	0.065	2.24	0.107
		12	3.93[e]	0.084	4.65	0.122
8	Minimum process water	0	5.13	0.025	0.58	0.063
		6	–	0.036	–	0.068
		12	4.54	0.053	3.59	0.068
9	Maximum process water	0	4.62	0.044	1.57[g]	0.080
		6	3.56[e]	0.104	4.39	0.091
		12	3.62[e]	0.135	9.29	0.100

[a] Samples stored in air
[b] Sum of mean peak area ratios for headspace vapor components 2, 18, 17 and 23 (identified in Table 3)
[c] Sum of mean peak area ratios for volatile concentrate components 16, 19, 23, 24, 29, 44, 45 and 47 (identified in Table 3)
[d] Samples stored in nitrogen
[e] Significantly different from hidden standard at 0.01 level
[f] Significantly different from hidden standard at 0.05 level
[g] Samples stored 12 months in N_2 at −18°C

from small (Product 5) and large (Product 6) raw potato pieces contained slightly higher levels of volatile oxidation products than the other samples at zero time.

Changes during storage in nitrogen

As in previous studies of potato flake stability (Sapers et al., 1972), nitrogen-packed potato flakes stored at 23°C showed little evidence of deterioration. After 12 months in storage, the trained taste panel found no significant differences between the products stored in nitrogen at 23°C and the nitrogen packed conventional flakes (Product 1) stored at −18°C.

Levels of 2- and 3-methylbutanal in the headspace vapor of the nitrogen packed flakes showed little change during storage for one year at 23°C (Table 2).

Changes during storage in air

All nine potato flake products showed evidence of oxidation during 12 months storage in air at 23°C. As is seen in Table 2, the development of hay-like off-flavors was accompanied by increases in the levels of the volatile components previously associated with potato flake oxidation. Typical changes in individual volatile components during storage are shown in Table 5. Increases in the lower boiling oxidation products determined by headspace vapor analysis were not as great as increases in higher boiling oxidation products determined in volatile concentrates. Among the latter, peaks 19, 44, 45 and 47 were especially conspicuous in severely oxidized samples, sometimes exceeding peak 16 (hexanal) in size and rate of increase. Peak 44, an unknown component, was not previously detected in oxidized potato flakes although a component having the same retention time did appear as a major oxidation product in explosion puffed dehydrated potatoes (Sapers et al., 1971).

To determine the degree of association between potato flake flavor and volatile formation during storage, correlation coefficients were computed for the regression of flavor score against levels of individual oxidation products, different combinations of oxidation products and the sums reported in Table 2. All corresponding GLC and sensory data were included in the calculations except those for Product 3 and for Product 2 at 6 months, the former being objectionably sweet and the latter having an atypical off-flavor probably arising from a raw material defect which was carried through the process.

It can be seen in Table 6 that there is a high negative correlation between flavor score and oxidation product level for all peaks except hexanal (HV 23, VC 16) and benzaldehyde (VC 45). Correlation coefficients were slightly higher when flavor scores were correlated with combi-

Table 3—Volatile components of potato flakes indicative of flavor changes during storage

Source	Component no.	Identity[a]	Associated with
Headspace vapor	2	Pentane	Oxidation
	8	Propanal	Oxidation
	14	2- and 3-Methylbutanal	Nonenz. browning
	17	Pentanal	Oxidation
	23	Hexanal	Oxidation
Volatile conc	16	Hexanal	Oxidation
	19	2-Pentenal[b]	Oxidation
	23	2-Pentylfuran & 2-hexenal	Oxidation
	24	Unknown	Oxidation
	29	Unknown	Oxidation
	40	Furfural	Nonenz. browning
	44	Unknown	Oxidation
	45	Benzaldehyde	Oxidation & nonenz. browning
	47	Unknown	Oxidation
	51	Phenylacetaldehyde	Nonenz. browning

[a] Sapers et al. (1972)
[b] Tentative

Table 4—Levels of hexanal and benzaldehyde in volatile concentrates prepared from new potato flakes stored in bulk at room temperature

Flake product	Variable	Days in bulk storage	Mean peak area ratio	
			Hexanal	Benzaldehyde
3	High sugar raw material	19–22	0.22	0.56
		31	0.40	0.84
7	Potato pieces held before cooking	24	0.22	0.38
		32	0.60	0.91
8	Minimum process water	10–12	0.17	0.32
		30	0.37	0.50

Table 5—Major volatile oxidation products in potato flakes stored in air at 23°C

Flake product	Variable	Storage time (months)	Mean peak area ratio[a]													
			Headspace vapor component					Volatile concentrate component								
			2	8	17	23	Sum	16	19	23	24	29	44	45	47	Sum
1	Conventional process	0	0.012	0.005	0.004	0.016	0.037	0.22	0.02	0.01	0.01	T	T	0.43	T	0.69
		6	0.016	0.005	0.003	0.016	0.040	0.24	0.11	0.12	0.06	0.02	0.14	1.17	0.17	2.03
		12	0.015	0.007	0.005	0.019	0.045	0.29	0.26	0.13	0.13	0.14	0.10	1.56	0.27	2.87
2	Cull raw material	0	0.010	0.004	0.005	0.026	0.045	0.21	0.03	0.02	0.03	T	T	.43	0.01	0.73
		6	0.025	0.012	0.004	0.022	0.063	0.42	0.63	0.17	0.17	0.24	0.70	1.06	0.52	3.91
		12	0.026	0.021	0.010	0.042	0.098	0.76	0.91	0.20	0.23	0.46	1.20	1.92	1.20	6.88

[a] Components identified in Table 3; T = Trace.

Table 6—Correlation of flavor score with levels of volatile oxidation products in potato flake samples stored in air at 23°C

Component[a]	Correlation coefficient (−) Flavor score vs. Log peak area ratio	Flavor score vs. Peak area ratio
HV 23	0.805	0.741
HV Sum[b]	0.885	0.917
VC 16	0.763	0.723
VC 19	0.883	0.894
VC 23	0.766	0.836
VC 24	0.812	0.792
VC 29	0.861	0.873
VC 44	0.805	0.879
VC 45	0.532	0.641
VC 47	0.772	0.865
VC 19 + 47	0.824	0.901
VC 29 + 47	0.803	0.892
VC Sum[c]	0.860	0.871

[a] Components identified in Table 3
[b] HV Peaks 2, 8, 17, 23
[c] VC Peaks 16, 19, 23, 24, 29, 44, 45, 47

nations of volatile oxidation products, especially components 19 and 29 with 47; comparable correlation coefficients were obtained when the sums of the major headspace vapor and volatile concentrate oxidation products were substituted for the individual component levels or simple combinations. Some improvement was obtained with most oxidation products and their combinations when flavor scores were correlated with the logarithm of component levels. In view of the similarity of the correlation coefficients for a number of volatile oxidation products, one cannot demonstrate a cause and effect relationship between any single component and the oxidized off-flavor. However, the use of these components (and their sums) as objective indices of flake oxidation is validated by their high degree of correlation with off-flavor level.

Effects of raw material and processing

The extent of oxidation as determined by sensory evaluation and the production of volatile oxidation products (Table 2) varied considerably among the experimental samples.

Potato flakes prepared from cull raw material (Product 2) had slight-moderate off-flavor levels after 6 and 12 months storage in air at 23°C and contained substantially higher levels of volatile oxidation products than did the conventional flakes. The instability of this product may be due to the presence of pro-oxidant substances in the fragments of sprouts, rot and other defects which were in contact with the mash throughout the process. Such defects should be excluded from the product through careful selection and trimming of the raw material.

Flakes produced from unpeeled tubers (Product 4) were more highly oxidized than the other potato flake products after 6 and 12 months of storage. The instability of Product 4 is noteworthy since peel fragments may be carried through the commercial flake process intentionally (Hollis and Borders, 1965) or as the result of poor peeling and trimming procedures. The original intent of this experiment was to determine whether potato flakes could be stabilized by naturally occurring water soluble antioxidants found in the peel (Pratt and Watts, 1964). It is apparent, however, that this effect, if it occurs, is countered by the pro-oxidant activity of other peel components leached into the mash.

Flakes produced from potato pieces cooked and cooled with the maximum possible rate of process water turnover (Product 9) were similar in stability to the flakes from unpeeled potatoes, having slightly lower levels of higher boiling volatile oxidation products and a slight-moderate off-flavor level. The susceptibility of these flakes to oxidation may be due to the extraction of water soluble antioxidants (e.g., amino acids, quercetin and caffeic acid) during cooking and cooling. In contrast, the flakes produced with a minimum rate of process water turnover (Product 8) were at least as stable as the conventional flakes.

Prolonged holding of the peeled potatoes prior to cooking (Product 7) had a small effect on stability and is probably not a major factor affecting shelflife. The piece size of the subdivided raw potatoes (Products 5 and 6) did not appear to influence potato flake stability. Likewise, the sugar content of the raw material (Product 3) had no effect on the sensitivity of flakes to oxidation. However, the high sugar product was downgraded by the taste panel, possibly because of its conspicuous atypical sweetness.

The extent of oxidation in the potato flake products after storage showed no consistent relationship to the extent of oxidation initially or after brief bulk storage. Oxidation also appeared unrelated to product moisture content or equilibrium relative humidity. The most highly oxidized flakes had somewhat lower levels of SO_2 and higher levels of volatile browning products. Heat damage to these products during drying could have destabilized them through the loss of antioxidants or the formation of pro-oxidants.

It can be concluded that the stability of air-packed potato flakes stored at 23°C is decreased by the presence of peel, by the use of defective raw material and by a high rate of process water turnover during cooking and cooling.

The effects on potato flake stability of the drying operation, moisture content and packaging are under investigation and will be the subject of future publications.

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PILOT PLANT EVALUATION OF INDIVIDUAL QUICK BLANCHING (IQB) FOR VEGETABLES

INTRODUCTION

CONVENTIONAL WATER and steam blanching of vegetables can result in nutrient loss and high organic solids in plant effluent (Holdsworth, 1968). Steam blanching has been found to cause less nutrient loss and lower effluent volumes than water blanching (Harris and von Loesecke, 1960; Lee, 1958). Lazar et al. (1971) described a modification to steam blanching, the Individual Quick Blanch (IQB) process, which gave less effluent than conventional steam blanching. With IQB, blanching takes place in two steps: a heating step and a holding step. Heating is done in a condensing steam unit with food particles one layer deep, and enough heat is added to raise the mass average temperature of the product high enough to inactivate enzymes (above 190°F). The second step is adiabatic holding, where the pieces leaving the heating section achieve a uniform temperature, and sufficient time is provided to inactivate enzymes and to achieve the desired texture.

Lazar et al. (1971) also showed that "preconditioning" carrots prior to blanching by warming and partial drying could significantly reduce effluent. Partial drying provided a surface that absorbed condensing steam, and prewarming reduced the sensible heat required for blanching.

Previous work measured the internal temperature, peroxidase inactivation and volume of effluent for IQB blanching of carrots (Lazar et al., 1971; Lund et al., 1972). In this work the effect of IQB blanching and preconditioning were evaluated under pilot-plant operating conditions using green beans, lima beans, Brussels sprouts and peas. The pilot plant was installed at a frozen food plant and was operated as nearly as possible at commercial conditions. Product quality, overall yield, solids retention and effluent volumes and strengths were measured for IQB and conventional steam blanching. The IQB products were compared with conventionally steam-blanched samples and commercially blanched products.

EXPERIMENTAL

Equipment

Details of the pilot size IQB blancher are shown in Figure 1. The four units were basically stainless steel wire mesh conveyors with adjustable speed drives: (1) a preconditioning section for partially heating and drying before blanching; (2) a condensing steam heating section; (3) an adiabatic holding section; and (4) a cooling section.

The preconditioning section (7 in. × 75 in.) was heated by an air supply arranged so that air could flow over or through the belt. The walls, cover and bottom of the unit were insulated. Residence times could be varied from 2–10 min. Hot air, without recycling, was used for warming and drying.

The insulated heater section (9 in. × 50 in.) could be adjusted to give residence times between 15 sec and 20 min. The cloth curtains at the entrance and exit reduced heat loss due to air leakage. Effluent was collected from drains installed under the belt.

The insulated and steam traced holding section was connected to the heater by an insulated transition. This section could be varied in length by using different length covers and taking product off the side of the belt. In the experiments with green peas a modification was made whereby the length could be varied by sliding the holder under the heater as shown in Figure 1. This modification allowed a shorter holder length and hence heavier belt loading (6 lb/ft²). Cloth curtains were used at the entrance and exit to curtail heat loss, and drains under the belt were used for collecting effluent. Residence times from 30 sec to 10 min with product belt loadings up to 6 lb/ft² were possible.

Air could be passed either through or over the cooling conveyor (11 in. × 60 in.). Fog sprays were used to aid in evaporative cooling and to reduce evaporative loss of product. Product residence times between 30 sec and 6 min were possible.

Procedure, sampling and analyses

The pilot-plant blancher was installed at Patterson Frozen Foods, Patterson, Calif. Green beans, lima beans and Brussels sprouts were run during the months of August and September, 1971. The experiments with green peas were done at this Laboratory during April and May, 1972.

These vegetables were blanched in the pilot plant at three different conditions: (1) conventional steam blanching; (2) IQB blanching; and (3) IQB blanching with preconditioning. Product style and operating conditions for the pilot plant are shown in Table 1.

The temperatures shown are those of the space above the product on the heater and holder belts. By eliminating most sources of air draft the heater temperature (211°F) was kept close to that of condensing steam (212°F). The holder temperature depended on the mass average temperature of the product leaving the heater. During a run the holder temperature fluctuated (185–200°F), probably owing to the fact that air drafts could not be completely excluded from entering this chamber, which did not have steam injected into it and which was shorter than the heater. The holder temperature was never allowed below 185°F during a run.

The air temperature, air flow rate and bed depth (single layer) in the preconditioner were set and the residence time varied to achieve the desired dehydration. So that the effect of preconditioning could be clearly observed for a given product, IQB runs with and without preconditioning were made at approximately the same feed rates. The runs on green beans were made with air flowing through the preconditioner belt. All other runs were made with air flowing over the belt.

The blanching times shown in Table 2 for conventional steam blanching in the pilot plant

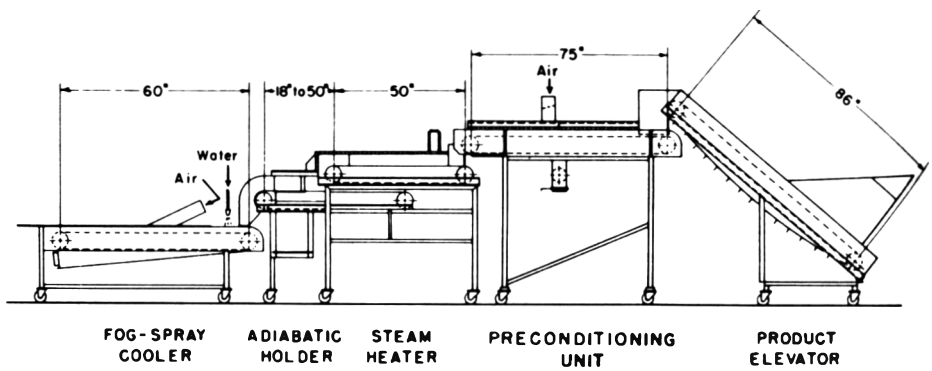

Fig. 1 – Diagram of the IQB pilot plant layout.

unit were those used in the production line at this plant. Samples of the raw material were taken to the Laboratory, and residual peroxidase was measured after heating and holding in an insulated container. Those heating and holding times that gave a peroxidase reduction comparable to that obtained with commercial blanching times were used in the pilot plant (Table 2).

Although some variations in cooling conditions were tried, cooling was not the primary concern of this work. For most experiments the cooling water, air flow rates and the residence time in the cooler were kept constant so that direct comparisons could be made between the different blanching conditions for each product.

For field operation, 50-lb lots of vegetables were taken directly from the processing line after washing, cutting, grading and inspection. Materials were carefully weighed at the feed and discharge to obtain an overall material balance. After blanching and cooling, samples were frozen in sealed plastic bags at −20°F. Samples of frozen product were also taken from the commercial processing line. The pilot-plant and commercial samples were evaluated by a panel after frozen storage (−10°F) for 1–3 months. Peas were blanched in the pilot plant under the same conditions as the other vegetables, except that 400-lb lots of peas were taken from the processing line prior to blanching, mixed with ice and transported in insulated containers to the Laboratory for experiments done within 24–72 hr.

Product was sampled at the feeder, after preconditioning, after blanching and at the discharge after cooling. These samples were used for total solids analyses.

Effluent was collected from the heater, holder and cooler. Total solids were determined by AOAC method 20.010 (1965) and total effluent volume was measured for each weighed lot of product blanched. A Beckman Total Carbon Analyzer (Model 915) was used to estimate chemical oxygen demand (COD) (American Public Health Assoc., 1965).

Peroxidase, chlorophyll and ascorbic acid levels in the frozen products were measured according to the methods described by Dietrich and Neumann (1965). Flavor differences were determined by duo-trio tests on cooked samples served under lighting conditions designed to minimize visual comparison. Color and appearance were evaluated by paired comparison of samples under daylight equivalent lamps.

Data are reported as the average of two or three replications at each condition—conventional, IQB and IQB with preconditioning. Owing to the small number of replications and the variability in raw material, no attempt was made to estimate experimental error.

Table 1—Operating conditions for IQB blancher

Feed rate and product style	30–300 lb/hr, 3/4 in. cut green beans, baby lima beans, Brussels sprouts (medium size), peas (field run)
Preconditioner	
Inlet air condition	220°F dry bulb, 60°F wet bulb
Air velocity	120 ft/min through belt; 800 ft/min over belt
Residence times	2.5–6.0 min
Heater	
Temperature	211°F
Residence times	1.3–2.2 min
Holder	
Temperature	185–200°F
Residence times	1.3–2.2 min
Cooler	
Air velocity	120 ft/min through belt; 800 ft/min over belt
Water flow	0.05–4.4 lb/lb feed
Residence times	3.3–5.5 min

Table 2—Summary of effect of different blanching conditions on products

	Heating time (min)	Holding time (min)	Preconditioning dehydration (%)	Gross yield (%)	Solids yield (%)
Green beans	2.5	0	0	92	91
	1.3	1.3	0	98	95
	1.3	1.3	9	97	94
Lima beans	3.0	0	0	96	98
	1.5	1.5	0	94	99
	1.5	1.5	14	92	98
Brussels sprouts	4.5	0	0	105	93
	3.0	2.0	0	110	96
	3.0	2.0	6	108	100
Green peas	1.5	0	0	81	94
	0.5	1.0	0	82	97
	0.5	1.0	9	78	91

RESULTS & DISCUSSION

Product yield

Loss of product in steam blanching arises from leaching of solids by condensate and evaporation of water. The former loss causes a decrease in yield by removal of cellular solids and fluid; while the latter loss is only water which can be countered in some products by water absorption.

Yields used in this work are defined as follows: Gross yield is the weight of product at discharge, after cooling, divided by the weight of feed. Solids yield is obtained from the total solids of the feed and blanched material:

$$\%\ \text{solids yield} = \frac{\left(\text{wt of blanched product} \times \%\ \text{solids in product}\right)}{\left(\text{wt of feed} \times \%\ \text{solids in feed}\right)} \times 100$$

$$= (\text{gross yield})\ \frac{(\%\ \text{solids in product})}{(\%\ \text{solids in feed})} \times 100$$

The data in Table 2 show gross and solids yields for the different blanching procedures used. While these yields do not show the exact nature of the changes in the product due to leaching, evaporation and absorption, they can help in identifying the predominating mechanism. When leaching losses predominate, the solids yield is less than the gross yield. When the solids yield is 100%, water absorption is indicated by gross yields above 100% and evaporative loss is shown by gross yields below 100%.

Green beans appeared to be very sensitive to heat induced cell damage during blanching, and since IQB blancing reduced this heat exposure, gross and solids yields for IQB blanched green beans improved substantially over those of conventional blanching. Preconditioning did not markedly improve either the gross or solids yields, further indicating that green beans are very susceptible to irreversible cell damage.

Lima beans present a different picture in that leaching losses are not a major problem, and evaporation caused the major loss of product. This difference between green beans and lima beans is probably related to the fact that the latter are processed without cut surfaces, and their skin inhibits rehydration in cooling.

Table 3—Effluent produced in steam blanching under different conditions

Product	Heating time (min)	Holding time (min)	Feed rate (lb/hr)	Heater belt loading (lb/ft²)	Holder belt loading (lb/ft²)	Preconditioning dehydration (%)	Effluent ratio (lb/100 lb feed)	Total solids in effluent (%)	COD (ppm)	Blancher solids loss (%)
Green beans	2.5	0	64	1.0		0	29	1.1	13000	3.6
	1.3	1.3	56	0.5	0.8	0	31	0.61	7200	1.7
	1.3	1.3	39	0.3	0.5	9	19	0.33	3600	0.7
Lima beans	3.0	0	96	1.8		0	27	1.5	13000	1.2
	1.5	1.5	90	0.9	1.9	0	30	1.7	16000	1.6
	1.5	1.5	91	0.9	1.9	14	23	0.81	2800	0.6
Brussels sprouts	4.5	0	52	1.5		0	29	0.37	3900	0.9
	2.2	2.2	81	1.1	1.9	0	25	0.34	1600	0.7
	2.2	2.2	83	1.2	2.2	6	18	0.15	1600	0.2
Green peas	1.5	0	260	2.3		0	20	2.23	21000	2.3
	0.5	1.0	365	1.1	6.2	0	15	2.03	23000	1.6
	0.5	1.0	68	0.2	1.4	9	17	1.07	14000	0.9

IQB blanching of Brussels sprouts gave better gross and solids yields than conventional steam blanching. Brussels sprouts were the only product to gain weight in blanching, absorbing up to 10% of their weight in water. As with green beans, minimizing heat exposure with IQB treatment appeared to minimize cell damage and thus give a higher solids yield. Unlike the green beans, however, preconditioning Brussels sprouts did increase solids yield.

In the experiments with peas a lower ratio of cooling water was used, and as a result peas showed a large evaporative loss. Cooling the peas in a container of water rather than in an air-water stream eliminated the evaporative loss. Peas, like lima beans, have skins which probably inhibit rehydration, but apparently if they are soaked in water, any water lost in blanching or preconditioning can be returned. Preconditioning tended to aggravate cell damage as shown by the decrease in solids yield and the increase in skin splitting observed in the product.

Cell damage is inherent in any heating process. Heating and holding blanching conditions can be chosen for maximum enzyme inactivation while having minimum exposure time at the processing temperature. Maximum enzyme inactivation may not be the only criterion for adequate blanching, since texture, gas expulsion or some other factor may be the limiting condition. These experiments did not investigate different heating and holding times, and more work is required on each product to determine the optimum times for whatever final product characteristic is desired.

Effluent analysis

The solids in the blancher effluent come from cell damage and leaching. Table 3 summarizes the effect of the different blanching conditions on blancher effluent. The blancher solids loss (Table 3,) is calculated as follows:

$$\%\ \text{blancher solids loss} = \left[\frac{\%\ \text{solids in effluent}}{\%\ \text{solids in feed}}\right]\left[\text{effluent ratio}\right] \times 100$$

where the effluent ratio is defined as the weight of effluent per unit weight of feed.

Clearly, in these experiments the amount of product solids lost in the blancher is markedly reduced when preconditioning of the feed is used as compared to conventional blanching. The combination of IQB and preconditioning reduced losses as much as 81% for green beans, and 75, 61 and 53% for Brussels sprouts, peas and lima beans, respectively. However, preconditioning reduced gross yields compared to those obtained with IQB alone (Table 2). Preconditioning probably changed the cell structure in such a way that the blanched vegetables could not readily reabsorb the lost water. Even though preconditioning decreased markedly the solids lost in the blancher effluent for green beans and lima beans, this reduction did not show up as an increase in the solids yield of the product (Table 2). This discrepancy is probably a result of experimental error since a relatively large decrease in solids loss in the blancher effluent gives only a slight change in the solids yield of the product.

Ignoring sensible heat added to the feed and heat losses, a 12% dehyrdration is needed to absorb all the condensate produced in heating the product (see calculation below). The results of this work show that all of the water removed in preconditioning cannot readily be added back to the product; thus there is an optimum amount of dehydration which gives

Table 4—Solids loss in cooling[a]

	Blanching condition	Cooling water ratio (lb water/ lb feed)	Cooler residence time (min)	Product temp[b] (°F)	Total solids in cooler effluent (%)	Solids loss in cooler (%)
Green beans	IQB with Preconditioning	4.4	3.1	80	0.04	1.8
	IQB	1.0			0.32	1.6
Lima beans	IQB with Preconditioning	2.4	3.1	90	0.24	1.3
	IQB with Preconditioning	0.2			0.54	0.3
Brussels sprouts	IQB	1.0	7.0	95	0.05	1.3
	IQB with Preconditioning	0.7			0.16	0.5
Green peas	IQB	0.05	2.0	90	2.1	0.6

[a]Air passed through belt with green beans; with other vegetables air passed over the belt

[b]Product temperature was measured by placing a 200-g sample from the discharge into a Dewar flask and measuring the bulk temperature.

the maximum gross yield and minimum solids loss. Possibly, milder conditions of dehydration would give better product yields; therefore, air with a higher wet bulb temperature should be tested by operating the equipment with part of the air recycled. There is a maximum in the amount of dehydration; Lund (1972) reported that product quality for canning deteriorated when preconditioning dehydration exceeded 6.6% for green beans, 7% for peas and 18.5% for cut corn.

Except for lima beans, IQB without preconditioning also gave a reduction in solids loss when compared to conventional steam blanching. These reductions would probably be even larger if heavier belt loadings had been used in the conventional blanching experiments. Since the steam blancher in the production line of this freezing plant operated at a single-layer belt loading, it was decided to use single-layer loadings for conventional blanching in the pilot plant tests. A 1 lb/ft^2 belt loading is approximately a single layer for most vegetables. Steam blanchers can operate at much heavier loadings, as much as 4 lb/ft^2, and these heavier belt loadings give higher solids loss in the blancher (Lazar et al., 1971). Thus, the low belt loadings used for conventional blanching in these experiments compared the solids loss of conventional steam blanching at its most favorable condition to IQB blanching.

The effluent ratios reported in Table 3 include condensate produced from heat losses in the equipment and entrained condensate in the steam supply. The entrained condensate at the frozen food plant varied considerably and could not be determined reliably. Heat losses in the equipment in the absence of product were determined to be 6 lb/hr of condensate when steam of 100% quality was used.

If it is assumed that the heat capacity of vegetables is 1 BTU/lb°F, and if the latent heat of condensing steam is taken as 970 BTU/lb, then the amount of condensate produced in heating the product from 70°F to 190°F is approximately 0.12 lb/lb feed. Hence,

$$\text{effluent ratio} = \left[0.12 + \frac{6}{(\text{feed rate})}\right] 100 \frac{\text{lb}}{100 \text{ lb feed}}$$

The effluent ratios for green beans, lima beans and Brussels sprouts (measured at the frozen food plant) are much larger than theoretically calculated, because of the large amount of entrained condensate in the steam supply, while the effluent ratios for green peas (measured at the Laboratory with high quality steam) are relatively close to theoretical. Since the effluent ratio is highly dependent on the design and size of the equipment, this value may be used for equipment evaluation; however, it does not serve very well for a comparison of different blanching techniques. The blancher solids loss, which is probably not very dependent on the effluent ratio, is a better comparison of the different techniques of blanching.

In addition to the effluent due to heat loss, equipment design can also affect the amount of solids in the effluent by crushing or mechanically damaging the product during conveying. For example, in the heater of the pilot plant unit small particles were observed to fall through the mesh of the conveyor belt, and they were crushed on the pulleys as the belt passed over them. The condensate forming on the cold belt extracted soluble solids from these crushed particles, and these solids, as well as those leached directly from the product, were collected in the effluent. This was a particularly troublesome problem with green beans, peas, and lima beans. Alternative conveyor designs could eliminate the problem.

Analysis of the effluent draining from the cooling belt showed that extensive solids loss could occur even when using careful cooling with air and fog sprays (Table 4). The type of blanching may have some effect on the solids lost in cooling, and as a result direct comparisons between solids loss for different cooling water-to-feed ratios cannot be made. However, it is clear that cooling can contribute large losses of solids even at relatively low water-to-feed ratios, and thus any reduction in solids loss achieved by IQB and preconditioning could be negated during cooling. Also, it is clear from the gross yields obtained in the experiments with peas (Table 2) that air cooling with low water ratios can give a very high evaporative loss in the product. More data are needed to design cooling equipment which keeps both leaching and evaporative losses to a minimum. This subject is now being studied at this Laboratory.

Product quality

In the sensory evaluations of the products, large variability in the samples tested necessitated making several replications of the same comparisons. When the panel members could distinguish between samples, they did not consistently prefer one treatment over another or experimental samples over commercially blanched samples. In all cases the differences, if any, between products blanched at different conditions were very small. The variation in raw product quality characteristics from hour-to-hour during the season apparently was sufficient to mask any differences arising from blanching treatments.

Table 5 shows the results of the chemical analyses done on the frozen products. In all cases the products were adequately blanched as described by Dietrich and Neumann (1965). The IQB blanched peas showed a higher residual peroxidase than those conventionally blanched, but when IQB with preconditioning was used, the residual peroxidase was about the same as in conventional blanching. Apparently preconditioning can substantially reduce the time necessary for enzyme inactivation. The differences shown in chlorophyll conversion and ascorbic acid content were probably due to variations in raw material.

CONCLUSIONS

The results of these pilot-plant experiments show that the IQB blanching technique adequately blanched vegetables without any discernible decrease in product quality. In addition IQB reduced the solids content of blancher effluent, thereby increasing the amount of solids in the product.

Preconditioning the feed with a

Table 5—Chemical analyses of product

	Type of blanching	Residual peroxidase (%)	Chlorophyll conversion (%)	Ascorbic acid in product (mg/100g)
Green beans	Conventional	0.9	18	8
	IQB	1.4	18	11
	IQB with preconditioning	1.1	15	7
Lima beans	Conventional	0.0	(not measured)	16
	IQB	0.0	(not measured)	24
	IQB with preconditioning	0.0	(not measured)	22
Brussels sprouts	Conventional	0.0	45	47
	IQB	0.3	31	46
	IQB with preconditioning	0.0	33	43
Green peas	Conventional	0.4	7	21
	IQB	2.4	7	18
	IQB with preconditioning	0.3	7	20

5–10% dehydration markedly reduced the product solids lost with blancher effluent without damaging product quality. The cost of preconditioning must be balanced against the cost of waste disposal to determine the practicality of preconditioning before blanching.

The results of this work also indicate that an IQB blancher as described here would not be much larger than a conventional steam blancher. The feed capacity of any steam blancher is proportional to the belt loading. Since a single-layer belt loading is the maximum desired in the heater, the capacity of an IQB blancher is limited by the heater. For example, in the pilot plant unit 365 lb/hr of peas could be blanched but only 81 lb/hr of Brussels sprouts, because the Brussels sprouts required four times as much heating as did the peas. Using the heating and holding times shown in Table 2, and IQB commercial blancher with 1 and 6 lb/ft^2 belt loadings on the heater and holder, respectively, a 4-ft wide conveyor belt, and a feed capacity of 1 ton/hr of Brussels sprouts would require an 18-ft long heater and a 3-ft long holder. A 4-ft wide conventional steam blancher for Brussels sprouts using the blanching time shown in Table 2, having a 1 ton/hr feed capacity and having a 2 lb/ft^2 belt loading would be 19 ft long. Of course, this same equipment, IQB or conventional, would be able to blanch 4 tons/hr of green peas. Work is under way on a compact steam blancher design using the IQB principle to reduce floor space requirements significantly below those for conventional steam blanching.

It was observed during these experiments that the amount of effluent and solids leaving a steam blancher depended also on blancher design factors, such as insulation, air draft at the ends, the steam condensate flow through the product, steam quality and type of conveyor belt and pulleys. In order to reduce product solids loss each of these design characteristics must be included in such a way that steam condensate and its contact with the product will be a minimum.

The product solids lost in blanching cannot be considered separately from those lost in cooling. The data on solids loss in cooling indicate that it can contribute a higher product loss than blanching when a high water ratio is used, and air cooling with a low water-to-feed ratio gives a large evaporative loss.

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Reference to a company or product name does not imply approval or recommendation of the product by the USDA to the exclusion of others that may be suitable.

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NUTRITIVE CONTENT OF CANNED TOMATO JUICE AND WHOLE KERNEL CORN

INTRODUCTION

CURRENT INTEREST in nutritional labeling has focused attention on the adequacy of available data on nutrient content of foods. Although several food processors have collected and privately published nutritional data on their products, most of the information in the technical literature was obtained 25–30 yr ago. Since that time there have been many changes in the industry that could have resulted in an altered nutrient content. The commercial varieties of many fruits and vegetables grown for canning purposes have undergone substantial changes. Tomato varieties widely used 10–15 yr ago are no longer to be found. Varietal types suitable for mechanical harvesting have been almost universally adopted. There have also been changes in commercial processing and handling practices that could have affected vitamin retention.

To carry out a nation-wide nutritional survey of all of the great variety of canned products available today would represent a massive undertaking. However, data obtained on a national sampling of selected products can provide an indication of the applicability of the older survey literature to today's canned foods. With this objective in mind, the National Canners Association Research Laboratories in cooperation with interested government agencies as sponsors, are restudying selected products using sampling methods directly comparable to older survey procedures. Sampling and analysis of canned tomato juice and whole kernel corn have been completed. Resurveys of canned green beans, peaches and sweet potatoes are in progress.

Earlier nutrition programs

Canning industry-sponsored research developed the bulk of available data on the nutrient content of canned foods. The history of these programs has been outlined by Cameron (1954) and the resulting publications have been recorded in detail. The first industry program was carried out from 1922–1937 resulting in 17 papers. The second major effort was begun in 1941 and was essentially complete by about 1950. It generated approximately 50 literature contributions presenting data on the nutrient content of canned foods and the effects on nutrient retention of canning and warehousing practices.

Most of the actual nutrient content data were obtained from surveys of the canned foods produced during the 1941–1943 seasons. Data on the proximate composition and mineral content were obtained by Kramer (1946). Pressley et al. (1944) determined the ascorbic acid and carotene content of 61 samples of yellow whole kernel corn and 79 samples of tomato juice; Ives et al. (1944) covered the thiamin and niacin content; Thompson et al. (1944) reported on riboflavin and pantothenic acid; and Hinman et al. (1947) furnished additional analyses of carotene, ascorbic acid and thiamin in canned yellow whole kernel corn.

A review of the literature published since about 1950 failed to reveal additional data on the nutrient content of canned corn and tomato juice as produced in this country. Nutritional data collections published since that time have presumably relied primarily on the data referenced above for their canned food entries. Probably the most widely quoted among these compilations, the USDA Agriculture Handbook No. 8 Composition of Foods (Watt and Merrill, 1963), utilized data from a wide variety of published and unpublished sources but must have relied heavily on the above references for information on canned foods. More recently Orr (1969) compiled values for pantothenic acid, vitamins B_6 and B_{12}.

To investigate possible changes in the

Table 1—Mineral recoveries from whole kernel corn by atomic absorption

Element	Level added (ppm)	No. test	Percent recovery Range	Average
Calcium	25.0	5	97.4–109.6	104.1
Chromium	0.05	3	80.5– 86.8	84.5
Copper	0.10	5	87.7–100.5	94.0
Iron	2.0	5	96.4–106.7	101.4
Magnesium	20.0	4	70.0–111.0	93.6
Manganese	0.1	4	95.0–105.0	98.5
Zinc	0.5	5	80.1– 90.4	86.0

Table 2—Inter-laboratory comparison on identical samples

Nutrient	Berkeley		Washington		Number of comparison
Carotene IU/100g	696	(225)*	663	(108)*	7
Protein (N × 6.25)%	0.77	(0.089)	0.76	(0.093)	7
Total solids %	6.3	(0.618)	6.4	(0.764)	7
Ascorbic acid mg/100g	14.3	(6.176)	14.3	(6.516)	14
Ash %	1.15	(0.042)	1.17	(0.043)	7
Fat %	0.05	(0.0045)	0.05	(0.0058)	4

* Standard deviation

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nutritive value of canned foods that may have occurred since these earlier surveys, it is appropriate that strictly comparable sampling designs be employed. Improved methodology should of course be utilized for the analytical work. Fortunately published descriptions of the 1941–1950 program together with those portions of the original records that remain on file at the National Canners Association provided sufficient detail to permit the design of a truly comparable survey.

EXPERIMENTAL

Sampling

Tomato juice from the 1969 season was sampled at approximately the same level as that used in the 1941–1943 survey. Each sample consisted of six No. 303 cans (industry designation, 303 × 407) or three "46 oz" (404 × 700 or "#3 cylinder"). A total of 130 samples was collected from the major producing areas in proportion to their fraction of the total national production. They were analyzed after approximately 6–9 months of storage.

For the resurvey of nutrient values in canned yellow whole kernel corn, the distribution of samples among the Eastern, Midwestern and Northwestern production areas was based upon 1970 production statistics. A total of 54 samples was analyzed of which 39 were brine pack and 15 were vacuum pack. Of the 39 brine packed samples, 27 were processed by continuous rotary retorting procedures and the remainder by stationary retorting methods. Fourteen of the 15 vacuum-packed samples were processed by continuous rotary methods.

Table 3—Vitamin content of commercially canned corn and tomato juice

		Whole kernel corn[a]					
		Brine pack		Vacuum pack		Tomato juice[a]	
		39 samples		15 samples		130 samples	
Ascorbic Acid	mg	–		–		13.4	(4.96)
Thiamin	mg	0.028	(0.010)*	0.045	(0.021)*		
Riboflavin	mg	0.061	(0.009)	0.077	(0.007)		
Vitamin B_6	mg	0.037	(0.018)	0.055	(0.020)		
Niacin	mg	1.03	(0.10)	1.28	(0.11)		
Vitamin A	I.U.	196	(52)	207	(40)	690	(163)

[a] 100g total can contents
* Standard deviation

Table 4—Proximate composition of commercial production

	Whole kernel corn[a]					
	Brine pack		Vacuum pack		Tomato juice[a]	
	39 samples		15 samples		130 samples	
Moisture, %	81.7	(1.46)*	76.4	(1.58)*	93.5	(0.78)*
Ash, %	0.92	(0.13)	1.1	(0.135)	1.22	(0.11)
Fat, %	0.52	(0.13)	0.73	(0.110)	0.052	(0.015)
Protein, %	1.92	(0.18)	2.55	(0.130)	0.77	(0.08)
Carbohydrate, %	14.9	(1.4)	19.2	(1.45)	4.36	(0.55)
Food energy,[b](Cal)	62	(5.4)	81	(5.6)	19	(1.62)

[a] 100g total can contents
[b] Calculated using the following factors: corn, 1g protein = 2.44 calories; 1g fat = 8.37 calories; 1g carbohydrate = 3.57 calories. Tomato juice, 1g protein = 3.36 calories; 1g fat = 8.37 calories; 1g carbohydrate = 3.60 calories (Atwater system, USDA Handbook No. 8, Table 6, Watt & Merrill, 1963).
* Standard deviation

Table 5—Mineral content of commercial production

	Whole kernel corn[a]					
	Brine pack		Vacuum pack		Tomato juice[a]	
	39 samples		15 samples		129 samples	
Calcium, mg	3.5	(1.27)*	4.8	(1.43)*	9.2	(3.1)*
Magnesium, mg	12.8	(2.88)	19.3	(4.65)	10.1	(1.5)
Phosphorus, mg	41.0	(3.43)	54.5	(5.53)	17.3	(2.4)
Sodium, mg	210.9	(49.2)	264.5	(57.42)	–	
Potassium, mg	117.8	(9.17)	157.1	(16.65)	–	
Iron, mg	0.22	(0.078)	0.3	(0.18)	0.55	(0.31)
Zinc, mg	0.28	(0.071)	0.37	(0.089)	–	
Manganese, mg	0.033	(0.0118)	0.067	(0.0269)	–	
Copper, mg	0.023	(0.0070)	0.029	(0.0059)	0.104	(0.063)
Chromium, mg	0.0024	(0.00092)	0.0037	(0.00075)	–	
Selenium, mg	0.00024	(0.00012)	0.00020	(0.00011)	–	

[a] 100g total can contents
* Standard deviation

Analytical methods

Most of the analytical methods employed were from the Association of Official Analytical Chemists (AOAC, 1970).

Major constituents. Moisture was determined on 10-g samples of corn by the method of the AOAC Sec. 32.004, total solids in tomato juice from refractive index measurements utilizing correlations obtained in the NCA Berkeley Laboratory (Lamb, 1969; AOAC Sections 32.008, 32.009 and 32.010). Ash determinations utilized the method of AOAC Sec. 31.013 and protein was determined as in AOAC Sec. 2.051.

There is no "official" method specified in the AOAC for the determination of fat in fruit and vegetable products. The method under Animal Feed (Sec. 7.048) was used with modifications. 25g of well blended sample was weighed out and mixed with 4g asbestos and 50–100 ml water. The sample was filtered and the residue dried in 70°C vacuum oven. Disposable aluminum dish, filter paper and sample were removed from the vacuum oven and cut into small pieces. All pieces were extracted in a Soxhlet apparatus with petroleum ether for at least 16 hr and the residue dried to constant weight in an air oven at 100°C.

Total carbohydrate was calculated by subtracting the total of all other constituents from 100. Food energy calculations were made using factors from the Atwater system: USDA Handbook No. 8 Table 6, (Watt and Merrill, 1963). Calories are reported as the nearest whole number of calories per 100g of edible product.

Vitamins. Carotene was determined by the method described in the AOAC Sec. 39.014–39.023. Recovery tests using pure beta carotene averaged approximately 80%. The values reported were corrected for the average recovery. Ascorbic acid was determined by titration with 2,6-dichloroindophenol (NCA, 1968). Riboflavin was extracted and determined according to the AOAC Sec. 39.039–39.042. Recoveries were close to 100% and no correction was made. Niacin was determined by microbioassay using the organism *Lactobacillus plantarum* ATCC 8014 with titrimetric procedures. (AOAC Sec. 39.102–104). Thiamin was assayed according to the AOAC Sec. 39.024–39.030. Recoveries averaged 90% and results were corrected for this recovery.

The AOAC procedure lists four alternate enzyme preparations for liberating thiamin from plant material. A comparison was made of the amount of thiamin extracted using Takadiastase (two preparations) Mylase P (two preparations)

and Diastase. On raw corn Mylase P gave values of 0.00–0.04 mg/100g whereas a value of 0.15 mg/100g is reported in USDA Handbook No. 8 (Watt and Merrill, 1963). The amount of thiamin found in a different lot of raw corn using Takadiastase averaged about 0.10 mg/100g. Since Takadiastase is one of the recommended enzymes and had been used extensively in the 1942–45 survey it was used in these studies. One factor in its favor is its stability and reproducibility which may not be true of some of the other enzymes. It would appear that different enzymes should be studied with each product and that efforts should be made to standardize the use of a specific enzyme for a given product.

Total vitamin B_6 was run according to the Association of Vitamin Chemistry, Inc. (1966) using *Saccharomyces carlsbergensis* ATCC 9080.

Mineral. Phosphorus was determined by the molybdovanadate spectrophotometric method (AOAC Sec. 22.040). Selenium was determined fluorometrically (Hoffman et al., 1968). Calcium, chromium, copper, iron, magnesium, manganese and zinc were determined on the ash by atomic absorption spectrophotometry. The recoveries obtained on each element are shown in Table 1.

Sodium and potassium were determined on the ash by flame emission spectrophotometry. Recovery tests were run at the 600 ppm level for each element. Average recovery of six runs was 98.5% for sodium and 99.9% for potassium.

Interlaboratory comparisons. To detect and eliminate as many inter-laboratory differences as possible in advance of the analytical work on the survey samples, preliminary analyses were carried out in both laboratories, applying all of the analytical methods to identical sample materials. It was not always possible to ensure absolute homogeneity of these collaborative samples. In general, they consisted of cans of tomato juice having the same code. In most instances, several cans of the same code were composited separately in each laboratory and analyzed on approximately the same date. Results were compared and procedural details modified to eliminate or minimize any differences in results. Samples were also exchanged in the course of the regular analytical work as a "quality control check" on the conduct of the methods in both laboratories.

Average results obtained on a number of individual comparisons are shown in Table 2. Agreement between the laboratories on independent determinations of protein, total solids, ash and fat was very good. There was some difficulty in eliminating internal variation and inter-laboratory variation on the carotene determinations. Difficulties were experienced by the laboratories in obtaining pure carotene standards and slightly different procedures were used by the two laboratories. In spite of these difficulties, however, the bias between laboratories as indicated by these inter-laboratory comparisons was less than 5% of the reported values.

Special attention was devoted in ensuring the absence of laboratory differences in the ascorbic acid analyses since as the work progressed a "geographical effect" in ascorbic acid became apparent. Frequent samples were interchanged between the two laboratories. Agreement was satisfactory using the indophenol dye titration method and no evidence of a bias between laboratories is apparent.

RESULTS & DISCUSSION

Nutrient content of recent commercial production

The results of the resurvey of the nutrient content of canned tomato juice and whole kernel corn are presented in Tables 3, 4 and 5 which present the vitamin, proximate composition and mineral content of these products. The mean value for each nutrient is followed by the standard deviation of a single sample.

The probable advent of some form of nutrient labeling lends great significance to the question of normal variability of nutrient content. There are suprisingly few data available displaying variability of individual packages of foods.

In many situations it is more practical and economical to prepare composite samples. Since these can usually be constituted so as to encompass the most significant variables associated with the sampled population, data obtained from such composites can be just as representative as that from multiple analyses of smaller units. Information on individual package variability is sacrificed, but this has not previously been required.

The standard deviations in this work are also those of composite samples and not individual containers. For our purposes, this form of sampling was most desirable since it provided direct comparisons with earlier surveys. It should be recognized however, that compositing six

Table 6—Variability of nutrient in terms of the mean, and as percentages of the RDA. The percent RDA furnished in one cup is also shown

	Whole kernel corn						Tomato juice		
	Brine pack (39 samples)			Vacuum pack (15 samples)			130 samples		
Nutrient	% RDA[a]	Coef. var.	S.D. % RDA	% RDA	Coef. var.	S.D. % RDA	% RDA	Coef. var.	S.D. % RDA
Ascorbic Acid							54	37	20
Thiamin	5	36	2	8	47	4		–	
Riboflavin	9	15	1	12	9	1		–	
Vitamin B_6	5	49	2	7	36	3		–	
Niacin	14	10	1	18	9	2		–	
Vitamin A	10	27	3	11	19	2	34	24	8
Moisture		2			2			1	
Ash		14			12			9	
Fat		25			15			29	
Protein	7	9	1	10	5	1	3	10	0
Carbohydrate		9			8			13	
Food Energy	6	9	1	7	7	1		9	0
Calcium	1	36	0	2	30	1	3	34	1
Magnesium	9	23	2	14	24	3	7	15	1
Phosphorus	13	8	1	17	10	2	5	14	1
Sodium		23			22			–	
Potassium		8			11			–	
Iron	6	36	2	8	60	5	13	56	8
Zinc		25			24			–	
Maganese		36			40			–	
Copper		30			20			61	
Chromium		38			47				
Selenium		50			55				

[a] Recommended Dietary Allowances: Reference 22–35 yr-old male. National Academy of Sciences (1968)

Table 7—Yearly variation of ascorbic acid content of canned tomato juice

	No. of samples examined	Ascorbic acid (Milligrams per 100 ml) Max	Min	Avg
1960	30	26.1	9.4	16.0
1961	29	22.2	5.5	13.5
1962	35	18.8	6.4	12.6
1963	31	20.2	6.4	14.2
1964	26	19.2	3.0	13.8
1965	26	22.5	5.0	14.2
1966	29	22.4	6.9	15.1
1967	32	31.4	5.2	16.0
1968	39	21.7	3.2	12.8
1969	34	27.5	5.8	15.4
1970	32	18.9	6.3	12.5
1971	38	25.3	4.9	12.5

consumer-size packages (three in the case of 46 ox. tomato juice) will conceal some package-to-package variability. The variability in these surveys is more nearly comparable to that of multiple can samples representing different code lots of the same product.

In Table 6 we have expressed the standard deviations of each nutrient in each product as a percent of the mean. Expressed in these terms, the vitamin and mineral content of these products is seen to exhibit considerable variation, while the "proximate constitutents" and food energy content are, by comparison, relatively consistent from one sample to another.

As an index of the nutritional significance of these variations, we have also tabulated the standard deviation of each nutrient in terms of the percent of the Recommended Dietary Allowance furnished in a one-cup portion of the product. The "reference man" is the 22–35 year old male in NAS Publication Number 1694 (National Academy of Sciences, 1968). Weights of the one-cup portions were estimated from the specific gravity values of each product as follows: tomato juice, 1.208; corn, brinepack, 1.06; and corn, vacuum pack, 1.08. Since most fruits and vegetables furnish only modest fractions of the Recommended Dietary Allowance for many of the nutrients, sample-to-sample variability within this frame of reference, is of little nutritional importance. A possible exception is the ascorbic acid content of tomato juice. This product is a very significant source of vitamin C. This nutrient is, however, subject to marked variability attributable in large part to seasonal and environmental factors.

Figure 1 displays the distribution of the Beta carotene values obtained in the present survey of tomato juice, and provides a graphic comparison of regional averages (Western tomatoes compared with Eastern and Midwestern) as well as average values from the 1941–1943 survey.

The average carotene content of the 1969 samples was somewhat lower than that of the 1941–1943 data. Differences in methodology between the two surveys could account for a portion of this difference. Since carotene is well retained during canning, variability of carotene content must be attributed to variations in the raw product.

Niacin in corn is also well retained and the frequency distribution for this nutrient in brine packed corn is given in Figure 2.

The niacin results approximate a normal distribution and indicate the variations in the vitamin may also be attributed to variation in the raw product. The average niacin content was somewhat higher than that of the 1941–1943 data.

In Figure 3 we have diagrammed the distribution of ascorbic acid in canned tomato juice. Regional averages (Eastern-Midwestern vs. Western) and the 1941–1943 averages are also shown. The difference between the mean ascorbic acid content of the Eastern-Midwestern producing areas and that of the Western area is real, statistically significant ($t = 4.66$; $p \leq 0.001$), and not attributable to methodology. The many factors that can affect the ascorbic acid content of canned tomato juice have been well studied (Cameron et al., 1955; Harris and Von Loesecke, 1960). Varietal and climatic factors can play very important roles, as well as the performance of individual canning operations. Operations which tend to incorporate air into the juice can be detrimental if it is not removed prior to heating. Traces of copper derived from brass fittings can also be detrimental. The type of container can play a role to the extent that it affects

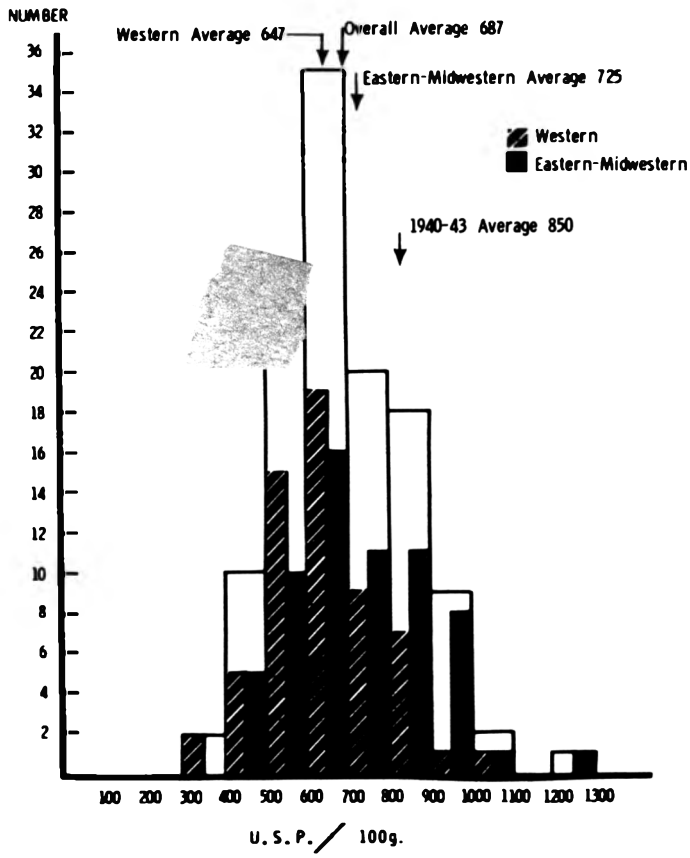

Fig. 1—Distribution of beta carotene in Eastern (Washington) and Western (Berkeley) tomato juice samples.

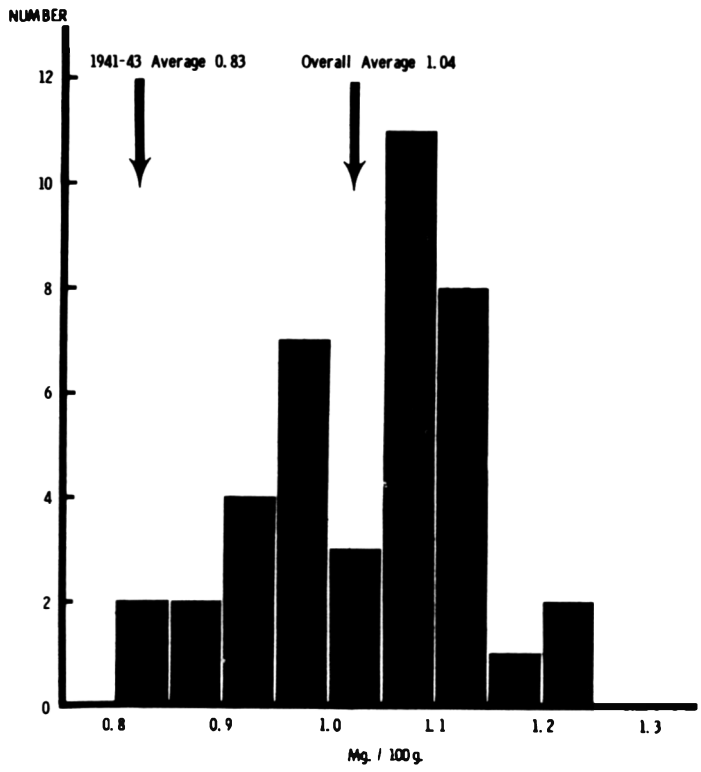

Fig. 2—Distribution of niacin in yellow kernel brine packed corn.

the oxygen available in the headspace of the can. A plain-bodied tinplate container diminishes headspace oxygen by the normal detinning action taking place during and immediately after processing. Thus, the plain tinplate can provides somewhat less oxygen for reaction with the ascorbic acid. The effect of the container on ascorbic acid content is very small.

The ascorbic acid content of tomato juice exhibits considerable yearly variation. Table 7 displays yearly variation in one region as determined in annual cuttings for the last 12 years. Yearly means have ranged between 12.5 and 16.0 mg per 100/ml.

The vitamin content of corn produced in the three major growing areas was compared on a regional basis with the results indicated in Table 8. Differences among regions are inconsequential. The Vitamin A content of corn produced in the Northwest is somewhat lower than that from other sections of the country, but the difference is likely to be within yearly variation.

Differences between stationary and agitating retorting procedures are minor (Table 9). Thiamin was slightly higher in the samples cooked in the agitating retorts. The difference is of no consequence from a nutritional point of view, but it is probably real since thiamin is a heat liable vitamin and agitating cookers achieve commercial sterility with less total heat input into the can as compared with stationary retorting methods for these products.

Table 8—Vitamin composition of canned corn from different regions. (1971 brine pack)

	No. of Samples	Thiamin mg/100g	Riboflavin mg/100g	Niacin mg/100g	Vit. B_6 mg/100g	Vit. A IU/100g
East	5	0.025 (0.008)*	0.057 (0.007)*	1.02 (0.13)*	0.024 (0.006)*	196 (20)*
Midwest	24	0.029 (0.009)	0.062 (0.010)	1.07 (0.09)	0.039 (0.022)	208 (54)
Northwest	10	0.027 (0.009)	0.061 (0.009)	1.01 (0.11)	0.031 (0.018)	167 (48)
Overall	39	0.028 (0.010)	0.061 (0.009)	1.04 (0.10)	0.037 (0.018)	196 (52)

* Standard deviation

Table 9—Difference between processes. (1971 brine pack)

Process	No. of samples	Thiamin mg/100g	Riboflavin mg/100g	Niacin mg/100g	Vit. B_6 mg/100g	Vit. A IU/100g
Stationary	12	0.025 (0.010)*	0.060 (0.007)*	1.03 (0.10)*	0.038 (0.017)*	203 (62)*
Agitating	27	0.029 (0.007)	0.061 (0.009)	1.05 (0.10)	0.037 (0.023)	193 (46)
Overall	39	0.028 (0.010)	0.061 (0.009)	1.04 (0.10)	0.037 (0.018)	196 (52)

* Standard deviation

Table 10—Retention of thiamin, niacin and vitamin B_6 in whole kernel brine and vacuum pack corn[a]

Vitamin & Product	Raw		Continuous cooker			Retort		
	Wet	Dry	Wet	Dry	% Retention	Wet	Dry	% Retention
Thiamin								
Brine pack	0.098	0.421	0.015	0.090	21.5	0.015	0.089	21.0
Vac. pack	0.109	0.409	0.044	0.168	41.2	–	–	–
Niacin								
Brine pack	1.53	6.59	1.01	5.86	89.4	1.02	5.93	90.3
Vac. pack	1.55	5.82	1.43	5.49	94.5	–	–	–
Vitamin B_6								
Brine pack	0.151	0.649	0.019	0.112	17.3	0.019	0.113	17.4
Vac. pack	0.161	0.606	0.034	0.130	21.3	–	–	–

[a] Values expressed as mg per 100g

Retention of nutrients during the canning of corn

An experiment was performed at a corn packing plant in the Northwest to determine the retention of thiamin, niacin and vitamin B_6 during the canning of yellow whole kernel corn. Corn of the Jubilee variety grown within a 20-mi radius of Junction City, Oregon, was husked, washed, trimmed and sorted into three maturities, designated as Fancy, Choice and Standard. Only the two top maturity grades were used for canning. Fancy grade was canned in No. 303 cans as whole kernel brine pack. Choice grade corn was canned as vacuum pack in 75-oz (60 x 600) cans.

The corn in this plant was not blanched before canning; hence, the kernels remained cold until they were filled into the cans. The washing was done with cold water. A considerable amount of starchy material was removed in the washing procedure. Raw samples were taken after washing, just before filling into cans.

The No. 303 cans were filled with hot brine just prior to steam flow closure. These cans were run directly to an FMC continuous cooker in which they were cooked 17.4 min at 250°F. They were then water cooled to 90–100°F. For each sampling of raw corn, 24 cans were removed before entering the continuous cooker and placed in a still retort where

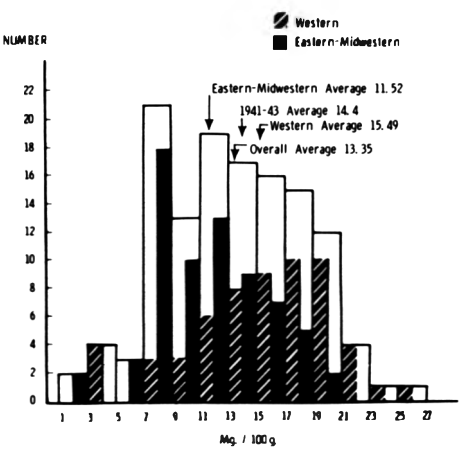

Fig. 3—Distribution of ascorbic acid in Eastern (Washington) and Western (Berkeley) tomato juice samples.

Table 11—Nutrients in one cup portions. Resurvey results compared with "Handbook 8" values

Nutrient	Product[a]	HB8	Present	% RDA Present	Difference % RDA[b]
Calories	TJ	46	46		0
	BC	165	181		+1
	VC	212	237		+1
Protein, g	TJ	2.18	1.87	3	0
	BC	4.76	4.81	7	0
	VC	6.39	6.52	10	0
Fat, g	TJ	0.24	0.13		
	BC	1.50	1.30		
	VC	1.28	1.87		
Carbohydrate, g	TJ	10.46	10.60		
	BC	39.37	37.36		
	VC	52.37	49.05		
Ash, g	TJ	2.68	1.49		
	BC	2.55	2.31		
	VC	2.55	2.81		
Calcium, mg	TJ	17.0	22.4	3	+1
	BC	10.0	8.8	1	0
	VC	7.66	12.3	2	+1
Phosphorus, mg	TJ	43.8	42.1	5	0
	BC	120.4	102.8	13	−2
	VC	186.5	139.2	17	−6
Iron, mg	TJ	2.19	1.34	13	−4
	BC	1.00	0.55	6	−5
	VC	1.28	0.77	8	−5
Sodium, mg	BC	492	529		
	VC	602	677		
Potassium, mg	BC	243	296		
	VC	247	401		
Vitamin A, IU	TJ	1945	1678	34	−5
	BC	677	492	10	−4
	VC	894	530	11	−7
Thiamin, mg	BC	0.075	0.070	5	0
	VC	0.077	0.115	8	+3
Riboflavin, mg	BC	0.13	0.15	9	+1
	VC	0.15	0.20	12	+3
Niacin, mg	BC	2.26	2.61	14	+ 2
	VC	2.81	3.27	18	+ 3
Ascorbic acid, mg	TJ	38.9	32.6	54	−10

[a] Nutrients in one cup: (TJ) Tomato juice, 243g; (BC) brine pack corn, 250g; (VC) vacuum pack corn, 255g.
[b] Recommended Dietary Allowances: Reference 22–35 yr-old male. National Academy of Sciences (1968)

they were processed for 30.0 min at 250°F followed by water cooling to 90–100°F.

The vacuum-packed samples were filled into 75-oz cans, cold brine added and vacuum closed. They were preheated in an exhaust box to a temperature of about 160°F and then processed for 19.3 min at 252°F in an FMC continuous cooker.

Samples of raw corn were preserved by appropriate procedures and transported to the Berkeley Laboratory for analysis. Samples were preserved of the entire contents of the processed cans within a few days after processing. Moisture and salt were determined on all raw and processed samples and the results calculated both on the wet ("as is") and the dry (salt-free solids) basis. Retention was calculated on the dry basis.

Five retention experiments were performed for each of the canning variables described above. Average values are shown in Table 10.

The results show no difference in retention for any of the three vitamins processed in a still retort and in a continous cooker. Higher retentions of all three vitamins were obtained in the vacuum packed cans than in the brine packed. The differences were the most pronounced in the case of thiamin. Retention of niacin was high in all instances, whereas, the retention of thiamin and vitamin B_6 indicated a considerable amount of thermal degradation of these vitamins, somewhat more of vitamin B_6 than of thiamin.

Comparison with earlier data

The USDA Handbook No. 8 (Watt et al., 1963) has figured prominately in public discussions of nutrient labeling proposals, and was frequently mentioned in comments filed in response to the Food and Drug Administration's nutrient labeling proposal (37 *Federal Register* 6493). Several interested parties have suggested that a nutritional labeling scheme based upon Handbook No. 8 provided the most practical basis for solving the many difficult technological problems associated with any nutritional labeling plan. Critics have referred to the compilation as "out of date", and some have suggested that the tabulated values no longer correspond to current food production.

The data obtained in the present survey provide the first opportunity to make valid comparisons between Handbook No. 8 values and average nutrient content figures obtained from representative nationwide sampling efforts.

Table 11 presents the results of this comparison. Nutrients tabulated in USDA Handbook No. 8 are compared on a "one cup" basis with the results of our resurvey. As an index of nutritional significance, the difference between the nutrient value of one-cup portions has also been expressed as percent of the Recommended Dietary Allowance (National Academy of Sciences, 1968) using the 22–35 yr age bracket as the "reference man." One-cup portions probably represent a generous serving in many households but for canned fruits and vegetables provide the most convenient basis for consumer use in making comparison among nutritional values. The last column in Table 11 presents the algebraic difference after subtracting Handbook No. 8 averages from present values.

The data provide for 25 comparisons. In 13 of these the difference represents 1% or less of the RDA. Differences amounting to less than 0.5% of the RDA are listed as 0. In only one instance was the difference as much as 10% of the RDA. The Handbook No. 8 value for ascorbic acid in canned tomato juice is 16 mg per 100g. It is interesting to observe that the mean ascorbic acid value in the earlier National Canners Association survey of 1941–1943 was 14.4 mg per 100g

(Cameron and Esty, 1950). On the basis of a one-cup portion, the difference between the 1941–1943 average and that obtained from the 1969 season represents approximately 4% of the RDA for the reference man. Detailed comparison of our 1941–1943 results on other nutrients reveal differences of the same order of magnitude as those in Table 11, since the original data sources are usually identical.

CONCLUSIONS

AFTER AN INTERVAL of approximately 30 yr, a systematic resurvey of the nutrient content of canned tomato juice and whole kernel corn has been carried out on a nationwide basis with sampling proportioned among the major producing areas in accordance with production statistics. Although there have been extensive changes in varietal type and processing methods during this interval, they have not resulted in a marked change in the nutrient content of these products.

Comparison of the nutrient values listed in Handbook No. 8 for these products reveal no differences that could not be attributed to seasonal variations. A possible exception is the ascorbic acid content of tomato juice, which for the 1969 season was found to be somewhat lower than the listed value. The "resurvey" value, however, was almost identical with the national average obtained in the 1941–1943 NCA–CMI nutrition program.

The sample-to-sample variability in this survey is essentially that among "small lots" of the canned product since each sample consisted of a composite of three or six consumer size containers. In terms of mean values (coefficient of variation) the variability of food energy content and the proximate constitutents tends to be small in comparison with that of the vitamin and some trace minerals. The coefficients of variability for many of the vitamins and minerals are fairly large however, and this variability would be of great significance in any nutrient labeling scheme involving enforcement provisions based upon percent departure from average values.

In the intense discussion of various nutritional labeling schemes, some have overlooked the statistical truism that mean values derived from adequate sampling programs are relatively stable although the population may encompass wide ranges of variability. It is for this reason that nutrient declarations based upon properly constituted average values provide the most practicable solution to the technical difficulties inherent in nutritional labeling.

The findings of the present survey should not discourage further efforts to survey the nutrient content of the current production. Adequate nutritional data are especially needed on many of the new products, especially many of the formulated or specialty items among processed foods.

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COMPOSITION OF THREE FOOD PRODUCTS CONTAINING DEFATTED CORN GERM FLOUR

INTRODUCTION

GERM ACCOUNTS for 10–12% of the total products resulting from dry milling of corn (Wall et al., 1971). Compositional data and nutritional studies on corn germ have been reviewed in detail and summarized by Gardner et al. (1971). Wall et al. (1971) studied the effect of processing on protein efficiency ratios. These investigators showed that corn germ can serve as a good protein source when drying temperatures are minimal and oil is removed by solvent extraction. At present, defatted germ is used almost exclusively as a component in animal feeds (Wall et al., 1971).

Commercial dry-milled corn germ was recently examined as a source of flour for human consumption (Blessin et al., 1972). That work resulted in a solvent-extracted flour containing approximately 25% protein. Taste panel evaluations of cookies, corn muffins and beef patties having various levels of defatted corn germ flour were satisfactory (Blessin et al., 1972). In this paper, we present detailed information on the effect of defatted germ flour on the composition of these three food products.

MATERIALS & METHODS

Materials

The germ fraction used in this study was prepared by dry milling yellow dent corn in a commercial plant. Standard dry-milling techniques were followed during the separation. The germ fraction was dried at the plant to a moisture content of 3% in a commercial dryer operating at an air temperature of 99°C. Slight toasting of the germ occurred during drying. Defatted corn germ flour was prepared by a previously described procedure (Blessin et al., 1972).

Ground beef for the patties was purchased at a local supermarket. Cuts of fat and lean

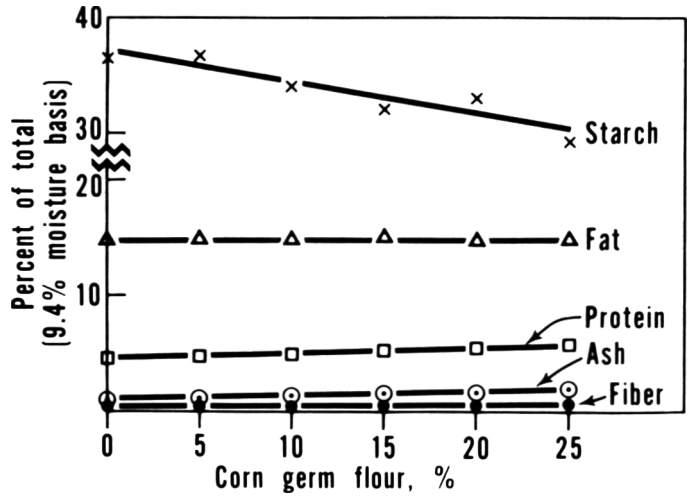

Fig. 1—Starch content and proximate composition of baked cookies containing defatted corn germ flour.

Table 1—Amino acid composition of defatted corn germ flour

Amino acid	g/100g Amino acids
Lysine	5.9
Histidine	3.2
Ammonia	1.7
Arginine	9.4
Aspartic acid	8.8
Threonine	4.1
Serine	5.0
Glutamic acid	15.0
Proline	5.0
Glycine	6.5
Alanine	7.0
Cystine	1.4
Valine	5.6
Methionine	2.1
Isoleucine	3.4
Leucine	7.2
Tyrosine	3.3
Phenylalanine	4.1
Tryptophan	1.2

Table 2—Essential amino acid patterns of defatted corn germ flour and hen's egg proteins

	g amino acid/100g Protein		Mg amino acid/g total essential amino acids	
Amino acid	Germ flour	Hen's egg	Germ flour	Hen's egg
Isoleucine	3.4	6.6	89	129
Leucine	7.2	8.8	188	172
Lysine	5.9	6.4	154	125
Phenylalanine	4.1	5.8	107	114
Tyrosine	3.3	4.2	86	81
Cystine	1.4	2.4	37	46
Methionine	2.1	3.1	55	61
Threonine	4.1	5.1	107	99
Tryptophan	1.2	1.6	31	31
Valine	5.6	7.3	146	141
Total essential amino acids	38.3	51.3		

Table 3–Amino acid composition of baked cookies

Amino acid	Control	5%[a]	10%	15%	20%	25%
	g/100g amino acids					
Lysine	1.4	1.8	2.2	2.4	2.9	3.1
Histidine	1.8	2.1	2.2	2.3	2.3	2.4
Ammonia	3.9	3.8	3.7	3.6	3.4	3.1
Arginine	3.4	3.8	4.3	4.6	5.0	5.4
Aspartic acid	3.9	4.2	4.4	4.9	5.5	5.7
Threonine	2.5	2.6	2.7	2.9	3.1	3.2
Serine	4.6	4.5	4.6	4.8	4.8	4.9
Glutamic acid	34.6	33.3	32.1	30.4	29.6	27.8
Proline	11.8	11.0	10.0	9.3	9.0	8.7
Glycine	3.4	3.5	3.7	3.9	4.3	4.6
Alanine	2.8	3.1	3.6	3.9	4.2	4.3
Cystine	1.4	1.4	1.7	1.6	1.8	1.7
Valine	4.3	4.2	4.4	4.6	4.8	4.9
Methionine	1.8	1.9	1.5	1.8	1.5	2.0
Isoleucine	3.6	3.5	3.6	3.9	3.7	3.7
Leucine	6.6	6.8	6.6	7.0	7.1	7.2
Tyrosine	3.2	3.1	3.2	2.9	2.6	2.6
Phenylalanine	4.5	4.5	4.6	4.1	3.5	3.7
Tryptophan	0.5	0.7	0.7	0.8	1.0	1.1

[a]Percentages of defatted corn germ flour in the total flour fraction

Table 5–Amino acid composition of baked muffins

Amino acid	Control	5%[a]	10%	15%	20%	25%
	g/100g amino acids					
Lysine	3.5	3.6	3.7	3.8	3.9	4.0
Histidine	2.2	2.4	2.4	2.4	2.4	2.5
Ammonia	2.6	2.6	2.5	2.7	2.6	2.7
Arginine	4.0	4.0	4.3	4.5	4.6	4.5
Aspartic acid	5.7	6.1	6.2	6.4	6.6	6.6
Threonine	3.3	3.4	3.4	3.4	3.4	3.4
Serine	5.2	5.1	5.2	5.1	5.1	5.2
Glutamic acid	23.8	23.6	22.7	22.5	22.3	21.7
Proline	9.4	9.3	8.6	8.3	8.1	8.0
Glycine	3.0	3.1	3.2	3.4	3.3	3.4
Alamine	4.9	5.0	5.0	5.1	5.2	5.3
Cystine	1.5	1.3	1.7	1.6	1.4	1.5
Valine	5.0	5.0	5.0	5.1	5.1	5.2
Methionine	2.3	2.4	2.6	2.6	2.4	2.2
Isoleucine	4.0	3.7	3.9	4.0	3.9	4.0
Leucine	9.5	9.5	9.6	9.8	9.6	9.7
Tyrosine	4.3	4.1	4.3	3.9	4.2	4.2
Phenylalanine	5.0	4.8	4.7	4.5	4.7	4.7
Tryptophan	0.9	1.0	1.0	1.0	1.0	1.0

[a]Percentages of defatted corn germ flour in the total flour fraction

beef, from a side which graded USDA Choice, were ground twice in a commercial meat grinder. Beef was selected so that the final product contained approximately 20% fat.

Materials needed to make the cookies and muffins followed specifications listed in the formula or recipe.

Methods

Cookies were prepared according to Method 10-50 (AACC, 1962). Percentages of defatted germ flour in the total flour fraction ranged from 0–25% in 5% increments.

Muffins were prepared by a previously described yellow corn bread recipe (Blessin et al., 1972) obtained from The Quaker Oats Company, Chicago, IL 60654. Defatted germ flour was blended at 0, 5, 10, 15, 20 and 25% levels with all-purpose wheat flour in this recipe.

Defatted germ flour was added to ground beef as 1, 3, 5 and 10% of the total uncooked weight. The flour was thoroughly mixed with ground beef by hand kneading in a plastic bag. Uniform thickness was assured by rolling the mixture with a rolling pin between stainless steel strips 12-mm high and 37.5-cm long. Patties were cut with a stainless steel mold 10 cm in diameter. A conventional electric oven with an overhead element was preheated to maximum temperature before broiling the patties for 3 min; then they were turned over and broiled for 4 min. Weights were recorded before and after broiling. No salt was added to either the uncooked or broiled patties.

Samples of cookies, muffins and beef patties were sealed in plastic and stored in a freezer at −29°C until analyzed. Cookies and muffins were ground with a mortar and pestle to pass a 40-mesh screen. Beef patties were dried in an oven at 105°C for 113 hr to determine moisture and to modify the sample to a more suitable form. After removal of water, the residual meat product was broken into small pieces with a mortar and pestle. The ground cookies and muffins and the small pieces of beef patties were then equilibrated at room temperature and humidity. Samples were removed for the determination of fat, ash, starch and mineral constituents. The remaining portions of the foods were defatted with hexane. The defatted meat product was then ground to pass a 40-mesh screen. After defatted samples were equilibrated at room temperature and humidity, fiber, protein and amino acids were determined.

Moisture content of equilibrated samples was determined by drying in an oven for 2 hr at 130°C. For fat content, cookies and muffins were extracted for 7 hr, and the residual meat product for 17 hr, with hexane in a Soxhlet extraction apparatus. Ash was determined by ignition in a platinum dish at 575°C for at least 16 hr. Starch was measured by a polarimetric method with 90% dimethyl sulfoxide (DMSO) as the starch solvent (Garcia and Wolf, 1972). A 1-g sample of cookie or muffin was ground in 90% DMSO. The extracted starch was precipitated and then separated from optically active constituents. The precipitated starch was redissolved in 90% DMSO before measurement of the starch by optical rotation (Garcia et al., 1972b). Amylose content of the starch was measured to verify that observed optical rotation was due to starch. An approximate 3-g sample was wet-ashed with concentrated nitric acid and redissolved in 4% HCl. From this solution K, Mg, Ca, Na and Fe were determined by atomic absorption techniques (Garcia et al., 1972a). A colorimetric molybdenum procedure was followed for phosphorus (AACC, 1957). Fiber and protein were measured by standard methods (AACC, 1962).

Amino acid analyses, other than tryptophan, were carried out on hydrolysates obtained by refluxing 100-mg samples of defatted material in 250 ml of constant boiling hydrochloric acid for 24 hr under nitrogen atmosphere. Norleucine was added as an internal standard to each sample before hydrolysis. Amino acids were quantitatively determined with a Phoenix automatic amino acid analyzer (Model K-8000) by the Benson and Patterson (1965) accelerated procedure. Amino acid concentrations were calculated according to a computer technique developed by Cavins and Friedman (1968). Tryptophan was determined with the Phoenix analyzer by the $Ba(OH)_2$ hydrolysis procedure described by Knox et al

Table 4–Mineral composition of baked cookies

Cookie[b]	Mineral constituent[a]					
	Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Sodium (%)	Iron (ppm)
0 (control)	0.05	0.05	0.012	0.011	0.39	21
5	0.12	0.11	0.035	0.012	0.45	27
10	0.18	0.18	0.056	0.011	0.37	29
15	0.22	0.22	0.078	0.011	0.38	31
20	0.29	0.31	0.108	0.012	0.41	33
25	0.33	0.36	0.117	0.011	0.42	38

[a]All values reported on a 9.4% moisture basis.

[b]Percentages of defatted corn germ flour in the total flour fraction

Table 6—Mineral composition of baked muffins

Muffin[b]	Mineral constituent[a] Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Sodium (%)	Iron (ppm)
0 (control)	0.23	0.16	0.021	0.22	0.68	53
5	0.24	0.20	0.033	0.24	0.70	54
10	0.27	0.22	0.042	0.23	0.86	55
15	0.29	0.24	0.053	0.18	0.60	57
20	0.31	0.27	0.061	0.19	0.65	59
25	0.33	0.28	0.074	0.19	0.57	60

[a]All values reported on a 27.4% moisture basis

[b]Percentages of defatted corn germ flour in the total flour fraction

(1970). Compositional data are reported at mean moisture levels indicated in the various figures and tables.

RESULTS & DISCUSSION

Defatted germ flour

The commercial corn germ fraction, as received from the mill, contained approximately 18% protein, 23% fat, 3% fiber and 8% ash. From this fraction we prepared a defatted germ flour in 46% yield, which contained approximately 25% protein, 0.5% fat, 4% fiber and 10% ash. Additional compositional data on defatted germ flour have been published (Blessin et al., 1972). Distribution of amino acids in defatted germ flour is given in Table 1. Lysine level in defatted germ flour is higher than in any other milled fraction of corn (Inglett, 1970). Levels of the essential amino acids in defatted germ flour protein are compared to those in hen's egg protein in Table 2. On the basis of grams of amino acid per 100g of protein, defatted germ flour is lower than hen's egg in all essential amino acids but higher than the FAO provisional pattern (1965). The amino acid pattern of defatted germ flour protein compares more favorably with hen's egg protein when individual amino acids are reported as a portion of the total essential amino acids. Defatted germ flour is higher in lysine and somewhat lower in isoleucine and the sulfur amino acids. Since the level of sulfur amino acids in hen's egg protein is considered high (NRC, 1963), the lower values in defatted corn germ flour are not necessarily undesirable.

We developed data on the composition of cookies, muffins and beef patties containing various levels of defatted germ flour.

Cookies

Adding 25% defatted germ flour to wheat flour reduced starch content of baked cookies from 36 to 29% (Fig. 1). Fat content remained constant while ash content doubled. Fiber levels increased slightly. Although protein content increased only from 4.5 to 5.7%, composition of amino acids was altered significantly (Table 3). For example, in the control cookie, lysine accounted for 1.4% of the protein compared to 3.1% in the 25% defatted germ flour cookie. Tryptophan content of the protein doubled (from 0.5 to 1.1%) at the same time. Glutamic acid and proline values showed the most pronounced decreases. Since the control cookie was composed chiefly of flour, sugar and shortening, levels of mineral constituents, with the exception of sodium (due to added salt), were low (Table 4). Incorporation of 25% of defatted germ flour increased phosphorus and potassium levels approximately seven times and magnesium 10 times. Values for calcium and sodium did not change. Iron content was essentially doubled by increasing from 21 to 38 ppm.

Muffins

Starch content in muffins baked with 25% defatted germ flour was reduced by about 2% (from 41 to 39%) (Fig. 2). Both fat and protein content increased by 1%. Ash and fiber values remained essentially the same at all addition levels. Amino acid composition of the protein in all the muffins (Table 5) was similar although glutamic acid and proline accounted for less of the protein as the level of defatted corn germ flour increased. Baked muffins, formulated with baking powder, eggs and milk, were higher in mineral constituents (Table 6) than the cookies. Increasing levels of defatted germ flour increased phosphorus, potassium, magnesium and iron. However, the increases were of less magnitude than in the cookies. Calcium and sodium levels did not change.

Beef patties

Yield and proximate composition of broiled meat products changed as level of defatted germ flour increased (Fig. 3).

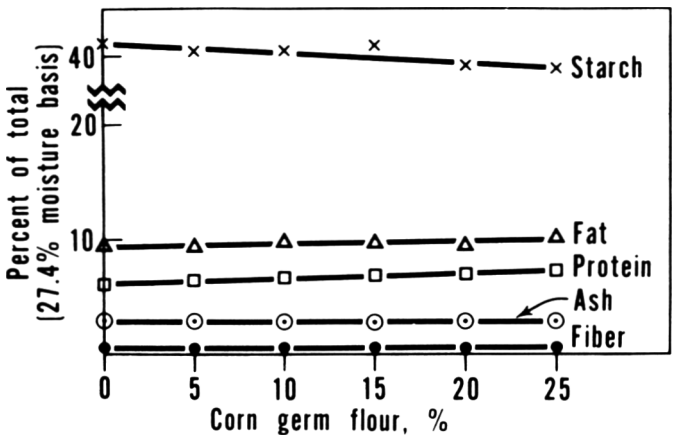

Fig. 2—Starch content and proximate composition of baked muffins containing defatted corn germ flour.

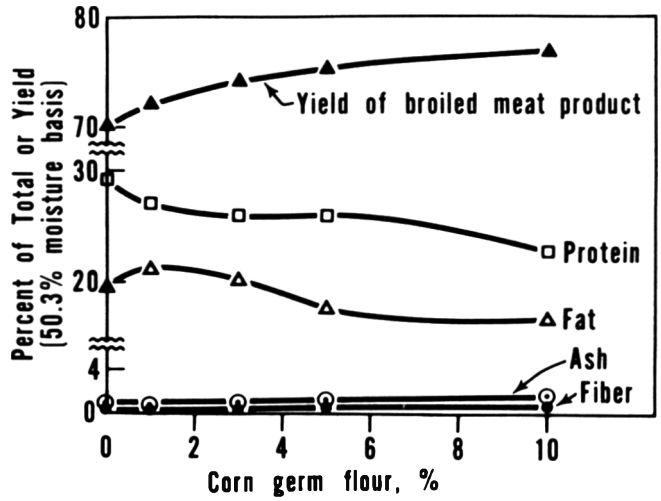

Fig. 3—Yield and proximate composition of broiled beef patties containing defatted corn germ flour.

Table 7—Yield and composition of broiled meat products related to uncooked material

Beef patty[a]	Broiled meat product[b] Weight (g)	Protein (g)	Fat (g)	Ash (g)	Fiber (g)
0 (control)	70.0	20.4	13.6	0.6	0.1
1	72.2	19.5	15.2	0.5	0.1
3	74.2	19.2	14.8	0.7	0.3
5	75.2	19.5	13.2	0.9	0.5
10	77.1	17.5	12.8	1.2	0.5

[a]Percent, by weight, of defatted corn germ flour added to uncooked beef
[b]Based on 100g uncooked material; data reported at 50.3% moisture

Table 8—Amino acid composition of broiled beef patties

Amino acid	Control	1%[a]	3%	5%	10%
	g/100g amino acids				
Lysine	8.3	8.2	8.0	7.9	7.5
Histidine	3.1	3.1	3.1	3.1	3.1
Ammonia	1.1	1.2	1.3	1.2	1.4
Arginine	6.6	6.7	6.6	6.6	6.5
Aspartic acid	8.7	8.7	8.7	8.8	8.8
Threonine	4.3	4.4	4.4	4.4	4.4
Serine	3.9	4.0	4.0	4.0	4.1
Glutamic acid	16.0	16.1	15.8	16.0	16.0
Proline	5.0	4.8	4.8	4.8	4.6
Glycine	6.5	6.6	6.4	6.4	6.4
Alanine	6.4	6.4	6.3	6.4	6.5
Cystine	0.9	1.0	1.0	1.1	1.2
Valine	5.0	5.0	5.0	5.1	5.2
Methionine	2.6	2.7	2.8	2.7	2.7
Isoleucine	4.7	4.8	4.7	4.7	4.6
Leucine	8.1	8.1	8.3	8.2	8.0
Tyrosine	3.6	3.3	3.5	3.4	3.7
Phenylalanine	4.2	4.0	4.4	4.1	4.3
Tryptophan	1.0	0.9	0.9	1.0	1.0

[a]Percent, by weight, of defatted corn germ flour added to uncooked beef

Table 9—Mineral composition of broiled beef patties

Beef patty[b]	Mineral constituent[a] Phosphorus (%)	Potassium (%)	Magnesium (%)	Calcium (%)	Sodium (%)	Iron (ppm)
0 (control)	0.25	0.39	0.024	0.009	0.16	35
1	0.26	0.41	0.038	0.008	0.16	35
3	0.33	0.50	0.063	0.007	0.15	41
5	0.41	0.60	0.097	0.008	0.18	51
10	0.52	0.75	0.161	0.007	0.13	56

[a]All values reported on a 50.3% moisture basis
[b]Percent, by weight, of defatted corn germ flour added to uncooked beef

Moisture contents of broiled products ranged from 49.6–51.0% with no discernible trend related to levels of defatted germ flour. Therefore, yield and compositional data were calculated at the mean moisture content of 50.3%. Yield increased from 70% in the control patty to 77% at the 10% defatted germ flour level. Protein and fat decreased, but ash and fiber increased. Addition of up to 5% defatted germ flour maintained acceptable levels of protein, fat, ash and fiber.

Data in Figure 3 show that protein and fat content were reduced when defatted germ flour was added to ground beef. The decreases are due to a greater yield of broiled meat product and a lower protein level in defatted germ flour rather than to an actual loss. This relationship is more readily seen when compositions of the meat products are compared on the basis of 100g of uncooked material (Table 7). The slight decrease in protein recovery and increase in ash and fiber were due to differences in composition of the defatted germ flour and ground beef. Recovery of fat was about the same for all meat products.

In general, amino acid composition of the protein in beef patties (Table 8) was not greatly altered by the addition of defatted germ flour at the levels studied.

Addition of 10% defatted germ flour to beef patties produced approximately a twofold increase in phosphorus and potassium and a sevenfold increase in magnesium when compared to the control (Table 9). As with cookies and muffins, calcium and sodium remained essentially the same. At the same time, iron increased from 35 to 56 ppm.

Compositional data reported here combined with that reported previously on cooking and baking characteristics and taste panel evaluations (Blessin et al., 1972), show that defatted corn germ flour has possibilities of serving as a protein and mineral supplement in a variety of foods.

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STUDIES ON THE UTILIZATION OF COCONUT MEAL
A New Enzymic-Chemical Method for Fiber Free Protein Extraction of Defatted Coconut Flour

INTRODUCTION

THE COCONUT PALM (*Cocos nucifera*) is indigenous or cultivated in nearly all tropical countries. In many countries of the world currently suffering from protein shortage coconuts are a major crop.

Coconut is economically and industrially most important for coconut oil production from the dried kernel by means of an expeller or hydraulic press or through pre-press solvent extraction (Woodroof, 1970).

Coconut meal (defatted coconut flour) has been studied for several years as another possible food grade protein source. The use of coconut meal as a sole or major protein source, however, has been limited by two major factors: (1) the heat treatment to which the copra is subjected either before or during the oil extraction reportedly lowers the nutritive value because the coconut proteins are sensitive to heat damage (Better and Davidsohn, 1958; Butterworth and Fox, 1963); (2) the high fiber content of the coconut meal interferes with the digestibility of the meal and thus the retention of nitrogen (Rama Rao et al., 1965) as well as limiting the use of the meal in diet formulations to relatively low levels (Daniel et al., 1968).

On the other hand, coconut proteins have been shown to have a fairly high nutritive value (Krishnamurthy et al., 1958; Loo, 1968) and to have a relatively favorable amino acid profile (Srinivasan et al., 1964).

Several methods for coconut oil extraction have been devised using mild temperatures. Some of the methods (wet processes) offer the possibility of extracting the soluble coconut proteins in the same operation (Rajasekharan, 1967); however, they have been criticized on the basis of being relatively uneconomical and that the soluble protein fraction does not have as high a nutritive value as the total coconut meal proteins (PAG, 1962).

Another approach has been to extract the protein from previously defatted coconut flour thus considerably lowering the fiber content. Several methods of protein extraction applicable to coconut meal have been reported using either a chemical process (such as NaOH, HCl, $CaCl_2$, NaCl) at different temperatures and concentrations (Chelliah and Baptist, 1969; Samson et al., 1971), or enzymatic treatments ranging from crude enzymes prepared by growing fungi in the original meal and later precipitating the enzymes in the filtrate of the harvested mycelium (Chandrasekaran and King, 1967) to commercial enzymes such as the cellulase preparation "Meicelase-P" (Rama Rao, 1969).

The objective of the present work was to devise a simple, low-cost, method for the protein extraction of commercial coconut meal—when the protein has, generally, undergone partial denaturation—which would be equally effective when applied to at least five coconut meal samples obtained through different commercial oil extraction techniques with the final intent of obtaining a product with a reduced fiber content, higher protein nutritive value and more desirable functional properties than the original coconut meal.

EXPERIMENTAL

Coconut meal samples

Five coconut meal samples obtained through different oil extraction techniques were used in the laboratory experiments.

Sample 1 was from solvent extracted expeller pre-pressed cake, nonpared. Sample 2 was from an expeller full pressed cake, nonpared. Samples 3 and 4 were direct solvent extracted, the former being pared while the latter was nonpared.

The material used for the production of the above four coconut meal samples was prepared from fresh ground coconut kernel immersed in coconut oil and dried under vacuum (29 in. Hg) at 55°C for several hours. The dehydrated ground coconut was then separated from the oil by centrifugation, resuspended in fresh coconut oil (containing some tenox 2 as an antioxidant), heated to 82°C for 15 min to insure sterilization and recentrifuged to remove the excess oil.

The above four coconut meal samples were prepared under the above conditions by the V.D. Anderson Co., Cleveland, Ohio (private communication).

Coconut meal sample 5 obtained from Heyman Process Corporation (Long Island City, N.Y.) was prepared by a method devised by the supplier (Heyman, 1968). No information was given as to the temperature to which the sample was subjected either before or during the oil extraction. Due to its availability this coconut meal (a large lot) was used for the pilot-plant protein extraction experiment reported here and the biological evaluation of the coconut meal proteins (Lachance and Molina, 1973).

Protein extraction

Chemical method. All coconut meal samples were subjected to a chemical protein extraction treatment similar to that proposed by Chelliah and Baptist (1969). The samples were subjected to a water treatment (using a 1:25 meal to water ratio) for 3 hr at 40 ± 1°C followed by a centrifugation step (1100 × G for 30 min) and resuspension of the residue in 25 parts (based on the original meal weight) of a 0.15% (wt/vol) HCl solution (mixture pH 2.04) for an acid treatment of 4 hr at 60°C after which the suspension was centrifuged (1100 × G for 30 min) and the residue discarded.

Enzymatic method. Five different technical grade, commercial proteases commonly used in the food industry: papain, bromelain, ficin (Nutritional Biochemicals Corp., Cleveland, Ohio), fungal prolase MT-7820 and bacterial prolase EB-21 (Walerstein Co., Morton Grove, Ill.) and two commercial carbohydrases: cellulase 4000 and hemicellulase Ce-100 (Takamine brand from Miles Laboratories, Inc., Elkhart, Ind.) were evaluated for the enzymatic protein extraction of coconut meal.

The optimum pH and temperature for maximum protein extractability were determined on the residue from a water treatment (1:25 meal to water ratio, at 40 ± 1°C for 3 hr with continuous shaking) of coconut meal sample 5 resuspended in 25 parts of distilled water (based on the original meal weight) using a fixed time (5 hr) and a fixed enzyme concentration (1% based on the original meal weight). The pH was adjusted with HCl or NaOH respectively. No buffers were used since it has been shown to be unnecessary in materials similar to coconut meal subjected to analogous enzymatic treatments (Sreekantiah et al., 1969). The carbohydrase action was evaluated by both protein extraction and reducing sugar production under the different pHs and temperatures tested.

The minimum enzyme concentration and minimum time of treatment required for maximum protein extractability were studied under the determined optimum pH and temperature of each enzyme.

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All centrifugations were done at 1100 × G for 30 min. Blanks containing boiled enzyme were examined at different pHs to determine the efficiency of the enzyme action.

Enzymic-chemical method. The enzymic-chemical method for the protein extraction of coconut meal as developed in the laboratory consisted of: (1) a standard water treatment (1:25 meal to water ratio at 40°C for 3 hr under continuous reciprocal agitation of 120 strokes per minute) followed by (2) an enzymatic (ficin) treatment of the water residue (under the optimum conditions determined for the enzyme) and finally (3) a complementary chemical treatment (at the minimum time, concentration and temperature determined for the maximum protein extractability by the dilute NaOH solution) of the enzyme treatment residue.

The pilot-plant scale experiment differed from the above laboratory scheme in that the original water treatment was eliminated and the meal to solvent ratio for both, the enzymatic and complementary chemical treatments, was increased up to the point where the fluidity of the suspension still was acceptable for the pumping and centrifuging operations (1:12.5 for the initial ficin treatment and 1:8.75 for the complementary alkaline treatment).

The enzymatic and complementary chemical treatments were carried out in a Lee 40 gal jacketed kettle with continuous agitation (at the max speed of 65 rpm) at the determined optimum conditions for each treatment.

After each treatment the suspension was pumped through a Waukesha IODO vari-speed pump to the Sharples horizontal centrifuge (Model P-600) in which the centrifugation steps were carried out. The chemical extract was neutralized (using HCl) after centrifuging.

Both the enzymic and the neutralized chemical extracts were combined and concentrated to 15–20% total solids in an Anhydro Laboratory vacuum evaporator (Model Lab-E with an evaporating capacity of 100 lb of water per hour) using a jacket temperature of 65–75°C and a vacuum of 28 in. Hg.

The concentrated coconut protein extract was then frozen (8 lb of concentrate per tray) for 2 days at −23°C and subsequently, freeze dried for 2 days in a Stokes Freeze drier (Model 902-020401) without the application of any internal chamber heat. The chamber temperature never exceeded 37°C and the whole operation was carried out at a pressure of 0.2 mm Hg.

Chemical analysis

Moisture, nitrogen (either macro or micro Kjeldahl), ash, oil (ether extract), crude fiber and total solids (in the case of the extracts) were determined in duplicate according to the AOAC (1965).

Protein estimations were carried out either by multiplying the nitrogen content of the samples by the customary conversion factor 6.25 or by the biuret (either macro or micro) colormetric method as described by Bailey (1967). [Although the USDA recommended conversion factor for coconut protein is 5.30 (Jones, 1931), here the factor used was 6.25 since the former was determined using only the coconut globulin and Samson et al., (1971) have shown that this protein fraction represents 61.9% of the total protein and further, these authors used the 6.25 factor.]

Total sugars were determined by the phenosulfuric acid method as described by Birch (1963). Total reducing sugars were determined by the neocuproine-HCl method as developed by Dygerts et al., (1965) and the results were expressed as glucose.

Table 1—Percent composition of defatted coconut flours (Dry basis)

Flour	Protein (N × 6.25)	Ash	Crude fiber	Carbohydrates[a]	Ether ext
1	20.91	6.40	9.65	62.90	0.14
2	21.01	5.55	13.45	53.96	6.03
3	22.10	5.74	11.50	60.30	0.36
4	20.95	5.23	13.38	56.29	4.15
5	21.90	5.05	11.85	47.35	13.85

[a]Carbohydrates by difference

RESULTS & DISCUSSION

THE PROXIMATE COMPOSITION of the five coconut meal samples used in the protein extraction experiments is given in Table 1.

Whereas the coconut meal samples contained similar amounts of protein (N × 6.25), ash, crude fiber and carbohydrates, the greatest variation was in their residual oil content. This variation offered the opportunity to study the effect of residual oil content on the protein extraction yields by the methods tested.

The protein extraction yields obtained when the coconut meal samples were subjected to the chemical extraction treatment were independent of their residual oil content. Contrary to the findings of Chelliah and Baptist (1969) a higher residual oil content did not correlate with higher protein extractability. Coconut meal sample 5 showed a much lower extractable protein figure (37%) than coconut meal samples 3 (67%) and 4 (69%).

On the other hand, the fact that the direct solvent extracted coconut meals (samples 3 and 4) showed a higher protein extractability than the expeller treated samples 1 (35%) and 2 (33%) and the sample (5) obtained by the Heyman Process (37%) suggests, again contrary to the findings of Chelliah and Baptist (1969), that the protein extractability of coconut meal by this chemical method is favored when the substrate has undergone solvent oil extraction. This phenomenon may be attributable to a lower temperature treatment during the oil extraction by the solvent method since Butterworth and Fox (1963) have clearly shown that the solubility of the coconut meal proteins, either in 6N NCl, 0.5M HCl, 0.5M NaCl or 0.02M NaOH, is considerably decreased as the temperature treatment of the material is increased. Similar results have been reported by Samson et al., (1971) who found that the coconut meal protein solubility in dilute acid, alkali or salt solutions was considerably lower when using a meal prepared from commercially desiccated coconut than when a carefully prepared (under controlled laboratory conditions) meal was used.

Such results clearly indicate that the use of a carefully (low temperature) prepared coconut meal is necessary in order to assure a high degree of protein extractability by a chemical method. Further, this factor could be considered as a major limitation to the possible commercial application of such a process to the currently produced coconut meals since, according to Woodroof (1970) they are obtained mainly through dry oil extraction techniques (e.g., expeller), where the protein has generally undergone partial denaturation.

Due to the inconsistency found in the chemical method when applied to all five of the commercial coconut meal samples, it was decided to examine the effect of enzymes for protein extraction.

Both proteases and carbohydrases were evaluated since the use of either of them has been reported to successfully extract protein from coconut meal and related materials (Rama Rao, 1969; Sreekantiah et al., 1969).

The maximum percentage of extracted protein obtained with each enzyme at its determined optimum pH and temperature is given in Table 2.

From these results it can be observed that, in general, under optimum pH and temperature the proteases gave higher extractable protein yields than the carbohydrases, thus indicating that the former rather than the latter are more efficient in

Table 2—Optimum conditions for enzymatic extraction as determined on the water extract residue

Enzymes[a]	pH	Temp °C	% Protein ext
Papain	7.5	50	25.8
Bromelain	6.5	50	35.2
Fungal prolase	5.0	50	27.7
Bact. prolase	7.0	50	34.4
Ficin	5.8	50	41.8
Cellulase	4.8	50	16.5
Hemicellulase	4.8	60	12.1

[a]Concentration 1%, time 5 hr

effecting protein extraction from coconut meal. No pH drift was observed during any of the enzymatic treatments.

Of the proteases evaluated ficin gave at all times tested (up to 6 hr), the best protein extractability. Based on these results ficin was chosen for all further studies.

Based on the original coconut meal weight, 0.5% ficin is the minimum concentration capable of providing maximum protein extractability in the relatively short treatment time of 2 to 2-1/2 hr.

Therefore, the conditions for the protein extraction of coconut meal using ficin were standardized as follows: 0.5% enzyme concentration, 2-1/2 hr of treatment and 50°C using the aqueous suspension of the water treatment residue without any pH adjustment.

The combined addition of ethylene diamine tetracetate (EDTA) and cysteine (0.001M and 0.005M respectively, in the suspension) did not increase the protein extraction yield although both reagents (at similar concentrations) have been reported to activate the enzyme (Glazer and Smith, 1971).

A 6 hr cellulase treatment (at its determined optimum pH and temperature and using an enzyme concentration of 1%) of the water treatment residue prior to the ficin treatment was found not to increase the protein extractability. These results indicate the limited ability of the carbohydrase not only to effect the protein extraction but, to facilitate the protein extractability by the proteolytic (ficin) treatment as well.

The protein material obtained through the ficin treatment proved to be partly heat coagulable and showed considerable precipitation when the pH of the extract was adjusted from 4.1 to 4.5.

Since by the water and ficin treatments only 60% of the original coconut meal protein was extracted (18% by the water treatment and 42% by the ficin treatment, as estimated by the biuret colorimetric determinations on each extract) it was decided to study the possibility of increasing the total protein extraction yield by using an enzymic-chemical extraction method.

When an acid or alkali treatment at 60°C for a time period up to 3 hr was used, the protein extraction yield obtained was consistently higher with the 0.075% NaOH solution (pH of mixture 9.8), than with the 0.15% (wt/vol) HCl solution (pH of mixture 2.04). These results are in accordance with those reported by Chelliah and Baptist (1969), who found a higher coconut meal protein extractability with dilute alkali than with dilute acid solutions, under similar conditions. Treatment for 1 hr under such conditions with the 0.075% NaOH solution was found sufficient to obtain maximum protein extractability.

Increasing the NaOH concentration up to 0.3% (mixture pH 10.6) did not increase the yield when the treatment was carried out under the same conditions for 1 hr although decreasing the NaOH concentration resulted in a drop in the protein extraction.

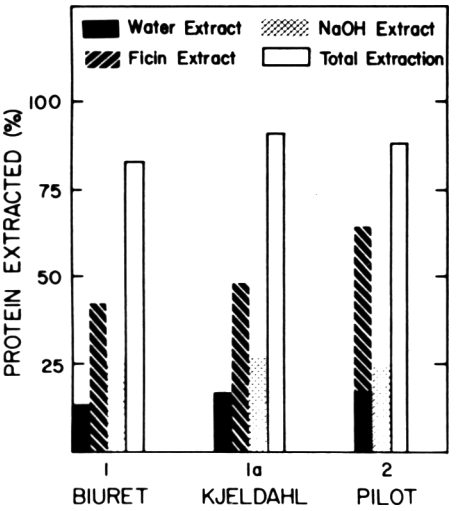

Fig. 1—Percentage protein extracted under original laboratory (1 and 1a) and modified pilot-plant (2) conditions.

Temperature during extraction was found to be an important factor since yields dropped considerably when the temperature was reduced (20° and 40°C); however, increasing the temperature to 70°C did not enhance protein extraction.

Similar temperature effects for the protein extraction of coconut meal had been reported by Chelliah and Baptist (1969) and Samson et al., (1971).

The slight yellow-brownish color which developed in the extract during the NaOH (0.075%) treatment for 1 hr at 60°C was easily reversed by neutralization, thus indicating that it is probably due to some flavone type of compound since such pigments are known to develop a yellow-brownish color under alkali conditions.

When the residue from the water treatment was further treated directly with 25 parts (based on the original meal weight) of 0.075% NaOH for 1 hr at 60°C, the protein extraction yield obtained (1.8 mg of protein per ml of extract) was relatively lower than that obtained when the same alkali treatment was carried out on the residue after the ficin extraction (2.2 mg of protein per ml of extract). This suggests that the protein extractability by the alkali treatment is being favored by the hydrolysis of the protein material occurring during the enzymatic treatment. If such is the case then it would be safe to assume that there is no need for the use of purified proteases to enhance the protein extractability by the enzymatic treatment (as suggested by Sreekantiah et al., 1969) since technical grade, commercial proteases effect the necessary hydrolysis of the protein material allowing the relatively easy solubilization of the remaining (possibly nondenatured) proteinaceous material through a complementary short-time alkali treatment.

The protein yield from this enzymic-chemical process (using coconut meal sample 5) expressed as percentage protein extracted was determined on the extracts by both the biuret colorimetric method and the Kjeldahl (N x 6.25) method, and the results are shown in Figure 1. The data indicate that the laboratory method was 91% efficient.

Evaluation of the enzymic-chemical method on coconut meal samples 1 to 4 resulted in similar protein extraction yields (81–86% as determined by the biuret colorimetric method), thus showing that the new enzymic-chemical method was generally applicable, under the same conditions, to the five different coconut meal samples tested.

In order to execute a pilot-plant scale experiment two modifications of the original laboratory process were deemed necessary because: (1) the total solids content of each of the extracts was too low to optimize drying of the combined extracts. The water extract had 1.4% total solids, the ficin extract 0.63% total solids and the NaOH extract 0.42% total solids; (2) the ficin treatment is carried out in an aqueous medium and so the possibility of eliminating the initial water extraction step without sacrificing protein extraction and yield was deemed possible.

In view of the above, the original laboratory method was modified as follows: The meal-to-water ratio was increased to 2:25 for a combined, one step, water-ficin treatment (2-1/2 hr, 50°C with an enzyme concentration of 0.5%). A higher meal-to-water ratio was found impossible since when a 3:25 ratio was used the fluidity of the suspension was lost thus making impossible the pumping and centrifuging operations. The total solids obtained under these new conditions were increased to 4%. The protein extraction yield was unaffected by these changes as shown in Figure 1.

The residue from the above treatment was then resuspended in the highest possible proportion of residue to NaOH (0.075%) solution which would flow relatively easy. A minimum of 17.5 parts of NaOH (0.075) solution (based on the original meal weight) was found necessary.

As can be seen in Figure 1, the protein yield of the alkali treatment under these new conditions, did not vary significantly from that obtained previously when 25 parts (based on the original meal weight) of the 0.075% NaOH solution were used to resuspend the ficin residue of 1 part of

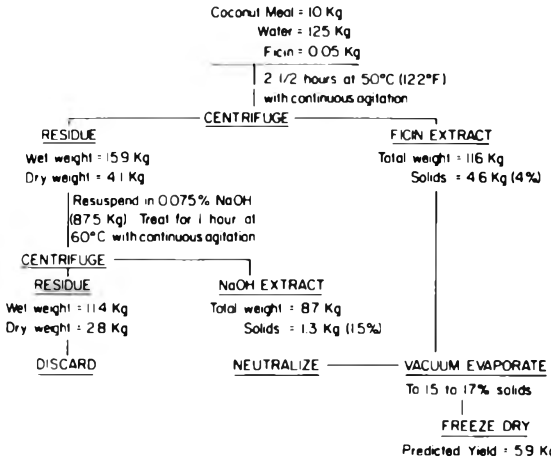

Fig. 2—Principal pilot-plant operations and material balance data obtained under the modified pilot-plant conditions.

Table 3—Percent composition of extract and residue

	Protein (N × 6.25)	Ash	Crude fiber	Carbohydrates[a]	Ether ext
Extract	31.80	8.62	0	43.73	15.85
Residue	2.38	16.21	43.60	37.76	0.05

[a]Carbohydrates by difference

the original coconut meal. The total solids, on the other hand, under the new conditions were 1.5%.

The principal pilot-plant operations utilized are outlined in Figure 2. Before being combined with the ficin extract for the vacuum evaporation step, the pH of the NaOH extract (pH 9.8) was adjusted to 6.85–6.9 with HCl. Vacuum evaporation increased the total solids to a range of 15–17%. The concentrate was then frozen and freeze dried.

The total solids recovered in the actual pilot-plant experiment (55%) were slightly lower than those predicted (59%) from laboratory experiments carried out under the pilot-plant conditions of the extraction. On the actual pilot-plant experiment the total extracted protein yield was estimated at 83% by the Kjeldahl method (N × 6.25).

Although the combined extracts were freeze dried in order to obtain a maximum amount of product, it should be pointed out that in the expert opinion of the pilot-plant personnel of T.J. Lipton Co. (Englewood Cliffs, N.J.) where the pilot-plant experiment was conducted, the concentrate of the combined extracts is considered a perfectly suitable material to be dried by other methods (e.g., spray drying).

The proximate composition of the freeze-dried extract and that of the final residue are given in Table 3.

It is significant that the fiber content of the final product as extracted was reduced to zero by the process which was the original objective. The residual oil content of the defatted meal appears to remain in the extract. In this respect, the oil (or ether extract) content of the extract would appear to be controlled only by the amount of the residual oil content of the meal itself thus making it possible to adjust it to a predetermined value by using a coconut meal containing the desired amount of residual oil.

The total protein content (N × 6.25) of the extract (31.8%) appears to be considerably higher than that of the original coconut meal sample 5 (21.9%) used in this extraction.

Among possible technological modifications to the protein extraction process described here, are: (1) the possibility of effecting the chemical extraction in the same suspension where the enzymatic treatment has been made thus avoiding the intermediate centrifugation step; and (2) the possibility of effecting the whole protein extraction using suitable equipment that would allow for the use of a higher meal-to-water ratio, thus eliminating the need of an evaporation step prior to drying.

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ISOLATION AND CHARACTERIZATION OF MICROORGANISMS INVOLVED IN THE FERMENTATION OF TRINIDAD'S CACAO BEANS

INTRODUCTION

THE FLAVOR AND AROMA of cocoa and chocolate depends greatly upon the manner in which the cacao beans have been fermented. Absence of the desired chocolate flavor from unfermented and slightly fermented beans has been realized by chocolate manufacturers.

Much has been published on the chemical and physical changes taking place in cacao beans during the process of fermentation (Forsyth and Quesnel, 1963; Rohan, 1963). As to the significance and role of microorganisms in this process, the majority of work has been carried out on the isolation and identification of yeasts during the early stages of fermentation (Knapp, 1937; Chatt, 1953). However, no real attempt thus far has been made to isolate and identify other microorganisms associated with this most important process and their major sources, creating a need for a comprehensive qualitative and quantitative investigation on the microflora involved in cacao bean fermentation.

Among the countries producing well fermented cacao beans, Trinidad has gained a worldwide reputation for its high quality and strong chocolate-flavored beans. Due to the availability of good facilities at the Cocoa Research Dept., University of the West Indies, Trinidad, it was therefore chosen as the site of this investigation. The purpose of this study was to isolate, identify and characterize the microflora of cacao beans before, during and after the process of fermentation at two commercial plantations. The major sources of microbial contamination of the beans were also investigated.

EXPERIMENTAL

Method of fermentation

Centeno Estate. Beans at this plantation were fermented in three wooden 2 × 2 × 1 m sweatboxes arranged in tiers, with each box having a sliding door to facilitate transfer of beans to the next box.

Matured cacao pods were cracked open by five to six employees using sharp machetes. The placenta containing 30–40 beans was removed from the pod and the beans were separated by hand. The fresh unfermented beans which possessed a very light pinkish color were taken to the fermentation house in jute bags.

Fresh beans were placed in the first sweatbox and covered with banana leaves. After 3 days, the beans which by then had a light brownish color and pleasant alcoholic aroma, were transferred into the middle sweatbox by opening the sliding door of the top box. The beans remained in the middle box until the fifth day of fermentation, at which time they

Table 1—Types of microorganisms isolated from various sources at both estates[a]

	Surface of the pods		Hands of employees		Machetes	
Isolates	C	S	C	S	C	S
Aerobacter aerogenes	–	+	+	+	+	+
Arthrobacter simplex	+	–	+	–	–	–
Azotomonas insolita	–	–	–	+	–	–
Bacillus cereus	+	+	+	+	+	+
Bacillus firmus	–	–	–	+	–	+
Bacillus megaterium	–	–	+	+	–	–
Cellulomonas cellasea	–	–	–	+	–	+
Corynebacterium fascians	+	–	–	–	–	–
Erwinia ananas	–	–	–	–	+	–
Escherichia coli	–	–	+	+	+	–
Lactobacillus fermenti	–	–	+	–	–	–
Leuconostoc mesenteroides	–	–	–	–	–	+
Microbacterium flavum	–	–	–	–	+	–
Micrococcus luteus	+	+	–	–	–	–
Micrococcus varians	–	+	–	–	–	+
Pedicoccus cerevisiae	–	–	–	–	+	–
Pseudomonas fluorescens	–	–	–	+	–	–
Sarcina lutea	–	+	–	–	+	+
Serratia marcescens	+	–	–	–	–	–
Staphylococcus epidermidis	–	–	+	–	+	–
Yeasts	–	–	+	+	+	+

[a]C = Centeno Estate; S = San Louis Estate. + = Present; – = Absent.

Table 2—Types of microorganisms isolated from the dried pulp materials of the sweatboxes at both estates[a]

	Depth (cm)					
	5		45		90	
Isolates	C	S	C	S	C	S
Acetobacter aceti	–	+	–	–	–	–
Acetobacter suboxydans	–	–	–	+	–	–
Azotomonas insolita	–	+	–	–	–	–
Bacillus coagulans	–	+	–	–	–	–
Bacillus pumilus	–	–	–	–	+	–
Bacillus stearothermophilus	–	–	–	+	+	–
Cellulomonas cellasea	–	–	–	+	–	–
Lactobacillus acidophilus	–	–	+	–	–	–
Lactobacillus bulgaricus	–	–	–	–	+	–
Lactobacillus casei	+	+	–	–	–	–
Lactobacillus fermenti	+	–	+	+	–	–
Lactobacillus lactis	–	+	–	+	–	+
Lactobacillus plantarum	+	+	–	–	–	–
Leuconostoc mesenteroides	+	+	–	–	–	+
Micrococcus conglomeratus	+	–	–	–	–	–
Micrococcus flavus	–	–	–	+	–	–
Micrococcus luteus	+	–	–	–	–	–
Pedicoccus acidilactici	–	–	–	–	–	+
Propionibacterium technicum	–	+	–	–	–	–
Streptococcus lactis	–	–	+	–	–	–
Streptococcus thermophilus	–	–	–	+	+	–
Zymomonas mobilis	–	–	+	+	+	+
Yeasts	+	+	+	+	+	+

[a]C = Centeno Estate; S = San Louis Estate. + = Present; – = Absent

were pushed down to the third and final box. After the seventh day, the sliding door of the bottom box was removed, and beans were spread on a drying tray approximately 4 × 14 × 0.5m. Drying was done mechanically by blowing hot-forced air through the bottom of the tray for 48 hr, during which foreign materials and moldy beans were removed and bean clumps were broken loose. The final stage of fermentation involved the polishing and grading of the beans.

San Louis Estate. Raw beans were placed in an old wooden sweatbox 3 × 3 × 2m, covered with banana leaves and allowed to ferment for 3 days, after which they were shovelled into the adjacent box (approximately the same size) until the fifth day of fermentation. At that time, the lightly fermented beans were transferred back to the origianl box and allowed to ferment for another 2 days for a total fermentation time of 7 days. The fermented beans were allowed to sun dry on the roof of the fermentation house for 2–3 days during which the beans were polished manually and foreign materials and moldy beans were removed.

Isolation of the microorganism

The surfaces of 14 healthy, matured cacao pods were sampled using sterile swab sticks soaked in 0.1% sterile peptone water. Each swab was then transferred into a test tube containing 5 ml of 0.1% sterile peptone water. The same technique was also used for examining the hands of four employees and their machetes. All samples were immediately taken to the laboratory for analysis.

Serial dilutions of each sample were prepared using dilution bottles containing 99 ml of 0.1% sterile peptone water. Plates of 10^{-2}, 10^{-3} and 10^{-4} dilutions were made in duplicate using the medium containing 0.5% tryptone (Difco), 0.5% yeast extract (Difco), 0.1% glucose, 0.1% K_2HPO_4, 0.1% $CaCO_3$, 1% pulp extract and 2% agar (TYGKCP) and incubated at 37°C for 72 hr. In a preliminary study, the growth of microorganisms from various sources was best supported by this medium. It was therefore chosen and used throughout this study.

The dried pulp material remaining on the sides and bottom of the sweatbox from previous fermentations was aseptically scraped and placed in sterile plastic bags. 20g of each sample were blended with 80 ml of 0.1% sterile peptone water for 2 min and serial dilutions made accordingly. The 10^{-2}, 10^{-3} and 10^{-4} dilutions were plated on TYGKCP agar and incubated at 28, 37 and 45°C for 72 hr under aerobic and anaerobic conditions.

The contents of 14 matured healthy pods obtained from both Estates were also examined by aseptically cracking the pods and removing approximately 10 fresh beans from each pod. The beans were blended with 90 ml of 0.1% sterile peptone water for 2 min and serial dilutions up to 10^{-3} were plated on TYGKCP agar and incubated at 28, 37 and 45°C for 72 hr under aerobic and anaerobic conditions.

At Centeno Estate, fermenting beans were first sampled at 0 hr, then at 8-hr intervals for the first 3 days and every 12 hr thereafter; beans at San Louis Estate were sampled every 24 hr for a period of 7 days. To determine the microbial content of the beans at various depths in the sweatbox, 10-g samples were taken at 5, 45 and 90 cm below the surface. The beans were blended in 90 ml of 0.1% sterile peptone water for 2 min and serial dilutions up to 10^{-4} prepared and plated with TYGKCP agar. All plates were then incubated under aerobic and anaerobic conditions at 28, 37 and 45°C for 72 hr.

To determine the microflora of dried beans, six 10-g samples were obtained from both Estates after 24 hr and completion of the drying period (48 hr). The same number of samples was also taken after the beans were polished. All samples were analyzed microbiologically using the procedure described earlier.

To examine the possible role of fruitflies (*Drosophila melanogaster*) which appeared in large numbers around the sweatboxes at Centeno Estate during the early stages of fermentation, four flies were trapped in plates of TYGKCP agar and allowed to move about for 2 min. They were then released and the plates were incubated at 28, 37 and 45°C for 72 hr.

After the incubation period, the poured plates having 10–100 colonies were selected, counted and recorded. All discernible colonies exhibiting different morphological characteristics on plates of TYGKCP agar incubated at different temperatures were transferred into tubes containing TYGKCP agar slants. Stock cultures were stored at 5°C and recultured every 2 wk.

At each sampling time the temperature of beans at the three different depths were measured using a Fisher all-metal thermometer. Initial pH and changes during the fermentation process were also measured using a Fisher brand pH meter.

Identification of the Isolates

Stock cultures were packed in a box containing a small amount of dry ice and flown back to The Pennsylvania State University for identification and characterization. Each isolate was first examined for ability to produce catalase, followed by determination of Gram characteristics. Isolates were then classified to genus and species according to the microscopic and biochemical examination described in *Bergey's Manual* (1957), *Manual of Microbiological Methods* (1957), *Laboratory Methods in Micro-*

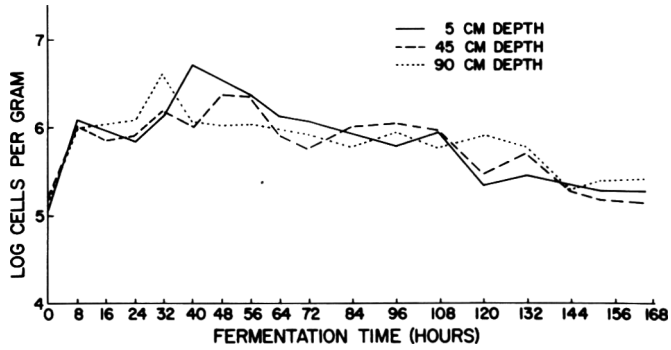


Fig. 1—Changes in microbial population per gram of bean at different depths of sweatbox during the fermentation period at Centeno Estate.

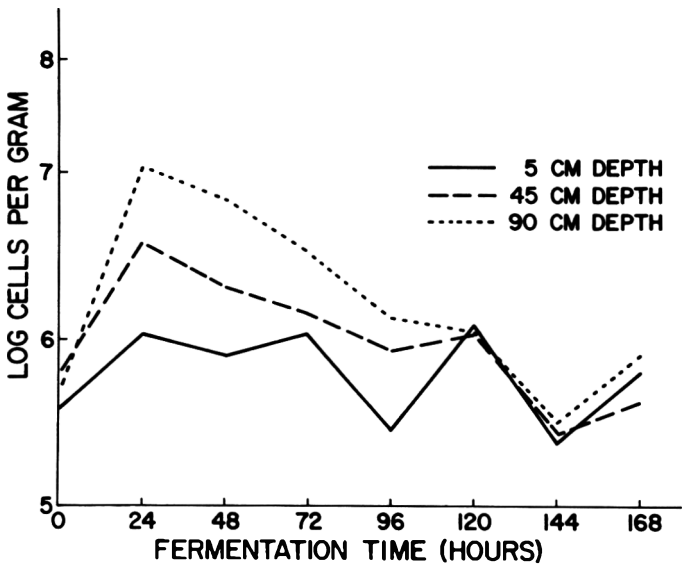


Fig. 2—Changes in microbial population per gram of bean at different depths of sweatbox during the fermentation period at San Louis Estate.

biology (1966) and *The Genera of Bacteria* (1967). The identification of isolates in doubt was confirmed using known cultures.

RESULTS & DISCUSSION

TAXONOMICAL STUDIES on 256 pure isolates resulted in the identification of 10 families of microorganisms: *Lactobacillaceae, Bacillaceae, Pseudomonadaceae, Micrococcaceae, Corynebacteriacea, Enterobacteriaceae, Propionibacteriaceae, Actinomycetaceae, Azotabacteraceae* and *Brevibacteriaceae.*

Table 1 lists the microorganisms isolated from the surfaces of the pods, hands of employees and their machetes at both Estates. *Bacillus cereus* was the only common organisms isolated from these sources. The most common microorganism isolated from the pod's surfaces was *Micrococcus luteus*, while *Escherichia coli, Aerobacter aerogenes, Bacillus megaterium* and yeasts were most common isolates from the hands of employees. The interior contents of healthy pods were found to be free from microorganisms confirming the findings of Knapp (1937).

The microflora of the dried pulp material scraped from the sides and bottom of the sweatboxes (Table 2) were found to

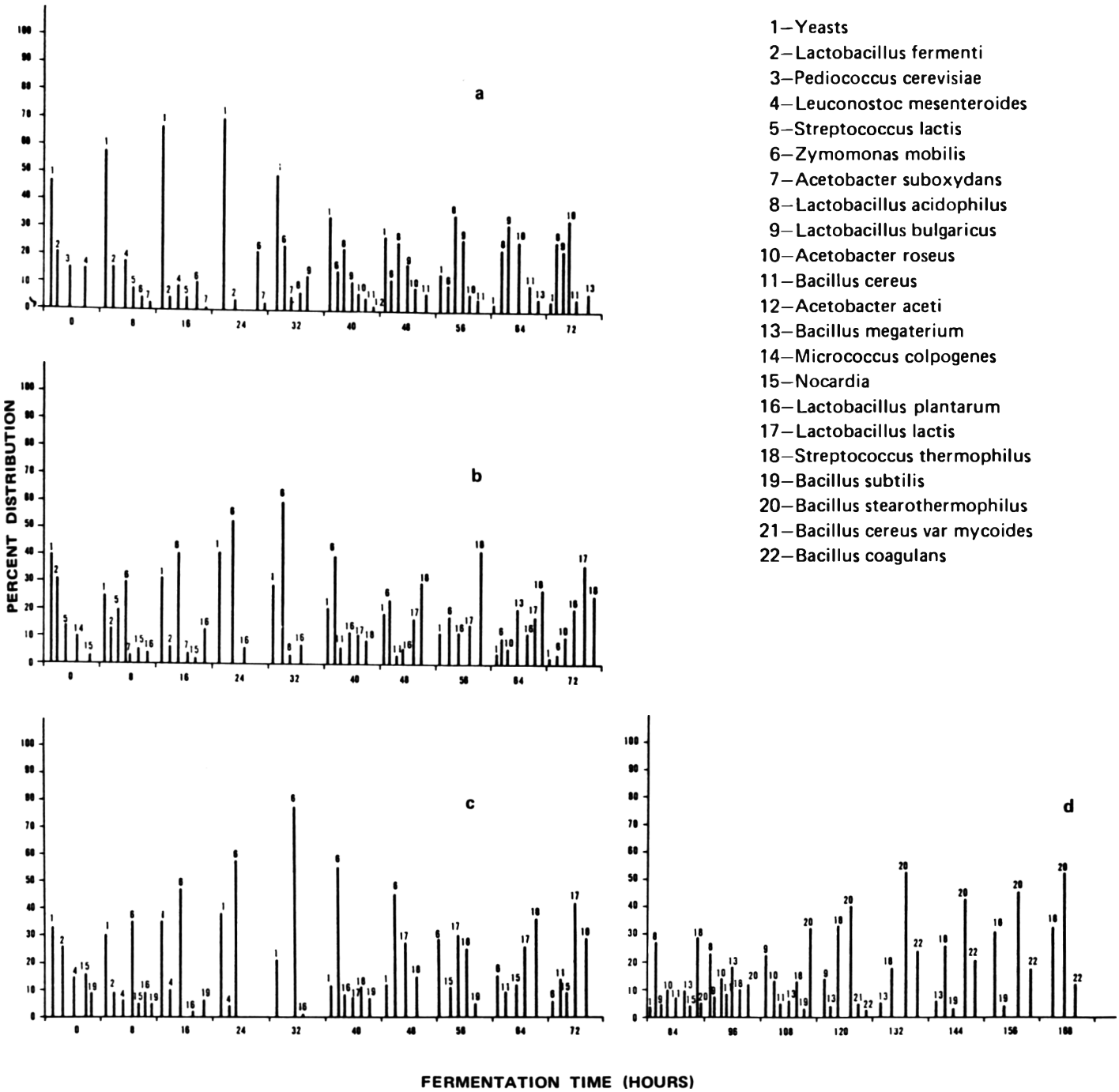


Fig. 3–Percentage distribution of microflora isolated from cacao beans at first 72 hr (a, b, c) and the later part (d) of the fermentation at Centeno Estate.

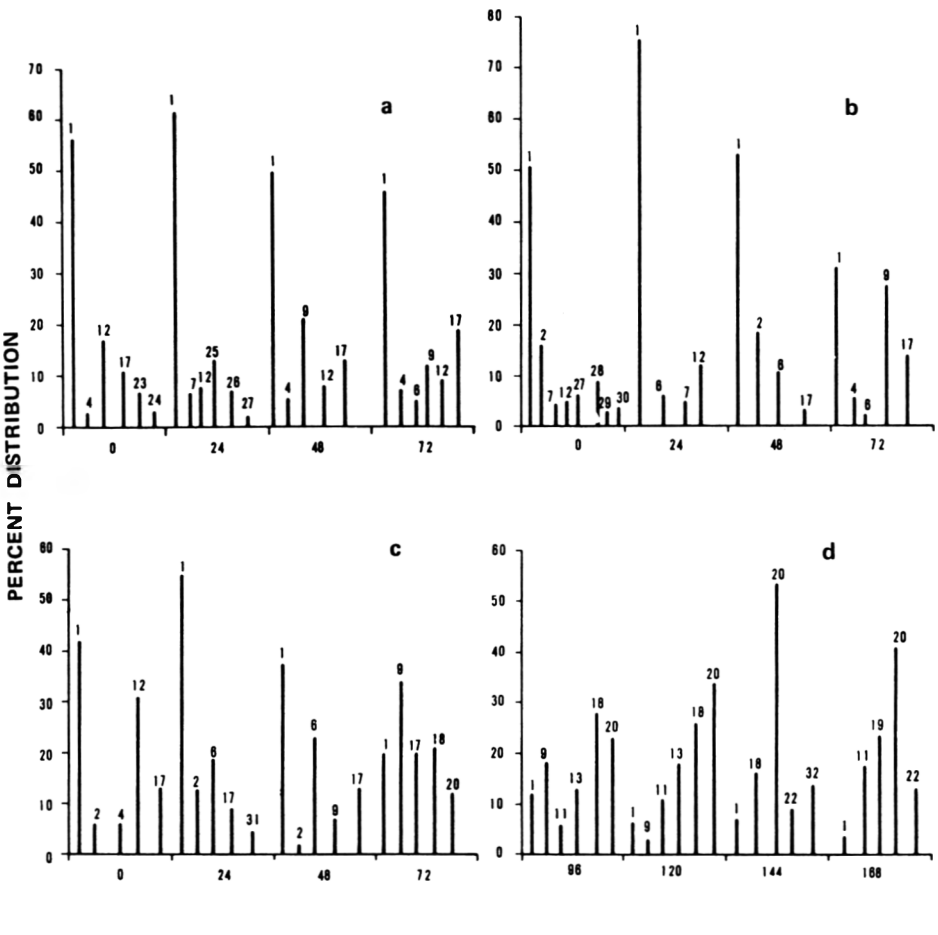
be quite different from those isolated from the above sources. Various species of homofermentative and heterofermentative lactic acid organisms such as *Lactobacillus casei, L. plantarum, L. fermenti* and *Leuconostoc mesenteroides* were present in samples obtained from different locations of the sweatboxes. While yeasts were isolated from different locations of the sweatboxes, *Zymomonas mobilis* was isolated only from the samples taken from the middle and bottom sweatboxes.

Changes in total viable numbers of microbioal population during fermentation at Centeno Estate are shown in Figure 1. At 0 hr the total counts ranged from 1.48 $\times 10^5$ to 1.62 $\times 10^5$ organisms/g of bean. The gradual increase in counts at all levels was evident during the first 32 hr of fermentation. From this point on, counts varied slightly and at the completion of the fermentation (7th day), the microbial counts ranged from 2.5 $\times 10^5$/g at 45 cm depth to 4.1 $\times 10^5$/g at the 90 cm depth. At 0 hr, counts at San Louis Estate ranged from 6.8 $\times 10^5$/g at the 5 cm depth to 8.3 $\times 10^5$/g of beans at 45 cm depth. An increase of approximately 1 log was noticed in counts at 5 and 45 cm depths after 24 hr, while the microbial population at 90 cm depth increased by approximately 2 logs. A decrease in microbial counts at all depths became evident after 24 hr of fermentation (Fig. 2).

Generally, the counts obtained from the San Louis Estate were higher than those obtained from Centeno Estate. This was attributed mainly to the time elapsed between the harvesting of the beans and their transfer to the fermentation houses. The beans at Centeno Estate were taken immediately to the fermentation house, while San Louis Estate beans were piled on banana leaves for a period of up to 5–6 hr prior to being transferred to the fermentation house.

The initial microbial populations recorded at both Estates agreed with Rombouts' (1952) findings who reported that the microbial population/g of Trinidad beans was 2.3 $\times 10^5$. However, our findings did not agree with the results of deCamargo et al. (1963) who showed a microbial population of 1.5 $\times 10^9$/g of Bahia beans. Such a difference is probably due to the type of beans used and the methods of fermentation practiced.

Taxonomical studies of isolates obtained from cacao beans during fermentation revealed the presence of a variety of microorganisms (Fig. 3 and 4). During the first 48 hr of fermentation, yeasts dominated the flora of the beans obtained from both sweatboxes except for the samples which were taken at 45 and 90 cm depths in the sweatbox at Centeno Estate. The dominant organism from these depths was *Z. mobilis* which accounted for 40 and 45% of the isolates up to 40 and 48 hr of fermentation, respectively. Domination by yeasts during early stages of fermentation at San Louis Estate, supports the findings of Rombouts (1952) who claimed over 90% of the total microorganisms isolated during the early stages of fermentation were yeasts. However, the occurrence and domination of *Z. mobilis* during the early stages of fermentation has not been reported previously. This organism was also isolated from the middle and bottom of the sweatbox at San Louis Estate, but did not account for a high percentage of the isolates. Such a difference in the occurrence of *Z. mobilis* in two methods of fermentation can be attributed to the amount of oxygen available in the fermenting heaps. This microorganism has an ability to grow in the absence of oxygen, and an aerobic environment will suppress and inhibit its growth. The tightly packed beans at Centeno Estate provided an almost anaerobic condition for the growth of *Z. mobilis*, whereas the loosely packed beans at San Louis Estate did not create such a condition. Furthermore, the conversion of pulp sugar to ethanol and CO_2 by yeasts as reported by Quesnel (1968) will also result in the creation of an anaerobic condition during the first 2 days of fermentation which induces the growth of *Z. mobilis*. The profuse ability of *Z. mobilis* to ferment sugar (*Bergey's Manual*, 1957;



1—Yeasts
2—Lactobacillus fermenti
4—Leuconostoc mesenteroides
6—Zymomonas mobilis
9—Lactobacillus bulgaricus
11—Bacillus cereus
12—Acetobacter aceti
13—Bacillus megaterium
17—Lactobacillus lactis
18—Streptococcus thermophilus
19—Bacillus subtilis
20—Bacillus stearothermophilus
22—Bacillus coagulans
23—Xanthomonas citri
24—Micrococcus ureae
25—Arthrobacter insolita
26—Lactobacillus casei
27—Micrococcus flavus
28—Pediococcus acidilactici
29—Cellulomonas cellasea
30—Brevibacterium ammoniagenes
31—Corynebacterium xerosis
32—Bacillus pumilus

Fig. 4—Percentage distribution of microflora isolated from cacao benas at first 72 hr (a,b,c) and the later part (d) of the fermentation at San Louis Estate.

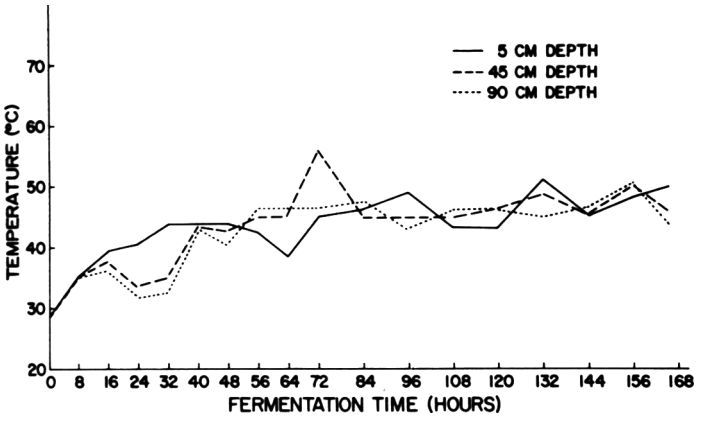


Fig. 5—The changes in temperature (°C) at different depths of sweatbox during the fermentation period at Centeno Estate.

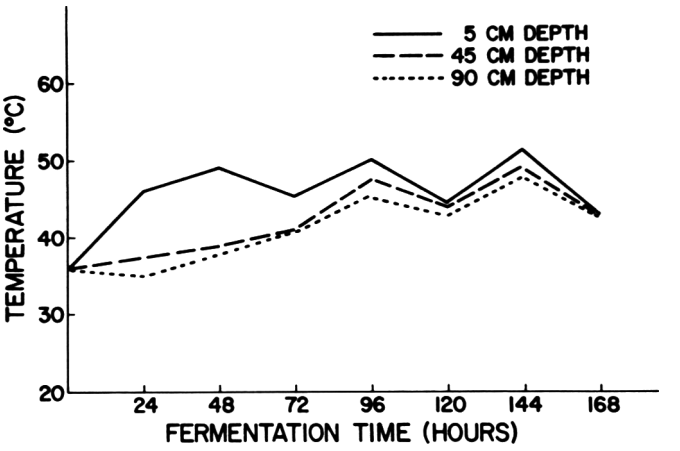


Fig. 6—The changes in temperature (°C) at different depths of sweatbox during the fermentation period at San Louis Estate.

Verachtert, 1970) strongly suggests that this organism can be quite important in the conversion of pulp sugar to alcohol, a fermentation previously credited almost solely to yeasts.

Different species of lactic acid organisms were found to be quite common at all three depths in the sweatboxes during the early stages of fermentation at Centeno and San Louis Estates. *L. fermenti, L. mesenteroides, L. plantarum* and *Streptococcus lactis* were quite abundant during the first 24 hr of fermentation. However, the more heat tolerant species *L. acidophilus, L. bulgaricus* and *L. lactis* appeared after 24 hr of fermentation and accounted for high percentages of the isolates at both Estates (Fig. 3 and 4). *L. bulgaricus* was isolated from 120-hr fermented beans and accounted for 14 and 4% of the isolates at Centeno and San Louis Estates. The concentrations of several organic acids in unfermented and fermented beans from Trinidad were reported by Weissberger et al. (1971). These investigators showed that well fermented beans (Trinidad and Ghana) had higher lactic acid concentrations than unfermented beans (Sanchez and Tabasco). These findings closely correlate with results obtained from this study and support the role of lactic acid organisms in cacao bean fermentation.

Acetic acid organisms which have been reported by Roelofsen and Giesberger (1958) and Rombouts (1952) to dominate the total microflora of the cacao beans during the first 2 days of fermentation, accounted for low percentages of the isolates obtained from two sweatboxes. The only acetic acid organisms isolated from the fermenting beans during the entirety of this investigation were *Acetobacter roseus, A. aceti* and *A. suboxydans*. The highest percentage (34%) of acetic acid organisms appeared in the samples taken at 72 hr of fermentation from the 5 cm depth of the sweatbox at Centeno Estate (Fig. 3). It is interesting to note that during the same period and location, 49% of the isolates proved to be lactic acid organisms. However, in San Louis Estate's sweatbox, only 9% of the isolates from the same period and depth were acetic acid organisms while 31% belonged to lactic acid flora (Fig. 4). The occurrence of these organisms gradually decreased and by 108 hr of fermentation, *A. roseus*, which is considered to be a thermotolerant strain, was the only acetic acid organism isolated from the fermenting beans at Centeno Estate.

The reasons for low occurrence of acetic acid organisms in this investigation are quite obvious. During the first 32 hr, the fermentations were being carried out under almost anaerobic conditions which made it extremely difficult, if not impossible, for the acetic acid organisms to grow and multiply. Furthermore, the ma-

Table 3—Types of microorganisms isolated from cacao beans during and after drying at Centeno and San Louis Estates[a]

Isolates	Centeno		San Louis	
	24 hr	48 hr	24 hr	48 hr
Aerobacter aerogenes	–	+	–	–
Arthrobacter oxydans	+	–	–	–
Azotomonas insolita	+	–	–	–
Bacillus cereus	–	+	–	–
Bacillus cereus var *mycoides*	–	–	–	+
Bacillus coagulans	+	–	+	+
Bacillus licheniformis	–	–	–	+
Bacillus megaterium	+	+	+	+
Bacillus pumilus	–	–	+	–
Bacillus stearothermophilus	+	+	+	+
Bacillus subtilis	+	+	+	+
Cellulomonas cellasea	–	+	–	–
Corynebacterium xerosis	–	+	–	–
Gaffkya tetragena	+	–	–	–
Lactobacillus bulgaricus	+	–	+	–
Micrococcus conglomeratus	–	–	+	–
Micrococcus flavus	–	+	–	–
Micrococcus luteus	+	–	+	+
Micrococcus roseus	+	+	–	–
Nocardia	+	+	–	–
Staphylococcus epidermidis	–	+	–	–
Streptococcus thermophilus	+	–	–	–
Yeasts	–	+	+	+

[a] + = Present; – = Absent

Table 4—Types of microorganisms isolated from polished cacao beans at Centeno and San Louis estates[a]

Isolates	Centeno	San Louis
Aerobacter aerogenes	+	+
Bacillus cereus var *mycoides*	+	–
Bacillus coagulans	+	+
Bacillus megaterium	+	–
Bacillus polymyxa	+	+
Bacillus pumilus	–	+
Bacillus stearothermophilus	+	+
Bacillus subtilis	+	+
Escherichia coli	–	+
Micrococcus flavus	+	–
Micrococcus luteus	–	+
Yeasts	+	+

[a]+ = Present; – = Absent

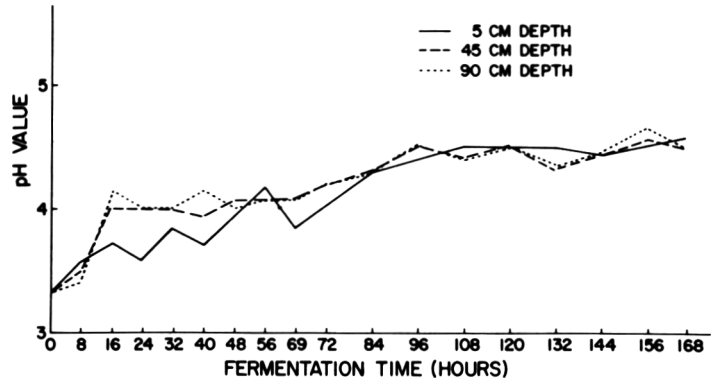


Fig. 7—The changes in pH at different depths of sweatbox during the fermentation period at Centeno Estate.

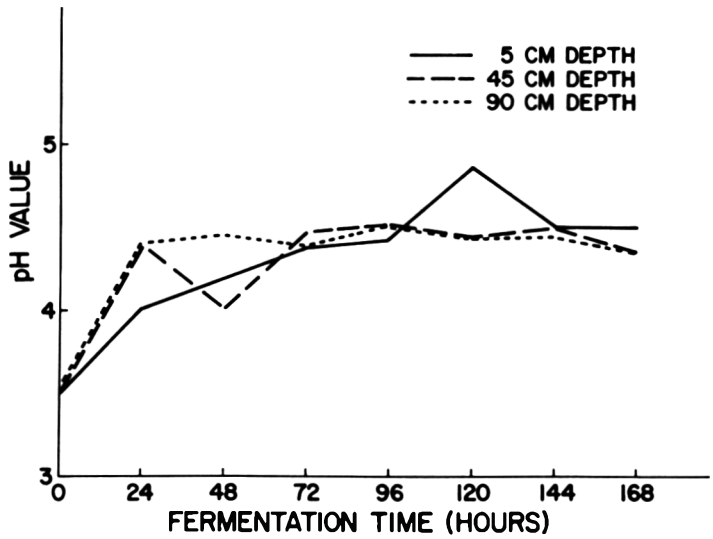


Fig. 8— The changes in pH at different depths of sweatbox during the fermentation period at San Louis Estate.

jority of acetic acid bacteria possess an optimum growth temperature of 30–35°C which is approximately 15°C lower than the temperatures recorded after 48 hr of fermentation in this study. Therefore, if such conditions prevail under normal fermentation practices, it would be rather difficult to accept the assumptions claimed by Roelofsen et al. (1958) and Rombouts (1953) who reported that acetic acid organisms play an important role in the fermentation of cacao beans during the early stages of fermentation.

After 96 hr of fermentation a gradual drop in the occurrence of lactic acid organisms became evident at both Estates, possibly due to the rise in temperature (Fig. 5 and 6). The higher temperatures which prevailed during the latter stages of fermentation resulted in sharp increases in the percentages of *Streptococcus thermophilus* and *Bacillus stearothermophilus*. These organisms accounted for 73% and 60% of the isolates at Centeno and San Louis Estates after 120 hr of fermentation. These along with other species of *Bacillus* dominated the microflora of the fermented beans at both Estates.

The role and significance of dried pulp material obtained from the sides and bottoms of the sweatboxes in the microbial inoculation of the beans becomes evident by a qualitative examination of microflora isolated from the fermenting beans at both Estates. After 8 hr of fermentation at Centeno Estate, a correlation existed between the microflora of fermenting beans and those isolated from the dried pulp material (Table 2 and Fig. 3). The common isolates from both sources were yeasts, *L. fermenti, L. mesenteroides, Z. mobilis, S. lactis* and *L. plantarum*. Such correlation was also evident between the flora of 24 hr fermented beans and the dried pulp material at San Louis Estate (Table 2 and Fig. 4). The microflora isolated from both sources were *A. suboxydans, A. aceti, Azotomonas insolita, Micrococcus flavus, Z. mobilis, L. lactis* and yeasts. It is interesting to note that with the exception of *L. fermenti, A. insolita* and yeasts, none of the above flora was isolated from pod surfaces, hands of employees or machetes.

Microbiological examination of the mechanically dried beans (Centeno Estate) and sun dried beans (San Louis Estate) resulted in the identification of 23 different microflora, a majority of which belonged to the genus *Bacillus* (Table 3). The presence of *M. flavus, M. roseus, M. luteus* and *Staphylococcus epidermidis* on dried Centeno beans can be attributed to contamination during the drying process. The common microflora isolated from the dried beans at both Estates were yeasts, *B. stearothermophilus, B. megaterium* and *B. subtilis*. A total of nine different flora were isolated from the mechanically polished beans at Centeno Estate. The same numbers of different types of organisms were also isolated from the manually polished beans at San Louis Estate. However, the only common flora isolated from both Estates were *A. aerogenes, B. stearothermophilus, B. coagulans, B. polymixa, B. subtilis* and yeasts (Table 4). These findings are generally in agreement with Barrile et al. (1971) who isolated several species of genus *Bacillus* and *Micrococcus* from unroasted cacao beans.

The changes in pH during the fermentation at Centeno and San Louis Estates are presented in Figures 7 and 8. The initial pH values (3.3–3.5) recorded at both

Table 5–Microorganisms isolated from the fruit flies (Drosophila melanogaster) at Centeno Estate[a]

Microorganisms	Flies 1	2	3	4
Acetobacter aceti	+	+	+	+
Acetobacter roseus	+	–	+	–
Acetobacter suboxydans	–	+	–	+
Arthrobacter simplex	–	–	+	–
Azotomonas insolita	–	–	–	+
Bacillus cereus	–	–	+	+
Bacillus licheniformis	–	+	–	–
Bacillus pumilus	–	+	–	–
Bacillus stearothermophilus	+	–	+	+
Lactobacillus fermenti	+	+	–	+
Propionibacterium freudenreichii	–	–	+	–
Propionibacterium shermanii	–	–	+	–
Streptococcus thermophilus	+	–	–	–
Yeasts	–	+	+	+

[a]+ = Present; – = Absent

Estates, generally agreed with those reported by Rombouts (1952) and de-Camargo (1963). However, the pH changes during the fermentation and that obtained from the fermented beans did not. The maximum pH measured at both Estates was 4.7 which was recorded after 156 and 120 hr of fermentation at the respective Estates. This was found to be much lower than pH 6.4 reported by Rombouts (1952) from fermented Trinidad beans. As to the pH values obtained at various depths of the sweatboxes, no major differences were observed at either plant. This was contrary to Rohan's (1963) findings who reported a higher pH value at the center of the fermentation heap in West African beans. Such disagreements can be due to the type of beans used, the size of the sweatbox and finally the size of the fermentation batch (heap). The gradual increase in pH of the pulp can be attributed to the dissimilation of the citric acid content by varieties of yeasts and lactic acid bacteria. As a result, the citric acid is replaced by the less readily dissociated lactic and acetic acids. It is interesting to note that the fine quality of beans obtained from both Estates does not support Saposhnikova's (1968) suggestion that a final pulp pH of less than 5.0 indicates defective fermentation.

The initial temperature of the heap recorded at Centeno Estate was found to be 28°C which was 8°C lower than that recorded at San Louis Estate. It gradually increased at both Estates and reached a maximum of 56°C after 72 hr at Centeno and 52°C after 144 hr at San Louis. The temperatures obtained at the top of the heap throughout the fermentation process at both Estates, generally proved to be higher than those recorded at the middle and the bottom. This was in agreement with Rohan's (1958) findings who recorded higher temperatures at the top of the West African fermenting beans. The overall temperature changes also agreed with those reported by Knapp (1937), Rombouts (1952) and Forsyth and Quesnel (1963) in Trinidad.

The significance of fruit flies (*Drosophila melanogaster*) in microbial inoculation of the beans during the early stages of the fermentation has been pointed out by Nicholls (1914). In this investigation, a total of 13 different microflora were isolated and identified from four fruit flies during the early stage of fermentation at Centeno Estate (Table 5). Except for *Propionibacterium shermanii* and *Propionibacterium freudenreichii*, the remaining microflora were isolated at one point or other during the fermentation process. Yeasts, *L. fermenti, A. roseus, A. aceti* and *A. suboxydans* which were isolated from the fermenting beans during the first 3 days of fermentation, were also isolated from the fruit flies. Therefore, it would be safe to assume that these flies can be important in the microbial inoculation of the fermenting beans.

CONCLUSIONS

IT IS CONCLUDED that results obtained from this investigation have for the first time provided qualitative and quantitative information as to the microbiology of cacao bean fermentation. The dried pulp material that remained on the sides and bottom of the fermentation boxes do contribute to the microbial inoculation of the beans. The isolation of *Z. mobilis* during the early stages of fermentation and its possible role in the conversion of pulp sugar to alcohol is of great significance and should be further investigated. It can also be concluded that during the course of fermentation, yeasts, *Z. mobilis*, homofermentative and heterofermentative lactic acid microorganisms and finally various species of genus *Bacillus* dominate the total microbial flora.

Further studies on the microflora of cacao beans at other cacao producing countries would provide a better understanding of the total flora involved in this most important process. Such information would hopefully lead into artificial inoculation of the beans and production of high quality cacao beans.

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IRON-FORTIFIED SYRUP BLENDS: PREPARATION, CHARACTERISTICS, APPLICATION

INTRODUCTION

IRON-DEFICIENT diets were reported for several million Americans in a food consumption survey made in 1965 (USDA, 1969a). The average diets of children below 3 yr of age contained only about 50% of the recommended dietary allowance (RDA) of iron. The American Medical Association's Committee on Iron Deficiency (AMA, 1968) issued a report that indicated a high incidence of iron deficiency in the United States in infants and pregnant women.

Human milk and cow's milk contain only minor amounts of iron. An infant's endowment of iron at birth only fulfills iron needs for 6 months (NRC, FNB, 1968). Unless iron is supplied by other sources at levels greater than that naturally present in foods, an iron deficiency prevails by 12 months. Infant feeding formulas are frequently prepared with cow's milk, water and sugar or corn syrup. Iron-fortified syrups were considered as a means to introduce iron into the infant's diet. The domestic donation program includes Type VI Corn Syrup Blend (USDA, 1969b) which is currently being purchased by USDA for food-help programs. Corn Syrup Blend contains a sweetener combination of 15% sucrose and 85% corn syrup, 40 minimum dextrose equivalent (DE). The solids level is adjusted to 75% or 40 degrees Baumé minimum. The product is clear, contains 0.5% maximum ash and is flavored with vanillin, U.S.P. and/or ethyl vanillin. In the proposed scheme for fortifying an infant feeding formula, 100 mg iron is added per pint of Corn Syrup Blend. Then 1.5 oz of the iron-fortified Corn Syrup Blend is used in preparing 1 qt of baby formula. Each 8-oz bottle of iron-fortified baby formula then contains approximately 2 mg iron.

The primary objective of the experiments reported here was the development of a stable iron-fortified syrup for use in infant feeding tests. On the basis of data developed in these studies, 1000 lb of Corn Syrup Blend containing 100 mg iron per pint as ferric ammonium citrate was commercially prepared for an infant feeding study. These tests are currently being conducted by Dr. Robert Berg, Chief of Pediatrics, Beth Israel Hospital, Roxbury, Mass.

Table 1—Characteristics of corn syrups and sugar used in iron-fortified syrups

Characteristic	RCS[a]	HCS[a]	Granulated sugar
DE	42	64	<1
Total solids, %	80.3	82	100
Baumé	43	43	–
Viscosity (cp),[b] 40°C	140,000	18,000	–
pH	5.0	5.0	7.0

[a]RCS = Regular conversion corn syrup; HCS = high-conversion corn syrup.
[b]Brookfield Model LVT viscometer.

EXPERIMENTAL

Materials and formulations

All supplies of corn syrups, sugar (sucrose) and iron salts from which the experimental iron-fortified syrups were prepared complied with commercial standards of identity (Tables 1 and 2).

Formulations for syrups intended as a carrier for the iron enrichment to infant feeding formulas followed the general pattern of Corn Syrup Blend. Formulations included an all-sugar syrup; RCSB, a blend of regular conversion corn syrup with 15% sugar (solids basis); and HCSB, a blend of high-conversion corn syrup with 15% sugar (solids basis). Each of these syrups was fortified with ferric ammonium citrate, ferric choline citrate, ferrous gluconate and ferrous sulfate, at a level of 100 mg iron per pint (about 0.015%). The syrups were flavored with 3 ppm of a 50/50 mixture of vanillin and ethyl vanillin. The all-sugar syrups were identified as A-1 to A-5, RCSB as B-1 to B-5 and HCSB as C-1 to C-5 (Table 3). The all-sugar syrups were formulated to contain 67% solids, whereas RCSB and HCSB contained 75%. The solids content was controlled at these levels to retard microbial growth during storage. The chemical and physical properties of these formulations are given in Table 3. This experimental design included three levels of DE, two solids levels, three viscosity levels, two ferric salts and two ferrous salts to find a combination of syrup and iron-type that was stable and that would enrich an infant feeding formula.

Preparation of iron-fortified syrups

The experiment iron-fortified syrups were made according to Figure 1. This general procedure was developed after a series of preliminary experiments. An initial approach was to blend the iron salt directly in the syrup solution, but this step was time consuming, and darkening resulted when ferrous sulfate was added in this manner. Iron salt solutions were then prepared with deaerated (boiled and cooled) water (100–400 ml) and added to the

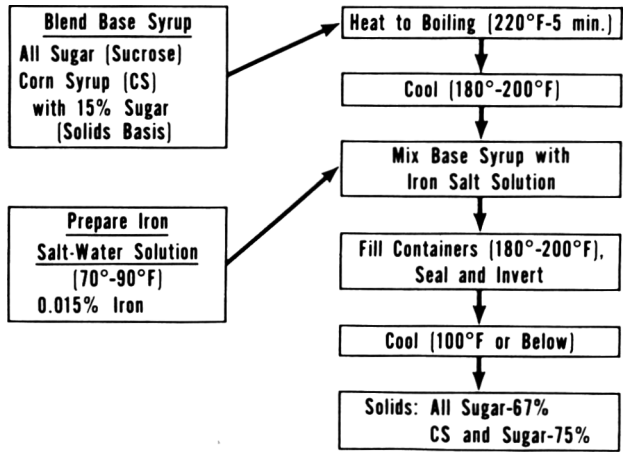

Fig. 1—Flow diagram of general method of preparing iron-fortified syrups.

Table 2– Characteristics of iron salts for syrup fortification

Iron salt	Chemical formula	Appearance	Contained iron (%)	Approximate solubility in cold water (g/100 ml)	Biological activity[a] ($FeSO_4$ = 100)	Approximate cost per lb[b] ($)
Ferric ammonium citrate	Undetermined[c]	Reddish brown	17	35	107	0.67
Ferric choline citrate	$C_{11}H_{24}O_{11}NFe$	Yellowish olive green	12	100	102	3.25
Ferrous gluconate	$C_{12}H_{22}FeO_{14} \cdot 2H_2O$	Yellowish gray	11.6	10	97	1.13
Ferrous sulfate	$FeSO_4 \cdot 7H_2O$	Pale bluish green	20.1	60	100	0.34

[a]Fritz et al. (1970)
[b]March 1972, 100-lb drum basis
[c]NH_3 (9%), Fe (16.3–18.5%) and $C_6H_8O_7 \cdot H_2O$ (ca. 65%)

syrup blend (4200–4600g). Deaerated water prevented iron solutions from clouding before adding them to the base syrup. The aqueous iron salt solution was prepared just before blending with the syrup because hydrolysis and oxidation to ferric sulfate, $Fe_2(SO_4)_3 \cdot nH_2O$ (Mellor, 1942), will occur during prolonged standing of aqueous ferrous sulfate solutions. The base syrups were mixed in gallon containers and heated in a laboratory autoclave to 220°F for 5 min. After the base syrup was cooled to 180–200°F, the aqueous iron was added through a funnel and mixed 30 sec with a "Lightnin" Model L stirrer. Adequate mixing of the iron salt solution with the base syrup was rapid. The iron-fortified syrups were then poured into pint bottles which were sealed, inverted and cooled. A second series of samples A-1 to C-5 was prepared with no heating step except to warm to 100°F to improve the flow characteristics during filling. Physical and chemical changes took place to the same extent as in the samples prepared by the process shown in Figure 1. The heating step is necessary to provide a reliable means of destroying spoilage microorganisms. The process must be carried out under strictly sanitary conditions.

Methods of analysis

Iron was measured by the thiocyanate method (Kolthoff and Sandell, 1949). DE was determined by the CIRF method (1959). Comparative apparent viscosity measurements were made with a Brookfield Model LVT viscometer. Other analyses followed standard procedures (AOAC, 1965). The samples were stored at 77°, 100° and 120°F. Changes in physical stability such as color changes or precipitation of iron from solution were determined by visual observation. Four 150-watt flood lamps were arranged to direct light reflected from a white backboard through the samples. Visual results were reported in previous work with wheat flour and macaroni products (ABA-MNF, 1972).

Flavor stability

The stored samples were withdrawn from storage after 0, 28 and 56 days at 120°F; after 182 days at 100°F; and after 182 and 365 days at 77°F for flavor evaluation by an experienced 16-member taste panel. At each tasting, only a single series was scored at a time, such as A-1 to A-5 or B-1 to B-5. Flavor comparisons were made between the coded, randomized stored samples A-2 to A-5 with the stored reference control A-1 of the same series. Each sample of a series was rated for flavor according to the 10-point scoresheet described by Bookwalter et al. (1968). A score of 10 described the samples as completely free from "off-flavors" while scores of 9 to 1 described the samples as containing increasing amounts of objectionable flavors. The primary objective was to determine difference or flavor change such as off-flavor development. Statistical evaluations were made on the experimental data after completion of each taste series. Analysis of variance, regression analysis (Snedecor and Cochran, 1968) and Duncan's multiple range test (Duncan, 1955) were utilized to examine results. Statistical significance is at the 5% probability-of-error level wherever the term significant appears in this paper.

Enrichment of infant formula

The experimental syrups were added to several food formulations to test their usefulness as a convenient, pourable iron-fortification medium. The iron-fortified syrup contained ingredients common to those in all applications considered. Syrups B-1, B-2, B-3, C-1, C-4 and C-5 (Table 3) were tested in an infant feeding formula provided by a local pediatrician:

Ingredients	Home procedure	Laboratory procedure (2/3 batch) (g)
Evaporated milk	1 can (13 fl oz)	282
Water	19 fl oz	370
Syrups (with or without iron)	3 tbsp	42

The iron-fortified syrup was mixed with water, which had been heated to 125°F, and the solution was then combined with the evaporated milk. The mixture was added to nursing bottles, which were placed in a sterilizer and heated for 25 min. The bottles were cooled and stored in a refrigerator overnight before testing.

Infant formulas with and without iron were rated for appearance and flavor by a 10-member taste panel. The formulas were also poured

Table 3–Composition and properties of syrups with iron (0.014–0.016%) and without iron

No.	Syrup and iron-type	Soluble solids (%)	Iron level (% Fe)[a]	pH	Viscosity[b] (cp)	DE
A-1[c]	Sugar and no iron	67.4	0	7.2	210	<1
A-2	Sugar + ferric ammonium citrate	66.9	0.016	7.2	240	<1
A-3	Sugar + ferric choline citrate	67.3	0.016	6.2	230	<1
A-4	Sugar + ferrous gluconate	67.3	0.016	6.2	240	<1
A-5	Sugar + ferrous sulfate	67.2	0.015	6.4	230	<1
B-1	RCSB[d] and no iron	76.3	0	5.2	11,000	37
B-2	RCSB + ferric ammonium citrate	76.4	0.015	5.8	12,000	37
B-3	RCSB + ferric choline citrate	76.2	0.015	5.0	11,000	38
B-4	RCSB + ferrous gluconate	76.4	0.014	5.0	11,000	38
B-5	RCSB + ferrous sulfate	76.4	0.015	5.0	10,000	38
C-1	HCSB[e] and no iron	75.5	0	5.1	2,800	54
C-2	HCSB + ferric ammonium citrate	75.5	0.016	5.9	2,900	54
C-3	HCSB + ferric choline citrate	76.0	0.015	5.2	2,700	54
C-4	HCSB + ferrous gluconate	76.0	0.014	4.8	2,500	53
C-5	HCSB + ferrous sulfate	75.5	0.015	5.0	2,500	54

[a]100 mg Fe/pint = 0.016% Fe in all-sugar syrup or 0.015% Fe in RCSB and HCSB.
[b]Brookfield Model LVT viscometer
[c]All class A syrups had a density of 625g/pint; the remainder, 665g/pint.
[d]Regular conversion corn syrup with 15% sugar (solids basis)
[e]High conversion corn syrup with 15% sugar (solids basis)

through a standard milk filter disc so that the filtrate could be tested for iron and any possible coagulation reactions which could affect nutritional value of the milk.

RESULTS AND DISCUSSION

Flavor stability

The average flavor score of syrups with iron (0.014–0.016%) and without iron after storage under various temperatures is shown in Table 4. Iron-fortified syrups were readily distinguished from unfortified syrups although the average flavor scores were usually 8 or above and no deleterious flavor development occurred. These flavor scores were subjected to analysis of variance. There were significant interactions of storage time with syrup-type, storage time with iron-type and iron-type with syrup-type. The interaction of storage time with syrup-type showed that samples containing RCSB or HCSB had significantly higher flavor scores than samples containing all sugar up to 182 days, but by 365 days at 77° there were no differences. The interaction of storage time with iron-type revealed that samples containing ferrous iron salts were significantly poorer than those with ferric iron salts after storage for 182 days at 77°F. At 365 days, flavor scores with all iron salts were similar. The interaction of iron-type with syrup-type showed that samples containing RCSB had higher flavor scores than those with all-sugar syrup regardless of iron-type. There were no differences between RCSB or HCSB containing ferric iron salts, but flavor scores were significantly higher for ferrous iron salts in combination with RCSB than with HCSB. With the all-sugar syrup, flavor scores were significantly lower with ferrous iron salts than with ferric iron salts. Although statistical differences in flavor were found, sometimes these had no practical importance since all flavor scores were 7 or above which indicated satisfactory flavor stability during storage.

Solution characteristics

The visual appearance, ranking by color and physical stability of syrups with 0.014–0.016% iron and without iron stored under conditions are listed in Table 5. In solutions of the freshly prepared syrups, ferric ammonium citrate usually produced a light amber color; ferric choline citrate, light yellow; ferrous gluconate, light amber; and ferrous sulfate, practically no color. All samples became darker during storage at 120°F for 56 days, at 100°F for 182 days and at 77°F for 365 days. The freshly prepared RCSB without iron was colorless but changed in color more than the all-sugar syrup during storage at the various conditions. The HCSB was generally similar or slightly darker in color than the RCSB after storage.

After each storage period, the samples were ranked from least color to most color. Presumably dark-colored samples could cause a greater color change in a food than the lighter colored samples. The light-colored samples resembled the base syrups closely. Samples in which iron had precipitated were coded "U" (unstable) but not ranked because they were considered unsuitable for food additives. Overall ranking at various storage conditions usually followed the pattern from least to most color as shown in Table 5: A-1, B-1, C-1, C-5, B-3, C-3, A-3, C-4, B-2, C-2, A-2 and A-4. Although the all-sugar syrup with no iron was usually lighter in color than RCSB or HCSB, the all-sugar syrups with the various iron salts were generally darker in color than the iron-fortified RCSB or HCSB.

In stable combinations, the fortified syrup containing ferrous sulfate was lightest, and those containing ferric ammonium citrate were darkest in color. All combinations of syrups with either ferric ammonium citrate or ferric choline citrate were stable. Ferrous gluconate was unstable, and a black precipitate formed when combined with RCSB. The all-sugar syrup containing ferrous gluconate became darker in color, but the solution was stable. The HCSB having ferrous gluconate was stable under all conditions, and color changes were minor. In the all-sugar syrup combined with ferrous sulfate, a reddish top layer was observed followed by development of a red precipitate; in RCSB a white precipitate changed to black, but in HCSB color changes were minor and the combination was stable.

When syrups were stored in half-full uncapped bottles, a partial dehydration occurred, and a protective surface film formed. Under these conditions, the precipitate in sample A-5 changed from red to slightly black, and no precipitates formed in B-4 and B-5. In the black precipitates, the iron form was ferrous and unchanged, but oxidation to ferric iron occurred in sample A-5 as evidenced by a red precipitate. This was confirmed by chemical means (Kolthoff and Sandell, 1949). The black precipitate was negative when tested for sulfide.

Although no positive identification was made, the reaction apparently requires water and oxygen. A greater tendency to entrap air was observed in RCSB which is more viscous than HCSB, and this tendency could account for the formation of black precipitate in RCSB and not in HCSB. The greater water attraction associated with increased DE, such as in HCSB, could also be a factor. The black precipitate could be either an intermediate hydrated ferrous sulfate complex or the hydrolysis product ferrous hydroxide, $Fe(OH)_2$ (Kolthoff et al., 1962). The

Table 4—Flavor stability of syrups with iron (0.014–0.016%) and without iron

No.	Syrup and iron-type	Initial	Mean flavor scores[a] Days storage at various temperatures: 120°F 28	120°F 56	100°F 182	77°F 182	77°F 365
A-1	Sugar and no iron	10.0	9.3	9.5	9.5	9.6	9.7
A-2	Sugar + ferric ammonium citrate	9.0	7.8	8.3	8.9	8.6	7.7
A-3	Sugar + ferric choline citrate	8.5	8.1	8.2	8.1	8.9	8.3
A-4	Sugar + ferrous gluconate	8.0	7.9	7.5	7.3	7.5	8.4
A-5	Sugar + ferrous sulfate	7.9	–	7.4	8.0	8.1	8.5
B-1	RCSB[b] and no iron	10.0	9.6	9.7	9.3	9.5	9.4
B-2	RCSB + ferric ammonium citrate	9.2	9.1	8.9	8.5	8.9	7.9
B-3	RCSB + ferric choline citrate	9.1	9.0	9.3	8.6	9.0	8.4
B-4	RCSB + ferrous gluconate	9.1	–	9.1	8.5	9.0	8.6
B-5	RCSB + ferrous sulfate	9.4	–	9.4	9.0	8.5	8.3
C-1	HCSB[b] and no iron	10.0	9.6	9.5	9.3	9.7	9.4
C-2	HCSB + ferric ammonium citrate	9.2	8.3	8.4	7.9	8.9	8.2
C-3	HCSB + ferric choline citrate	9.3	8.8	8.7	8.6	9.0	8.1
C-4	HCSB + ferrous gluconate	8.8	8.1	8.3	8.1	8.9	8.1
C-5	HCSB + ferrous sulfate	8.8	8.4	8.3	8.6	8.7	8.2

[a]Each mean based on scores from 14 to 16 panelists.
[b]See footnotes d and e, Table 3.

Table 5—Visual appearance ranking by color[a] and stability of bottled syrups with iron (0.014–0.016%) and without iron

		Days storage at various temperatures											
				120° F				100° F		77° F			
No.	Syrup and iron-type	0		56		56[b]		182		182		365	
A-1	Sugar and no iron	3	Very light straw	1	Straw	9	Dark yellow straw	1	Light straw	1	Very light straw	1	Light straw
A-2	Sugar + ferric ammonium citrate	14	Amber	12	Dark amber	14	Dark amber	12	Dark amber	9	Light amber	11	Dark amber
A-3	Sugar + ferric choline citrate	6	Very light yellow straw	7	Dark yellow	10	Dark yellow	5	Light yellow	5	Yellow straw	7	Light yellow
A-4	Sugar + ferrous gluconate	12	Light amber	11	Amber	12	Grayish pale amber	9	Light amber	12	Amber	12	Dark amber
A-5	Sugar + ferrous sulfate	U	Reddish top layer	U	Red precipitate	U	Slight black ppt	U	Red precipitate	U	Red precipitate	U	Red precipitate
B-1	RCSB[c] and no iron	1	Colorless	2	Light yellow straw	3	Yellow straw	2	Straw	2	Light straw	2	Light straw
B-2	RCSB + ferric ammonium citrate	11	Very light amber	8	Amber	7	Amber	10	Amber	8	Light amber	9	Amber (2 dark amber[d])
B-3	RCSB + ferric choline citrate	6	Very light yellow straw	6	Yellow	5	Dark yellow	5	Light yellow	5	Yellow straw	5	Light yellow
B-4	RCSB + ferrous gluconate	9	Very light amber	U	Black precipitate	12	Grayish pale amber	U	Black precipitate	U	Black precipitate	U	Black precipitate
B-5	RCSB + ferrous sulfate	2	Nearly colorless	U	Black precipitate	11	Grayish straw	U	Black precipitate	U	Black precipitate	U	Black precipitate
C-1	HCSB[c] and no iron	4	Very light straw	2	Light yellow straw	1	Yellow straw	3	Straw	3	Light straw	3	Straw
C-2	HCSB + ferric ammonium citrate	12	Light amber	9	Amber	7	Amber	11	Amber	10	Light amber	10	Amber
C-3	HCSB + ferric choline citrate	8	Yellow straw	5	Yellow	4	Light yellow	5	Light yellow	5	Yellow straw	6	Light yellow
C-4	HCSB + ferrous gluconate	10	Very light amber	10	Light amber	6	Very light amber	8	Light amber	11	Light amber	8	Light amber
C-5	HCSB + ferrous sulfate	5	Very light straw	4	Light yellow straw	2	Straw	4	Straw	4	Light straw	4	Straw (1 grayish straw[d])

[a]Ranking 1 (least) to 12–14 (most) solution color for each storage condition; unstable (U) samples unranked
[b]Uncapped bottles stored one-half full
[c]See footnotes d and e, Table 3.
[d]Capped bottles stored one-half full

black precipitate is unstable to acid. More air and water are necessary to form a red precipitate, which may be either hydrated ferric sulfate, $Fe_2(SO_4)_3 \cdot nH_2O$ (Mellor, 1942), or ferric hydroxide, $Fe(OH)_3$ (Kolthoff et al., 1962). Further studies are necessary to determine the exact nature of these reactions.

Iron-enriched infant feeding formulas

Infant feeding formulas were prepared with iron-fortified syrup (0.015% Fe, 100 mg Fe/pint). RCSB containing ferric ammonium citrate (B-2) and ferric choline citrate (B-3) and HCSB containing ferrous gluconate (C-4) and ferrous sulfate (C-5) were tested. Comparisons were made with the controls RCSB (B-1) and HCSB (C-1). Each formula was filtered through a standard milk filter disc. The creamy white appearance and other physical aspects of all infant formulas with or without iron were similar. The calculated iron level of approximately 2.3 mg/8 oz infant formula was met in each baby formula. This similarity of iron level and the absence of coagulated particles on the filter discs indicate that no reactions occurred during preparation to change particle size and block passage through the hole in a nipple. All samples were tasted and no differences were found.

Infant feeding formulas were prepared with B-2 (RCSB-ferric ammonium citrate), Corn Syrup Blend (Table 1), Staley crystal corn syrup and Karo light corn syrup. Coded, randomized samples were presented to a 10-member panel and rated for flavor and color differences. Seven of the panel indicated no difference in flavor; three indicated B-2 as having a slightly different flavor; one indicated Corn Syrup Blend as having a slightly different flavor. None of the panelists indicated a color difference in either of the infant feeding formulas. Apparently no major color or flavor differences are introduced when a suitable iron-fortified syrup is added to infant feeding formulas.

CONCLUSIONS

STABLE SYRUP blends can be formulated at iron levels of 0.015% (100 mg/pint) with either ferric ammonium citrate, ferric choline citrate, ferrous gluconate or ferrous sulfate for conveniently fortifying infant feeding formulas. Satisfactory functional properties in infant formulas can be attained most economically with either ferric ammonium citrate in regular conversion corn syrup with 15% sugar (RCSB) or ferrous sulfate in high-conversion corn syrup (HCSB) with 15% sugar.

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NEW COMPUTATIONAL PROCEDURE FOR DETERMINING THE APPARENT THERMAL DIFFUSIVITY OF A SOLID BODY APPROXIMATED WITH AN INFINITE SLAB

INTRODUCTION

IT HAS FREQUENTLY been required to predict temperature history curves of foods during various heating or cooling treatments in order to determine proper procedures for these treatments. The thermal diffusivity of food is a physical parameter required for this prediction and is defined as follows: thermal diffusivity = thermal conductivity/(density × specific heat).

Dickerson (1968), Hill et al. (1967), Reidy (1968) and Woodam and Nowrey (1968) compiled data on the thermal conductivity and/or diffusivity values of various foods reported in published literature. Although a number of these values are given in the literature, these values should be determined frequently since different farming and/or food processing practices result in different physical properties of foods.

Various research workers reported their procedures for determining thermal conductivity or diffusivity of sample material (Bakal, 1970; Ball and Olson, 1957; Charm, 1963; Dickerson, 1965; Filippov, 1966; Frechette and Zahradnik, 1966; Gruen and Walz, 1965; Hayakawa, 1969; Hill et al., 1967; Mathews and Hall, 1967; Massey and Sunderland, 1967; Morley, 1966; Myncke et al., 1964; Pflug et al., 1965; Picot, 1968; Somerton and Mossaheibi, 1967; Tait and Hills, 1964; Tye, 1968). Reidy (1968) reviewed various procedures, which were utilized to determine the conductivity or diffusivity values of foods. It is important to note that most of these procedures require expensive instruments: e.g., constant temperature bath with accurate temperature control, accurate temperature sensors and/or high speed temperature recorders.

One of the procedures, which has frequently been utilized by various investigators, is based on the application of the first term of a theoretical formula for heat conduction to an experimental temperature history curve (Charm, 1963; Pflug et al., 1965). This curve is usually obtained by plotting the common logarithm of temperature difference between food and ambient medium against heating or cooling time. One of the great advantages of the procedure is that a diffusivity value can be estimated without any complicated calculations.

The first term of a theoretical formula estimates accurately a portion of a temperature history curve. For determining a diffusivity value, the term should be applied to this portion. A procedure for selecting the portion was reported by Pflug et al., (1965).

The Biot numbers of sample material should be evaluated in most cases when data on temperature differences between food and surrounding medium are used to determine diffusivity values. For this evaluation, additional computation and/or additional heat transfer experiments are required.

When temperatures on the surface of sample food are used for determining thermal diffusivity values, there is no need for determining Biot numbers. However, there is great difficulty in measuring surface temperatures. All published procedures which utilize data on surface temperatures for diffusivity value determination, were developed by assuming a constant surface temperature during heat transfer experimentation.

In the present investigation, a new computational procedure was developed for determining thermal diffusivity values of solid materials without measuring temperatures on the surface of sample material.

EXPERIMENTAL

Development of computational procedure

A theoretical formula for heat conduction is required to determine the thermal diffusivity value of sample material. In the present investigation, a theoretical model imposing the following four assumptions is used.

(1) The geometrical configuration of a sample body is an infinite slab.
(2) Temperatures in the sample are uniformly equal to a constant temperature at the zero time of heating or cooling.

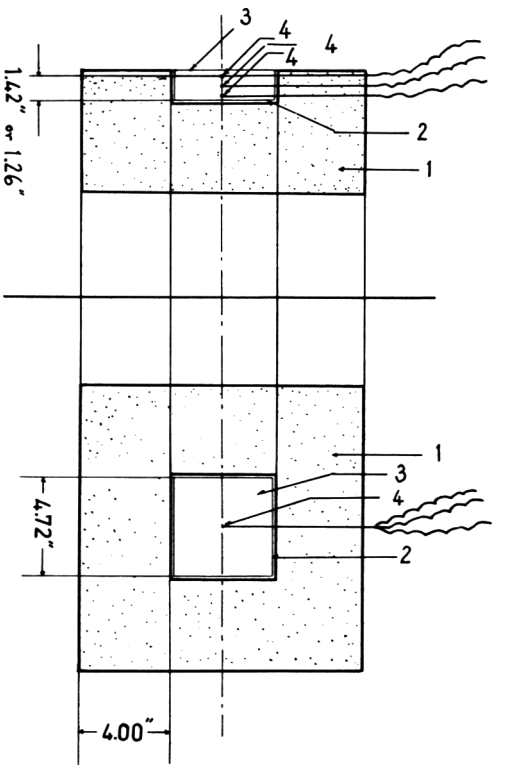

Fig. 1—Insulated rectangular cell used for determining the thermal diffusivity value of food: 1—Expanded polystyrene; 2—Polyethylene container; 3—Polyethylene cover; 4—Copper-constantan thermocouple junctions.

(3) One surface of a slab is thermally insulated and another surface is subjected to time variable surface temperatures. These variable temperatures are assumed to be known and a curve, which represents a relationship between these temperatures and heating or cooling time, is approximated with a combination of straight line segments.
(4) The thermal diffusivity of sample is temperature and location invariable.

The first assumption is imposed since a theoretical formula for heat conduction in the solid of this shape is relatively simple when it is compared with formulae for other geometrical shapes. Another reason for this assumption is that a sample holder, which satisfies it, may be easily fabricated.

The second assumption is imposed to simplify the derivation of the heat conduction formula and also to reduce computational work required for the determination of diffusivity. To satisfy this assumption, the sample must be placed in a constant temperature bath or chamber before heat transfer experimentation.

In most cases temperature variation on one surface of a slab is not identical to that on another surface when both its surfaces are exposed to heating or cooling medium: the reason for imposing the third assumption. It is important to note that the physical situation described in the third assumption can easily be simulated by using good insulation material. There is only one thermophysical parameter included in the theoretical formula which satisfies the third assumption. The thermal diffusivity of sample material can be determined through straight forward calculations when this formula is utilized. The above stated approximation of the surface temperature-time curve is imposed since any curve may be accurately represented with a combination of straight line segments.

The fourth assumption is imposed since the estimation of diffusivity becomes extremely complicated without this. It will be important to note that the diffusivity of a sample material does not vary greatly with changes in temperature in most cases when there is no phase change involved during heating or cooling (Carslaw and Jaeger, 1962).

A theoretical formula for heat conduction which satisfies the above stated assumptions is derived by using Duhamel's theorem (Carslaw and Jaeger, 1962). This formula is given below; its derivation is given in the Appendix:

$$v = T_{\bar{p}} + \frac{16\varrho^2}{\alpha\pi^3} \sum_{i=1}^{p} a_i \left[\sum_{i=1}^{\infty} Q_{pn(i-1)} - \sum_{i=1}^{\infty} Q_{pni} \right] \quad (1)$$

Where

$$Q_{pni} = (-1)^{n+1} \cdot \left\{ \cos(2n-1)\ \pi \times /(2\varrho) \right\} \cdot \left\{ 1/(2n-1)^3 \right\} \cdot \exp \left\{ -A_n (p-i)\ \Delta t \right\} \quad (1a)$$

$$A_n = \alpha(2n-1)^2\ \pi^2 /(2\varrho)^2 \quad (1b)$$

Equation (1) estimates temperature, v, at any location, x, in an infinite slab at the pΔt times of heating or cooling. This equation is utilized to determine the thermal diffusivity value of sample material. A procedure for determining this value is given below.

Thermal diffusivity value of sample. Temperatures of sample material are monitored experimentally at its internal locations and on its exposed surface. These temperatures are recorded at uniform time intervals (jΔt: j=1, 2, ..., p).

Equations (1, 1a and 1b) are used to calculate theoretical temperatures at the same time intervals in order to compare them with the recorded temperatures. For this calculation, experimental temperatures on the exposed surface are used together with various assumed thermal diffusivity values. For each value assumed, the sum of squares of differences between experimental and theoretical temperatures is calculated. The diffusivity value of sample material is determined by calculating a value, at which the sum of squares of differences becomes minimum. This calculation is accomplished by applying a five-point inverse interpolation formula (Salzer, 1951). This formula is for estimating the value of the independent variable at which the first order derivative of a dependent variable becomes a specified value. The computational procedure developed is similar to a procedure described by Mathews and Hall (1967), although their procedure is based on the numerical intergration of a heat conduction equation and is applicable only when the surface temperature of a slab is constant. The sum series in the brackets

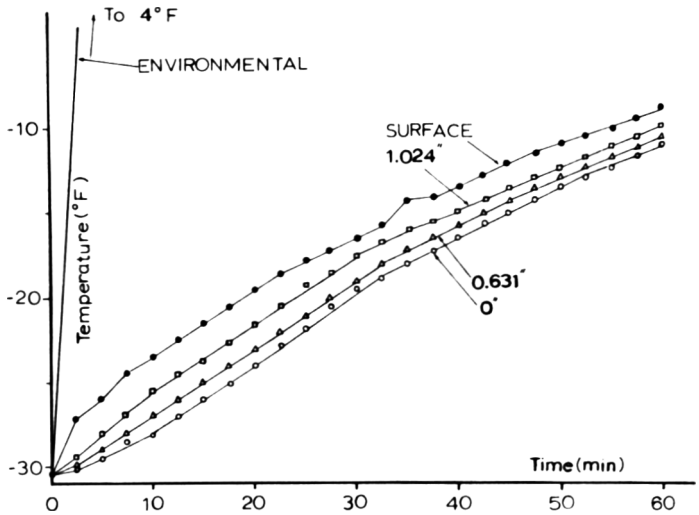

Fig. 2—Typical temperature history curves of ground beef meat which were monitored during heat transfer experimentation (Run No. 1).

of Eq. 1 are estimated by applying Euler's transformation (Hildebrand, 1956) since they are slowly convergent. A set of Fortran IV computer programs is prepared since massive computational work is required to calculate one diffusivity value.[1] This set of the programs print out determined diffusivity values and also produces three curves from each set of experimental data: (1) a curve which is obtained by plotting the sums of squares of differences between experimental and calculated temperatures and various diffusivity values assumed; (2) a temperature history curve of sample food, which is determined through heat transfer experimentation; and (3) a temperature history curve of sample food, which is calculated by using a determined diffusivity value. These curves are used to confirm visually estimated diffusivity values.

Surface temperature. Temperatures on the surface of sample material are quite difficult to be measured. The proposed computational procedure does not require measuring those temperatures since any time variable surface temperatures are assumed for its development. Temperatures, which are monitored at an internal location in an infinite slab, may be used as surface temperatures of an imaginary slab. The thickness of this imaginary slab is identical to a distance between this internal location and the insulated surface of the real slab.

Application of computational procedure

The developed procedure may be applied to determine the apparent thermal diffusivity value of solid material if the assumptions stated above are satisfied. The physical condition which is defined by these assumptions may be simulated through the use of insulated rectangular cells (Fig. 1). These cells are fabricated from expanded polystyrene slabs and from polyethylene containers. Expanded polystyrene slabs are used because of its excellent insulation properties. Its thermal conductivity is approximately 3×10^{-3} Btu/(ft°F hr) (ASHRAE, 1972). The design of these cells is determined after a series of heat transfer experimentations. It is observed that iso-temperature planes in grapefruit juice filled in each cell are parallel to its exposed surface, except the area close to the four sides of the cell. Therefore, heat transfer in the sample food in the cells may be approximated with that in an infinite slab. The polyethylene containers are used for holding sample material during heat transfer experiments. The physical dimensions of each container are: wall thickness = 0.08 in.; cross sectional area = 4.72 × 4.72 in.^{2}; depth = 1.42 in. or 1.26 in. The four sides and bottom end of the container are insulated with a 4-in. thick expanded polystyrene slab. Sample material is filled into the container and a polyethylene cover applied to prevent evaporative loss of moisture during heat transfer experiments. Thermocouple junctions are inserted from the side of the cell and placed on the central axis of the container. These junctions were made from 30 gauge copper-constantan thermocouple wire. Metal sheaths are not used to protect the thermocouples in order to minimize

[1] A copy of these programs is available upon request to the senior author.

heat loss or gain from surrounding medium to sample material. The physical stability of the thermocouples is maintained through the application of epoxy resin coatings. The thermocouples are glued firmly to the cell wall with epoxy resin. One thermocouple is placed approximately ¼ in. below the level up to which the sample is filled; others are located under this thermocouple. Temperatures monitored by these thermocouples are recorded by a multipoint temperature recorder (Honeywell Electronik 16).

RESULTS

PREPARED CELLS are used to determine the thermal diffusivity values of these samples: distilled water, 50% sugar aqueous solution, 40° Brix grapefruit juice and ground beef meat. In order to avoid convective movement of sample liquid, all determinations are done in sub-freezing temperature ranges. Each sample is carefully filled in a cell and placed in a high-low temperature chamber. Temperature in this chamber can be set at any one temperature between −100°F and +350°F. It is also possible to change the chamber temperature with time. Each cell is exposed to a constant chamber temperature. When a temperature distribution in the sample becomes uniform, it is exposed to time variable chamber temperatures. The temperatures of the sample are monitored by the thermocouples placed in the cell during this exposure.

Figure 2 shows typical temperature data obtained. These data represent transient temperature distribution in ground beef when environmental temperatures were maintained at 4 ± 0.5°F during an entire heat transfer experiment except during its initial time interval of 10 min. Diffusivity values are calculated by an IBM 360/67 computer through the application of the programs developed. About 0.2 min of computer time is required to calculate one diffusivity value. Diffusivity values obtained are given in Table 1 together with their mean values. Typical curves which were plotted by the computer are shown in Figures 3 and 4. Figure 3 clearly shows that the best estimate for the apparent thermal diffusivity value of frozen ground beef is approximately 0.0365 in.^{2}/min. This visually verifies the methematical validity of a diffusivity value (0.0362 in.^{2}/min) determined through the inverse interpolation. Figure 4 shows computer produced temperature history curves.

DISCUSSION

THE DIFFUSIVITY VALUES of ice, determined in the present investigation, are compared with those estimated from thermophysical properties of ice using the equation: thermal diffusivity = (thermal conductivity)/(sepcific heat × density). Thermal conductivity values were obtained from Lentz (1961) and specific heat and density values from a handbook (ASHRAE, 1972). The estimated diffusivity values of ice are 0.107–0.127 (in.^{2}/min) and are temperature dependent. The lower the temperature of ice, the larger the diffusivity values.

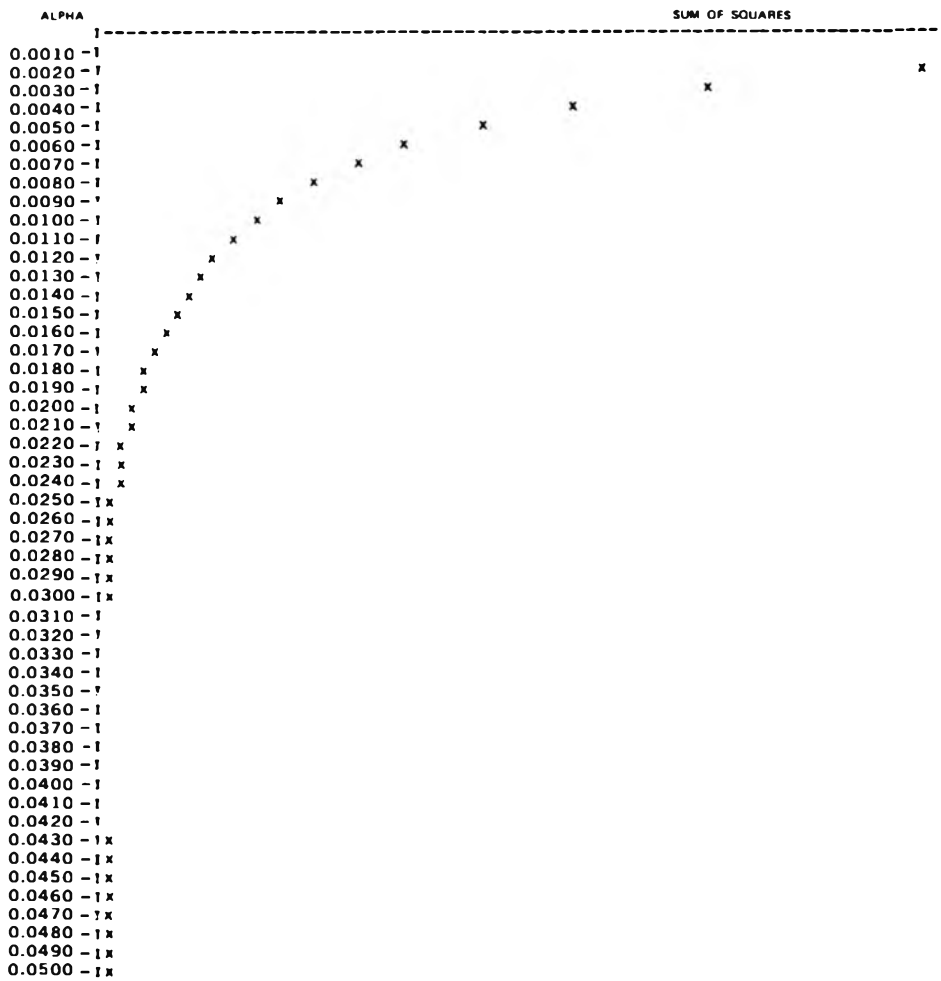

Fig. 3—Typical computer produced curve, which was obtained by plotting thermal diffusivity values assumed against the sums of squares of differences between the estimated and experimental temperatures of ground beef (Run No. 3, x = 1.024 in.).

The values determined in the present investigation are very close to these estimated values.

No diffusivity values of other samples, which are used in the present investigation, have been reported in published literature.

Therefore, the diffusivity values determined are compared with those of similar materials. The mean diffusivity value of concentrated grapefruit juice is compared with that of frozen concentrated orange juice (Keller and Ballard, 1956). The values estimated by the proposed procedure are from two to three times smaller than these published data. The close evaluation of a procedure used by Keller and Ballard (1956) reveals that their values are most likely over estimated since wood blocks, which are not good insulation material, are used to insulate two ends of the cylindrical cell used. The thermal conductivity of wood is approximately four to five times greater than that of expanded polystyrene (ASHRAE, 1972). It is also important to note that a heat conduction equation was erroneously utilized for determining diffusivities for their investigation. This formula was derived by assuming temperature independent thermophysical properties of sample material. However, they used it for estimating temperature variable diffusivity values. To estimate these values, a different heat conduction equation should be utilized (Carslaw and Jaeger, 1962).

The diffusivity values of 50% sugar solution, determined in the present investigation, are compared with those obtained by extrapolating those obtained by Keppler and Boose (1970). The latter values are 25–50% greater than the former values. It is unfortunate that in their investigations, Keppler and Boose erroneously applied a formula derived by Thomas (1957). It is important to note that Thomas's formula was obtained by imposing the following assumptions: (1) the geometrical shape of the sample solid is an infinite cylinder; (2) a constant heat flux is applied to the surface of sample solid; (3) a theoretical formula for heat conduction in sample, which consists of two terms, may be approximated with one term; and (4) thermal diffusivity is temperature independent.

The careful evaluation of a device used by Keppler and Boose (1970) reveals that most of these assumptions, which include the most critical assumption on a constant heat flux, are not satisfied. Therefore, the validity of their data is questionable.

The diffusivity values of ground beef are compared with those of unground beef. The diffusivity values of frozen beef are calculated from the thermal conductivities, specific heats and densities of frozen beef. Thermal conductivities and specific heats are obtained from a review by (Reidy, 1968) and densitities from a text book (Charm, 1970). Even though there is a formula for calculating the density of fresh meat (Pflug et al., 1965), this formula is not used since there are no published data available on the density values of various meat components in their frozen states. The mean temperatures of ground beef, T_m, given in Table 1 are in this range: $5^\circ F > T_m > -20^\circ F$. Conductivities and specific heat in the same temperature range are collected for various varities of beef meats and

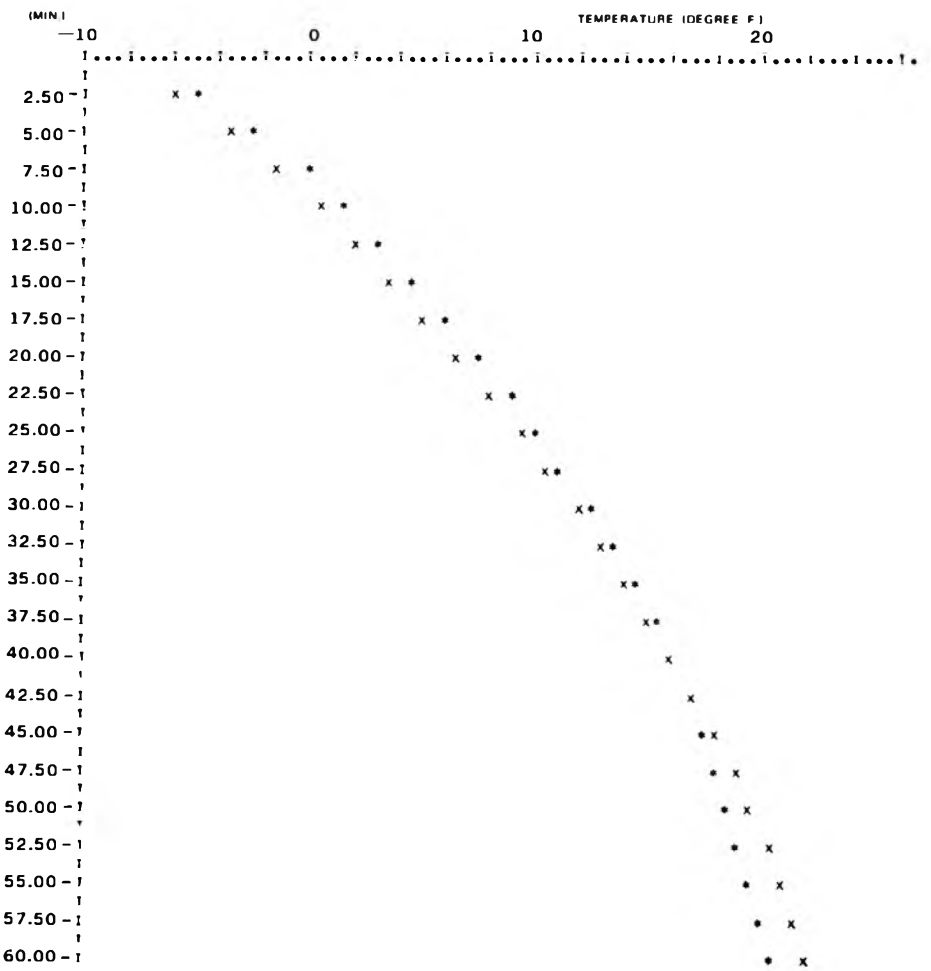

Fig. 4—Typical temperature history curves of ground beef (Run No. 3, x = 1.024 in.) which were produced by a digital computer. (•—•—• = Experimental; x—x—x = Estimated)

Table 1–Apparent thermal diffusivity values estimated by the proposed procedure[a]

Food	Run	ℓ = depth of cell (in.)[a]	x = location of thermocouple junction (in.)[b]	T_o = initial temp. of food (°F)	T_f = final temp. of food (°F)	T_m = mean of T_o and T_f (°F)	α = apparent thermal diffusivity of food (in.²/min)	Mean value of α (in.²/min)
Water, ice	1	1.260	0.866	−41.0	−1.0	−21.0	0.121	
			0.433		−1.0	−21.0	0.114	
			0		−1.1	−21.1	0.107	0.114
	2	1.260	0.866	2.0	24.2	13.1	0.102	
			0.433		24.1	13.1	0.0915	
			0		24.0	13.0	0.0900	0.0945
Conc grapefruit juice (40° Brix)	1	1.260	0.866	−36.0	−2.0	−19.0	0.00545	
			0.433		−2.8	−19.4	0.00899	
			0		−4.0	−20.0	0.00923	0.00789
	2	1.260	0.866	−40.0	−16.5	−28.3	0.00650	
			0.433		−17.2	−28.6	0.0106	
			0		−18.5	−29.3	0.0108	0.00930
Sucrose aqueous solution (50%)	1	1.260	0.866	−29.0	−17.0	−23.0	0.0311	
			0.591		−18.5	−23.8	0.0301	
			0		−20.0	−24.5	0.0237	0.00283
	2	1.260	0.866	−39.5	−13.5	−26.5	0.0229	
			0.591		−14.0	−26.8	0.0275	
			0		−15.0	−27.3	0.0245	0.00250
Ground beef meat	1	1.417	1.024	−30.5	−10.0	−20.3	0.0908	
			0.630		−10.5	−20.5	0.101	
			0		−11.0	−20.8	0.0971	0.0963
	2	1.417	1.024	1.5	−16.8	−7.7	0.0308	
			0.630		−16.0	−7.3	0.0668	
			0		−15.5	−7.0	0.0718	0.0565
	3	1.417	1.024	−7.0	20.5	6.8	0.0362	
			0.630		18.5	5.8	0.0429	
			0		17.0	5.0	0.0445	0.0412

[a]Values listed are converted from data measured in millimeters.
[b]Measured from an insulated end; converted from data measured in mm.

for various orientations of meat fibers. These values are:

Thermal conductivity = 0.640 ~ 0.900 Btu/(hr ft°F)
Specific heat = 0.45 ~ 0.75 Btu/(lb °F)

According to Charm (1970), the density of frozen meat is 60 lb/ft³. The range of thermal diffusivity values estimated from these values are: 0.0341–0.0987 (in²/min). It is observed that the mean diffusivity values of ground beef determined in the present investigation agree with the estimated values, although this agreement does not have any great significance because of the wide variation. Diffusivity values which are determined with Run No. 3 experiment, are considerably smaller than other diffusivity values. This might be caused by the relatively high temperature data used for this determination. It is most likely that a part of the heat transfered is consumed as the latent heat of fusion at these temperature.

The following trend is observed from published data on the diffusivity values of various frozen materials: These values decrease with increases in food temperatures within the ranges of subfreezing temperatures. This is also observed in the mean diffusivity values given in Table 1, except those of sucrose solutions. A relatively small difference in mean temperatures between the first and second runs of sucrose solutions would be a major reason of this exception.

There are considerable variations among diffusivity values which are evaluated at different locations in one sample material. We have experienced some difficulties in accurately measuring distances between thermocouple junctions and the inside bottom surface of each cell. Therefore, errors in diffusivity values determined are analyzed when there are errors in measuring these distances and also in measuring the depth of sample material in a cell. This analysis is accomplished by using computer programs, which are obtained through the slight modifications of the programs used to determine diffusivity values. It is observed that errors in measuring the distances stated above are at most 0.0394 in (1 mm). Therefore, errors in estimated diffusivity values are calculated by assuming that there are errors of ± 0.0394 in these measurements. Typical results of this calculation are shown in Table 2. It is observed that there are ± 30% or less errors in diffusivity values determined.

As stated previously it is not necessary to measure the temperature on the surface of a slab when the proposed computational procedure is used. Since surface temperatures are difficult to monitor, this characteristic of the proposed procedure is of great value for conducting heat transfer experimentation to determine diffusivity values. Any properly designed cells may be used to apply this procedure if they can simulate heat conduction in an infinite slab. The developed procedure may also be applied to determine a diffusivity value of an infinite slab whose both surfaces are exposed to a surrounding heat exchange medium. For this application, temperature variations on both surfaces must be identical. If this is satisfied, ℓ and x respectively represent a distance between real or imaginary surface and the central plane of the slab and that between the location of a thermocouple junction and the central plane.

Equation (1) is used to develop the proposed computa-

Table 2—Errors in thermal diffusivity values determined when there are ± 0.0394 in. errors in x and ℓ values measured.

Product	Run No.	ℓ (in.)	x (in.)	Errors in x values measured[a] (in.)	Percent errors in diffusivity values when there are the following errors in ℓ values measured[a]		
					−0.0394 in.	0 in.	+0.0394 in.
Conc grapefruit juice	1	1.260	0.866	−0.0394	−1.31	+11.79	+26.45
				0	−14.48	0.00	+15.05
				+0.0394	−27.35	−12.42	+3.64
Sucrose aqueous solution (50%)	1	1.260	0.591	−0.0394	−3.63	+4.50	+13.19
				0	−7.98	0.00	+8.72
				+0.0394	−12.90	−4.69	+4.01
Ground beef meat	3	1.417	0.630	−0.0394	−3.41	+3.77	+11.20
				0	−7.19	0.00	+7.39
				+0.0394	−10.99	−4.02	+3.37

[a]Negative and positive signs represent underestimated and overestimated values, respectively.

tional procedure. This equation was derived by assuming that the thermal diffusivity of a slab is temperature independent. It is well known that the diffusivity values of most foods are temperature dependent. Therefore, the range of temperature variations during a heat transfer experiment should be as narrow as possible to determine diffusivity values accurately.

The proposed procedure was used to determine diffusivity values of solid materials in the present investigation. This procedure may be applicable for determining diffusivity values of porous or glanular materials if the assumptions imposed for deriving Eq. (1) are satisfied. However, this application should be examined through further investigation.

CONCLUSION

A NEW PROCEDURE is developed for determining the apparent thermal diffusivity of solid material. The computational program prepared in the present investigation may be used whenever the assumptions, which are imposed to derive the theoretical formulas of heat conduction, are satisfied. It is suggested that the positions of thermocouple junctions, which are placed in a rectangular cell, should be accurately determined in order to estimate accurately thermal diffusivity values. The developed procedure uses the entire temperature history curve of sample material determined during heat transfer experimentation. Great advantages of the developed procedure are that sample food can be exposed to any time variable heating or cooling temperatures during this experimentation and that there is no need for monitoring surface temperatures.

APPENDIX

Derivation of Eq. (1): a formula for heat conduction in an infinite slab.

The thickness of an infinite slab is ℓ and its initial temperature is assumed to be zero. One surface of the slab, x = 0, is insulated and the other surface, x = ℓ, is subjected to a step change in its surface temperature at the zero time of heating or cooling. Transient temperatures, u, at any locations are calculated by the following equation (Carslaw and Jaeger, 1962):

$$u(x,t) = 1 - \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{(2n+1)} \cdot \exp\left[-\alpha (2n+1)^2 \pi^2 t/(4\ell^2)\right] \cdot \cos\left[(2n+1)\ \pi x/(2\ell)\right] \quad (a)$$

Equation (a) is used to derive a formula for calculating transient temperature distributions in an infinite slab when temperatures on its one surface change with time and when its other surface is insulated.

We assume that the time variable surface temperatures are represented with a set of straight line segments, Eq. (b):

$$\phi(t) = \begin{cases} a_1 t & 0 \leq t \leq \Delta t \\ & \Delta t \leq t \leq 2\Delta t \\ a_2 t + b_2 & \\ \vdots & \vdots \\ a_i t + b_i & (i-1)\Delta t \leq t \leq i\Delta t \\ \vdots & \vdots \\ a_p t + b_p & (p-1)\Delta t \leq t \leq p\Delta t \end{cases} \quad (b)$$

A formula for calculating a transient temperature distribution v (x, t), in the infinite slab at t = pΔt can be derived by applying Duhamel's theorem to Eq. (a) and (b):

$$v(x,t) = \int_0^{p\Delta t} \phi(\lambda) \frac{\partial}{\partial t} u(x, t-\lambda)\, d\lambda$$

$$= \frac{\alpha\pi}{\ell^2} \sum_{i=1}^{p} \int_{(i-1)\Delta t}^{i\Delta t} (a_i t + b_4) \cdot \sum_{n=0}^{\infty} (-1)^n \cdot (2n+1) \cdot \left[\cos\ (2n+1)\ \pi x/(2\ell)\right]$$

$$\cdot \exp\left[-\alpha(2n+1)^2 \pi^2 (t-\lambda)/(4\ell^2)\right] \cdot d\lambda$$

$$= \frac{\alpha\pi}{\ell^2} \sum_{i=1}^{p} a_i \sum_{n=0}^{\infty} (-1)^n (2n+1) \cos\left[(2n+1)\,\pi\, x/(2\ell)\right] \cdot \left\{\exp\left[-\alpha(2n+1)^2 \pi^2/(4\ell^2)\right] \cdot \left[p-(i-1)\right] \cdot \Delta t\right\}$$

$$- \exp\left\{\left[-\alpha(2n+1)^2 \pi^2/(4\ell^2)\right] \cdot \left[(p-i)\cdot\Delta t\right]\right\} \Big/ \left\{\left[\alpha(2n+1)^2 \pi^2/(4\ell^2)\right]^2 + \left[(\alpha\pi/\ell^2) \sum_{n=0}^{\infty} (-1)^n (2n+1)\right] \cdot \left[T_p \Big/ \left[\alpha(2n+1)^2/(4\ell^2)\right]\right] \cdot \cos\left[(2n+1)\,\pi x/(2\ell)\right] \quad (c)$$

In the above equations, T_p denotes the surface temperature when t = pΔt. The second sum series becomes equal to T_p because of the following equality:

$$\frac{4}{\pi} \sum_{n=0}^{\infty} \left[(-1)^n / (2n+1) \right] \cdot \cos \left[(2n+1)\, \pi x / (2\varrho) \right] = 1 \qquad (d)$$

Most computers have difficulties in arithmetic calculations which involve the numeral zero. Therefore, the sum index, n, in Eq. (c) is changed in order to avoid these calculations. The resultant formula is given below:

$$v(x,t) = T_p + (\alpha\pi / \varrho^2) \cdot \sum_{i=1}^{p} a_i \sum_{n=1}^{\infty} (-1)^{n+1} \cdot (2n-1) \cdot$$

$$\cdot \langle \exp\left[-\left\{ \alpha(2n-1)^2 \pi^2 / (4\varrho^2) \right\} \cdot \left\{ p - (i-1)\,\Delta t \right\} \right]$$

$$- \exp\left[-\left\{ \alpha (2n-1)^2 \pi^2 / (4\varrho^2) \right\} \cdot (p-i) \cdot \Delta t \right] \rangle$$

$$/ \left\{ \alpha(2n-1)^2 \pi^2 / (4\varrho^2) \right\}^2 \cdot$$

$$\cdot \cos \left[\left\{ (2n-1)\ \pi x \right\} / (2\varrho) \right] \qquad (e)$$

Eq. (e) becomes equal to Eq. (1) when Eq. (1a) and (1b) are substituted into Eq. (e).

Nomenclature

A_n	Expression defined by Eq. (1b).
a_1, a_2, a_i, a_p	Slopes of 1st, 2nd, ith and pth line segments respectively (°F/min).
b_2, b_i, b_p	Intercept coefficients of 2nd, ith and pth line segments respectively (°F).
i	Dummy summation index.
ϱ	Height of sample material filled in insulated cell (in).
n	Dummy summation index.
p	Number of line segments with which a temperature history curve on the uninsulated surface of sample material is approximated.
$Q_{pni}, Q_{pn(i-1)}$	Expressions obtained by entering j = i and j = (i − 1) into Eq. (1a) respectively.
Q_{pnj}	Expression defined by Eq. (1a)
T	Temperature variable (°F)
T_o	Initial temperature of sample material (°F)
T_f	Temperature of sample material when t = pΔt(°F)
T_m	$= (T_f + T_o)/2$ (°F).
T_p	Value of v on uninsulated surface of sample material when t = pΔt(°F)
t	Time variable (min).
Δt	Uniform time intervals (min).
v	$= T - T_o$ (F°)
x	Distance between thermocouple junction and insulated inside surface of cell (in).
α	Thermal diffusivity of sample material (in²/min).
λ	Dummy integration variable.

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TEMPERATURE DISTRIBUTIONS DURING HEAT/HOLD PROCESSING OF FOOD

INTRODUCTION

THE INDIVIDUAL Quick Blanch (IQB) method (Lazar et al., 1971) for blanching vegetables reduces the inequity of heat treatment that the outer portion of the vegetable receives compared to the center and reduces waste water generation in blanching. The method involves a two-stage process: (1) heating the piece to the appropriate mass average temperature and (2) adiabatically holding the piece at this mass average temperature until enzymes at the center are destroyed. Charts or procedures for estimating center and mass average temperature have been presented for the infinite slab and cube (Lund et al., 1972) but not for the sphere or infinite cylinder. It is the purpose of this paper to present solutions and charts derived from these solutions for estimating center and mass average temperature as a function of heating and holding time for the three basic geometries. The application of these charts for prediction of temperatures during blanching is illustrated. These charts could also be used for calculating temperatures in split cook processes (Martin and Wodicka, 1951) and calculating thermal properties of foods (Wadsworth and Spadaro, 1969). Combination of these solutions with kinetic parameters for nutrients and microorganisms would permit nutrient retention and lethality calculations for heat/hold processes.

THEORY

THE TEMPERATURE PROFILES for the three basic geometries (infinite slabs, infinite cylinders and spheres) were obtained by using the heating temperature profile as the initial temperature distribution for the adiabatic equilibration step. Thus, the three boundary conditions for the adiabatic hold are: (1) the heat flux at the surface is zero; (2) the heat flux at the center is zero due to symmetry; and (3) the initial temperature distribution is a function of heating time. Carslaw and Jaeger (1959) give the temperature profile during adiabatic equilibration for the infinite slab (page 118), infinite cylinder (page 204) and sphere (page 237). The initial temperature distribution has the three boundary conditions: (1) the heat flux at the center is zero; (2) the temperature at the surface is the heating medium temperature; and (3) the initial temperature throughout the piece is constant. These temperature profiles are the familiar heating temperature profiles given by Carslaw and Jaeger (1959) for infinite slabs (page 100), infinite cylinders (page 199) and spheres (page 233). Isotropic behavior is assumed for the product being considered. These solutions were combined for each geometry to give heat/hold temperature profiles.

The heat/hold solution for spheres is:

$$[(T-T_i)/(T_s-T_i)] - [(\langle T\rangle-T_i)/(T_s-T_i)] = [(T-\langle T\rangle)/(T_s-T_i)] = (2/\pi\rho)\sum_{n=1}^{\infty}\sum_{k=1}^{\infty}\exp(-\alpha_n^2 Fo_t - k^2\pi^2 Fo_\tau)\sin(\alpha_n\rho)\,[(\alpha_n^2+1)/\alpha_n^2]\,[(-1)^k/k]\,[(\sin(\alpha_n-k\pi)/\alpha_n-k\pi) - (\sin(\alpha_n+k\pi)/\alpha_n+k\pi)]. \quad (1)$$

The mass average temperature is given on page 233 of Carslaw and Jaeger (1959) and is:

$$(\langle T\rangle-T_i)/(T_s-T_i) = 1 - (6/\pi^2)\sum_{k=1}^{\infty}(1/k^2)\exp(-\pi^2 k^2 Fo_\tau). \quad (2)$$

Since the solution (Eq. 1) is indeterminant at the center ($\rho = 0$), L'Hopital's rule is applied as follows:

$$\lim_{\rho\to 0}\sin(\alpha_n\rho)/\rho = \lim_{\rho\to 0}\alpha_n\cos(\alpha_n\rho) = \alpha_n$$

The infinite cylinder solution is:

$$[(T-\langle T\rangle)/(T_s-T_i)] = -4\sum_{n=1}^{\infty}\sum_{k=1}^{\infty}J_0(\alpha_n\rho)\exp(-\alpha_n^2 Fo_t-\beta_k^2 Fo_\tau)/(\beta_k^2-\alpha_n^2)J_0(\alpha_n) \quad (3)$$

where α_n are the roots of: $J_1(\alpha) = 0$; and β_k are the roots of: $J_0(\beta) = 0$.

The mass average temperature for this geometry is:

$$(\langle T\rangle-T_i)/(T_s-T_i) = 1 - 4\sum_{k=1}^{\infty}(1/\beta_k^2)\exp(-\beta_k^2 Fo_\tau). \quad (4)$$

For infinite slabs, the solution of Lund et al. (1972) was modified to include the mass average temperature. The result is:

$$[(T-\langle T\rangle)/(T_s-T_i)] = (-4/\pi^2)\sum_{n=1}^{\infty}\sum_{k=0}^{\infty}\exp(-n^2\pi^2 Fo_t - (k+0.5)^2\pi^2 Fo_\tau)\cos(n\pi\rho)\,[(-1)^n/((k+0.5)^2-n^2)]. \quad (5)$$

The mass average temperature for this geometry is:

$$(\langle T\rangle-T_i)/(T_s-T_i) = 1 - (2/\pi^2)\sum_{k=0}^{\infty}(1/k+0.5)^2\exp[-(k+0.05)^2\pi^2 Fo_\tau]. \quad (6)$$

These equations were programmed on an IBM 1108 computer and the results used to establish charts for predicting temperatures as a function of heat/hold times.

RESULTS & DISCUSSION

FIGURES 1, 2 and 3 show the center and mass average temperatures for the sphere, infinite cylinder and infinite slab, respectively. In the linear regions, the temperature is described by the first term of Eq. 1–6, depending on the situation. The holding curves are presented for a few representative values of heating Fourier number Fo_τ. On adiabatic holding, the curve departs from the heating curve at the heating Fourier number (Fo_τ).

Although only solutions for the three basic geometries are given, other geometries can be obtained by multiplying the unaccomplished temperature profiles for the basic geometries together (Carslaw and Jaeger, 1959; Lund et al., 1972; Smith et al., 1967). Combining an infinite cylinder and infinite slab gives a finite cylinder. Also, combining three infinite slabs gives a cube or a right parallelopiped. Smith et al. (1967) give the first term for the finite cylinder and right parallelopiped temperature profiles.

Often a piece of food may be approximated by an infinite cylinder or infinite slab. Within 2% error, a finite cylinder is approximated by an infinite cylinder at $L/D \geq 5$ and is approximated by an infinite slab at $L/D \leq 0.08$. For the same

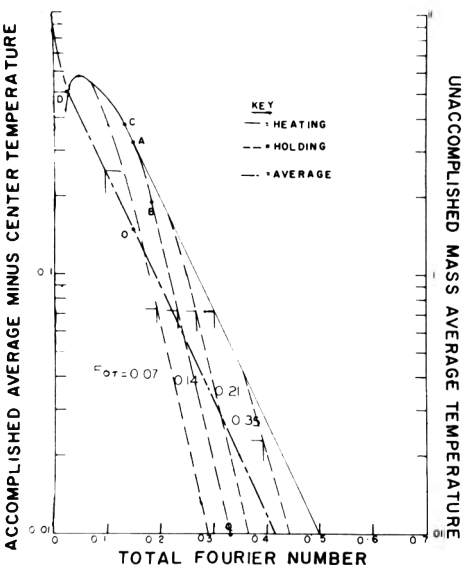

Fig. 1—Accomplished average temperature minus accomplished center temperature $(\langle T\rangle - T) / (T_s - T_i)$ vs. total Fourier number $(\kappa(t+\tau)/R^2)$ for heating (____) and heat/hold (---) in a sphere and unaccomplished average temperature $(T_s - \langle T\rangle) / (T_s - T_i)$ vs. heating Fourier number $(\kappa\tau/R^2)$ in a sphere (- - - -). The heat/hold curves are indexed with respect to heating Fourier number $(Fo_\tau = \kappa\tau/R^2)$.

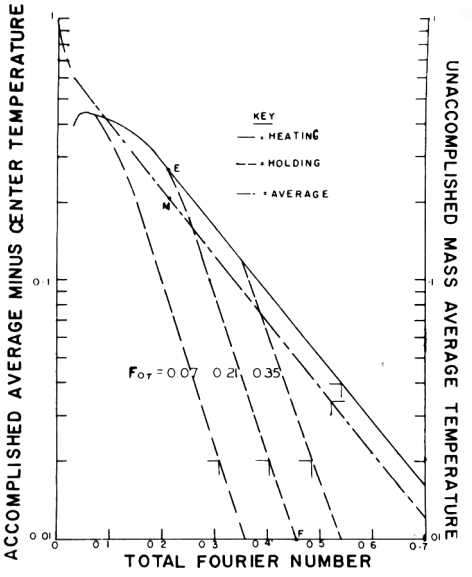

Fig. 2—Accomplished average temperature minus accomplished center temperature $(\langle T\rangle - T) / (T_s - T_i)$ vs. total Fourier number $(\kappa(t+\tau) / R^2)$ for heating (____) and heat/hold (----) in an infinite cylinder and unaccomplished average temperature $(T_s - \langle T\rangle) / (T_s - T_i)$ vs. heating Fourier number $(\kappa\tau/R^2)$ in an infinite cylinder (- - - -). The heat/hold curves are indexed with respect to heating Fourier number $(Fo_\tau = \kappa\tau/R^2)$.

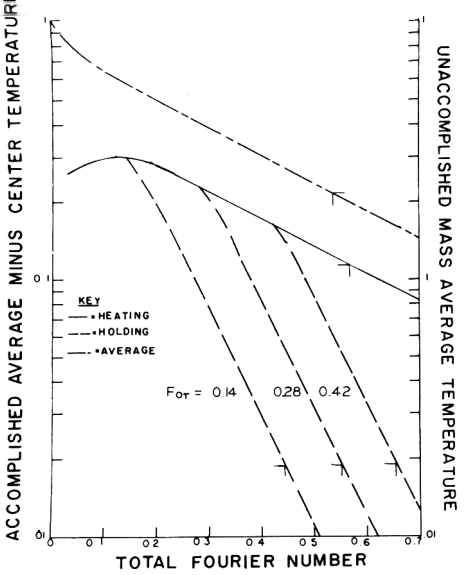

Fig. 3—Accomplished average temperature minus accomplished center temperature $(\langle T\rangle - T) / (T_s - T_i)$ vs. total Fourier number $(\kappa(t+\tau) / \lambda^2)$ for heating (____) and heat/hold (----) in an infinite slab and unaccomplished average temperature $(T_s - \langle T\rangle) / (T_s - T_i)$ vs. heating Fourier number $(\kappa\tau/\lambda^2)$ in an infinite slab (- - - -). The heat/hold curves are indexed with respect to heating Fourier number $(Fo_\tau = \kappa\tau/\lambda^2)$.

error, a brick-shaped object ($S_1 = S_2 > S_3$) is approximated by an infinite slab when $S_1/S_3 \geq 10$. Thus, slices of carrots, beets or potatoes can be approximated by an infinite slab while green beans can be approximated by an infinite cylinder.

To illustrate the application of the charts to blanching, two examples are presented. The first shows the relative heat/hold times for a given center and mass average temperature. The second shows the holding time and mass average temperature for a given heating time.

Example 1

Peas [thermal diffusivity (κ) = 1.61×10^{-6} ft^2/sec (Charm 1971) and R = 0.0156 ft = 3/16 in.] are blanched with steam (T_s = 212°F) so that the final center temperature is 190°F. The initial temperature is 72°F. What heat/hold process must be used to accomplish this center temperature? If the piece is equilibrated until the center temperature is within 1% of the mass average temperature, the mass average temperature will be approximately one degree Fahrenheit above the center temperature. Therefore, the average temperature should be 191°F after the heating process. The unaccomplished mass average temperature is:

$$(T_s - \langle T\rangle) / (T_s - T_i) = (212-191) / (212-72) = 0.150.$$

From Figure 1, point 0, the Fourier number for this temperature is:

$$Fo_\tau = \kappa\tau/R^2 = 0.145.$$

This gives a heating time of:

$$\tau = Fo_\tau R^2/\kappa = 0.145\,(0.0156)^2/1.61 \times 10^{-6} = 21.9 \text{ sec.}$$

Since the holding curves are parallel after 0.05 Fourier units, the appropriate curve for the heating Fourier number involved (0.145) is interpolated (curve ABQ) and the total Fourier number for equilibration to within 1% [$(\langle T\rangle - T) / (T_s - T_i)$ = 0.01] or a center temperature of 190°F is found on Figure 1, point Q. From this, the holding Fourier is:

$$Fo_t = Fo - Fo_\tau = 0.330 - 0.145 = 0.185.$$

The holding time is:

$$t = Fo_t R^2/\kappa = 28.0 \text{ sec.}$$

Example 2

Whole green beans [$\kappa = 1.71 \times 10^{-6}$ ft^2/sec (Woodams and Nowrey, 1968) and R = 3/16 in.] are blanched with steam (T_s = 212°F) for 30 sec and then held until equilibrated. The initial temperature is 72°F. What is the mass average temperature and what hold time is required to equilibrate the piece until the center temperature is within 1% of the mass average temperature? The Fourier heating number is:

$$Fo_\tau = 1.71 \times 10^{-6}(30)/(0.0156)^2 = 0.21.$$

From Figure 2, point M, the unaccomplished mass average temperature for this heating Fourier number is 0.21. The mass average temperature is:

$$\langle T\rangle = T_s - 0.21\,(T_s - T_i) = 212 - 0.21(212-72) = 183°\text{F}.$$

The hold time required to bring the center temperature to within 1% of the mass average temperature (points E to F) is:

$$t = (Fo - Fo_\tau)R^2/\kappa = (0.453 - 0.21)(0.0156)^2 / 1.71 \times 10^{-6} = 34.7 \text{ sec.}$$

These are just two ways to use the charts. Inactivation times for enzymes, microorganisms, etc. can be predicted by incorporating the kinetic data with the heat/hold or just heating temperature profiles (Lund et al., 1972; Lenz, 1972). Physical parameters can be predicted by using heating curves (Lund et al., 1972; Wadsworth and Spadaro, 1969; Dickerson and Read, 1968).

These charts apply for determining center temperatures where negligible resistance to heat transfer at the surface exists during the heating phase. For steam heating, the surface heat transfer coefficient is 1000–2000 Btu/hr ft^2 °F resulting in negligible resistance at the surface compared to resistance within the piece. For water blanching or deep bed blanching, this assumption may not hold. The equations are applicable to all positions in the piece although the charts presented here are for center temperatures only.

SYMBOLS

T = temperature at a point
T_S = steam or heating medium temperature
T_i = initial temperature
$\overline{(T)}$ = mass average temperature
$(T_S-T)/(T_S-T_i)$ = unaccomplished temperature
$(T-T_i)/(T_S-T_i)$ = accomplished temperature
$(T_S-\overline{(T)})/T_S-T_i)$ = unaccomplished mass average temperature
$(\overline{(T)}-T_i)/T_S-T_i)$ = accomplished mass average temperature (sometimes called the mass average temperature)
J_0, J_1 = Bessel functions of the zero and first order
r = radial distance to a desired point
x = linear distance to a desired point
R = radius of a sphere or cylinder
λ = half-width of a slab
D = diameter of a piece
L = total width of a piece
ρ = r/R or x/λ depending on the situation involved
κ = thermal diffusivity
t = holding time
τ = heating time
Fo_t = holding Fourier number = $\kappa t/R^2$ or $\kappa t/\lambda^2$
Fo_τ = heating Fourier number = $\kappa\tau/R^2$ or $\kappa\tau/\lambda^2$
Fo = total Fourier number = $Fo_t + Fo_\tau$
α, β = roots of the appropriate equations
S = length of a side

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IMPROVED REVERSE OSMOSIS PERMEATION BY HEATING

INTRODUCTION

REVERSE OSMOSIS (or hyperfiltration) is used to purify solvents, as in desalination, and to concentrate solutions, as in the dewatering of fruit juice. Membranes are used which ideally are permeable only to the solvent. Pressures of several hundred psi are applied to the solution to overcome osmotic pressure and to force solvent through the membrane. An obvious way of improving the performance of reverse osmosis equipment is to use the membrane under conditions which increase the permeation rate of solvent without increasing permeation of solute. It is also desirable to overcome concentration polarization, which increases osmotic pressure and is particularly detrimental in food systems (Merson and Ginnette, 1972). Concentration polarization is caused by the rejection of solute by the membrane. This causes solute to diffuse away from the membrane, where it is concentrated, toward the bulk of the solution, where it is more dilute. This steady state phenomenon should be distinguished from membrane fouling, which takes place when feed constituents adhere to the membrane causing a decrease in permeation rate over a period of time (Blatt et al., 1970).

One method to improve permeation of solvent is to heat the solution in the vicinity of the membrane. At higher temperatures the membrane permeability coefficient is higher allowing a greater rate of passage of solvent. Diffusion coefficients (D) in the solution also increase and viscosity coefficients (μ) decrease to decrease concentration polarization and thus improve solvent permeation.

Few data have been published on improved reverse osmosis by heating. Sourirajan (1970) has reported experimental results for pure water permeability increase for temperatures between 10° and 35°C. Solutions of 0.5M and 2.0M NaCl at a feed rate of 250 ml/min and 1500 psig operating pressure were tested. The permeation rate when multiplied by the solution viscosity at the operating temperature gave nearly constant values for each membrane tested. This finding for the small (7.6 cm^2) flat membranes of these experiments indicates that concentration polarization was slight, and that membrane resistance was the controlling factor.

In the present work two methods were tested for heating solutions flowing through cylindrical membrane tubes: (1) the entire feed to the system was preheated and (2) the membrane itself was heated from the outer surface. Heating the bulk of the solution allows for nearly uniform physical properties across the flow cross-section of the cylindrical membrane (if properties are not strongly concentration dependent). Heating the membrane wall, in general, leads to variable physical properties across the tube cross-section, and provides a more difficult physical situation to analyze mathematically. For certain solutions, however, this method may have the advantage of heating only the boundary film of the solution rather than the entire bulk. Heat sensitive materials would be less affected in this situation. Another advantage may accrue for certain solutions whose viscosity is strongly dependent on temperature. A decreased viscosity in the boundary layer may allow significantly greater mixing and hence reduce concentration polarization.

Theoretical predictions

Theories for predicting permeation flux in reverse osmosis for laminar flow in cylindrical membranes have been developed by DeFilippi and Goldsmith (1970) and by Sherwood et al. (1965). Both of these theories predict that heating the solution bulk will increase permeation rates.

The basic permeation flux expression used in these theories is the Merten (1963) equation for permeation velocity (v_w) through an ideal membrane,

$$v_w = A\,(\Delta P - \pi_w) \qquad (1)$$

where A is an empirical coefficient characteristic of the membrane, ΔP is the imposed pressure difference and π_w is the osmotic pressure of the solution at the membrane wall. For dilute solutions, π_w is proportional to the solution molality at the wall, c_w. Hence an increase in solute concentration near the membrane (concentration polarization) causes a decrease in permeation flux, and vice versa. The membrane coefficient A in Eq. (1) has an Arrhenius dependency on temperature (Lonsdale, 1966),

$$A = A_0 \exp(-H_0/RT) \qquad (2)$$

so that an increase in T will increase v_w. The constants A_0 and H_0 are determined

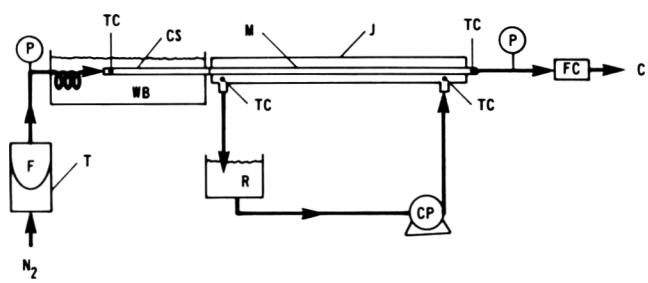

Fig. 1—Equipment. N_2 pressurized nitrogen source, T pressurized feed tank, F feed solution in neoprene pouch, P pressure gage, TC thermocouple, WB controlled temperature water bath and feed preheater, CS calming section, M tubular membrane inclined at 3° from horizontal, J concentric jacket for permeate collection or heating fluid, R reservoir for heating fluid, CP circulating pump, FC flow controller and C concentrated effluent.

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experimentally for each membrane.

The theories describe how flux toward the membrane causes concentration polarization. DeFilippi and Goldsmith (1970) utilized the film theory for the mass transfer relation between permeation velocity and wall concentration,

$$v_w = k \ln c_w/c_b \qquad (3)$$

in terms of the concentration of the solution (c) where the subscripts w and b refer to the quantity at the wall or in the bulk. For laminar flow the mass transfer coefficient k was provided by the correlation between the Sherwood number Sh = kd/ρD, and the Peclet number Pe = ūd/D,

$$Sh = 1.86\,(Pe\ d/L)^{1/3}\,(\mu_b/\mu_w)^{0.14} \qquad (4)$$

The tube diameter is d, the length is L, the average bulk velocity is ū, and ρ in the Sherwood number is the concentration of water in the fluid, assumed to be 1.00 g/ml. Eq. (1) to (4) may be used to calculate v_w at different temperatures.

Sherwood et al. (1965) and Brian (1966) used Yuan and Finkelstein's (1956) solution of the Navier-Stokes equations, which includes axial and transverse velocity components. A separation of variables technique enabled them to solve the convective diffusion equation. They also presented an approximate analytical solution in terms of the average solute concentration ($\bar{c}$) which applies not too far downstream:

$$\frac{c_w - \bar{c}}{\bar{c}} = \xi + 5\left[1 - \exp\left(-\sqrt{\xi/3}\right)\right] \qquad (5)$$

where $\xi = \delta/8\alpha^2$, $\alpha = 2D/v_w d$, and for uniform flux $\delta = v_w L/\bar{u}d$. A trial and error procedure enables one to solve Eq. (1), (2) and (5) for v_w for different temperatures. Since neither the density nor viscosity appears in this theory, the change in v_w is due to the dependence of D and A on T.

MATERIALS & METHODS

THE COMMERCIAL cellulose acetate tubular membranes (Type 510, Calgon-Havens Systems, San Diego, Calif.) were 220.4 cm long and 1.27 cm diam. Sucrose retention was 99.8% for each new membrane, decreasing after a few months to 99.2% at which time the membrane was discarded. The membrane tube was jacketed by a 3.7 cm diam concentric stainless steel pipe (Fig. 1). For experiments when the solution bulk was heated, permeated solvent was collected in the annulus between the membrane and pipe. For the experiments involving heating the boundary layer near the membrane only, hot water was circulated through this annulus. The outer stainless steel pipe was insulated to minimize heat losses to the surroundings.

A constant pressure of 500 psig was maintained in the tube with a stainless steel feed tank pressurized with nitrogen. The feed solution was contained inside a rubber pouch within the tank to separate the solution from the nitrogen. A differential flow controller (model 63-SDL, Moore Products Co., Spring House, Pa.) maintained a constant pressure differential across a needle valve providing a constant mass flow of solution. The incoming feed line was coiled and immersed in a water tank at constant temperature controlled by a laboratory type temperature controller. A 1-m long straight pipe of 9.4 mm inside diam connected the coil and the membrane inlet to allow fully developed laminar flow inside the membrane tube. Thermocouples measured the fluid temperature at four points: the feed inlet, the feed outlet, the outer fluid inlet, and the outer fluid outlet. Recorded temperature readings were accurate to ± 0.5°C. A refractometer was used to measure the sucrose solution concentration at the inlet and outlet to within 0.1 weight percent. Concentrate and permeate flow rates were measured with graduated cylinders and a timer.

The 15% sucrose solution was prepared with commercially available cane sugar and distilled water. The sugar solution was charged into the feed tank and the tank was pressurized to 500 psig. The flow rate was set by adjusting the needle valve, and measurements were taken after steady state was achieved. Further details are given by Monge (1972).

For the set of experiments with membrane I the range of flow rates was approximately 10–100 ml/min (Reynolds number range: approximately 10–120). At these low flow rates the concentration difference from inlet to outlet was large enough so that permeation rate could be determined indirectly from a mass balance using inlet and outlet concentrations. Another reason for using low flow rates is that concentration polarization is more pronounced under such conditions. For uniform temperature experiments the nearly horizontal stainless steel jacket was filled with water and the permeate was collected through an opening at the top of the jacket. For the heated boundary layer experiments, hot water (at 35 or 45°C) was pumped into the stainless steel jacket countercurrently to the feed solution which entered at 25°C. The flow rate of the heating fluid was large enough (over 400 kg/min) so that its temperature remained nearly constant. For this method of heating, permeation rates could not be measured directly in our equipment and it was necessary to calculate them from a mass balance.

Another series of experiments with a second membrane, II, extended the range of flow rates to about 600 ml/min, or to a Reynolds number of nearly 1000. For these trials, which were at

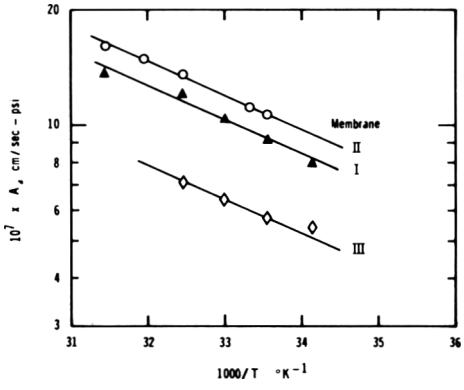

Fig. 2—Measured membrane coefficients. Apparent activation energy H_o is 2.2 kcal/mole.

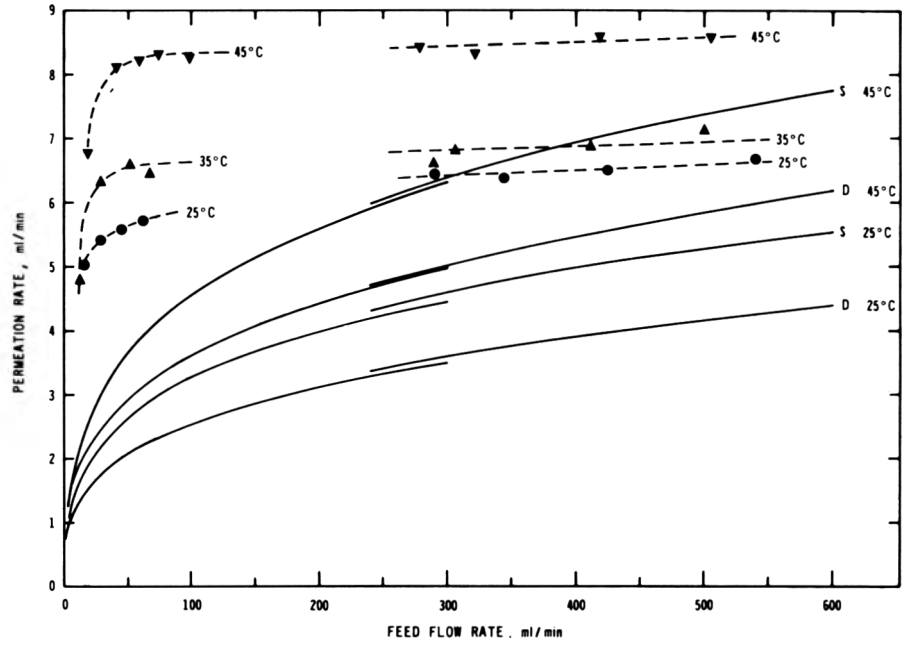

Fig. 3—Predicted and directly-measured permeation rates for two membranes. Experimental points and theoretical curves below feed flow rates of 250 ml/min refer to membrane I, those above to membrane II. S refers to the theory of Sherwood et al. (1965) and D refers to DeFilippi and Goldsmith (1970).

uniform temperature, permeation rates were measured directly by collecting the permeate in the stainless steel jacket.

To measure the membrane coefficient for different temperatures, heated distilled water was fed to the membrane. Permeation rates were measured as excess water from the pipe jacket. Values for the membrane coefficient were calculated by dividing the permeation rate by the membrane area and the hydrostatic pressure, and are presented as a function of temperature in Figure 2. Values of A from this graph were used in the theories to estimate permeation flux. Values for a third membrane, III, are included to show the uniformity of H_0. The differences in A are not unusual for membranes of the same type.

RESULTS & DISCUSSION

A PLOT of permeation rate versus feed flow rate is presented in Figure 3 for feed temperatures 25, 35 and 45°C. Values for the permeation rate increases at a flow rate of 60.0 ml/min are presented in Table 1. Larger increases (up to 20% at 35° and up to 55% at 45°C) were observed for lower flow rates where concentration polarization is expected to be more important.

Theoretical permeation rates were calculated using literature values of physical properties for sucrose solutions (Sourirajan, 1970). The predicted rates at the three temperatures are compared to the directly-measured rates in Figure 3. Both theories predict lower permeation rates than those experimentally obtained, the difference between theory and experiment being greater at the lower flow rates.

The difference could be caused by secondary flows at the entrance to the tube, variations in physical properties because of concentration gradients, or natural convection. Secondary flows could exist because of the slight increase in diameter at the junction of the delivery tubing and the membrane, or because of the membrane retaining ring at the edge of the membrane. However, secondary flows should be more important at the higher flow rates. The effect of concentration gradients on the velocity profile is neglected by the Sherwood et al. (1965) theory. Including this effect (which would result in higher viscosity near the membrane) would decrease the theoretical values further. The variation of physical properties is partially included in the DeFilippi and Goldsmith (1970) curve in Figure 3 because the viscosity and diffusion coefficients at the membrane wall were used. A more likely explanation of the differences between theory and experiment is natural convection caused by density gradients. Higher density fluid near the upper surface of the membrane may result in natural convection eddies superimposed upon the laminar flow. This effect would be less important at higher flow rates. The theoretical curves appear to approach extrapolated experimental lines asymptotically at higher flow rates.

For temperature increases, the theories predict increases in permeation rate of the same order of magnitude as those obtained in the experiments (Table 1). The calculations based on Eq. (1) to (5) show that permeation rate increases are caused mainly by increases in the diffusivity and the membrane coefficients. Viscosity and density effects are small in the DeFilippi and Goldsmith theory and do not appear in the Sherwood-Brian theory.

The two methods of heating are compared in Figure 4 and Table 1. The similarity in permeation rates indicates that the two methods may be used with nearly equal effectiveness. The small difference at 45°C is likely caused by the fact that the membrane is cooler than 45°C at the entrance when the feed enters at 25°C.

At the low flow rates for which the boundary layer heating method could be tested in our experiment (below 100 ml/min), we observed nearly complete temperature equilibrium at the tube outlet. This observation is in accord with temperature profiles calculated by a Graetz analysis (Monge, 1972). It is possible that for higher flow rates the thermal boundary layer would be thin enough that the desired increase in the membrane coefficient and the desired decrease in concentration polarization would be effected without heating the bulk of the solution. For most materials, however, the rate of heat transfer exceeds the rate of mass transfer and intermediate cooling might be necessary (between modules, for example).

Experimental error is estimated in two ways corresponding to the two methods of determining permeation rates. Errors were small when the permeating liquid was collected directly as for data in Figures 2 and 3 and column 2 of Table 1. Repeated experiments indicated precision to be on the order of ± 3% about the mean. Permeation rates calculated from the mass balance (Fig. 4 and column 3 of Table 1) are less accurate because of sensitivity to small errors in the refractometer readings. The probable error ranges shown in Figure 4 were based on a possible error of 0.1% (w/w) in the measurement of the outlet concentration. The possible error is greater for larger flow rates because the concentration differences from inlet to outlet were smaller.

Table 1—The effect of temperature increase on permeation rate[a]

Temperature increase	Experimental rate increase (%)		Predicted rate increase (%)	
	Heated feed	Heated membrane	Sherwood et al. (1965)	DeFilippi and Goldsmith (1970)
25–35°C	13	10	19	18
25–45°C	45	46	39	42

[a] Values are rate increases as percentages of the values at 25°C. Feed flow rate was 60.0 ml/min.

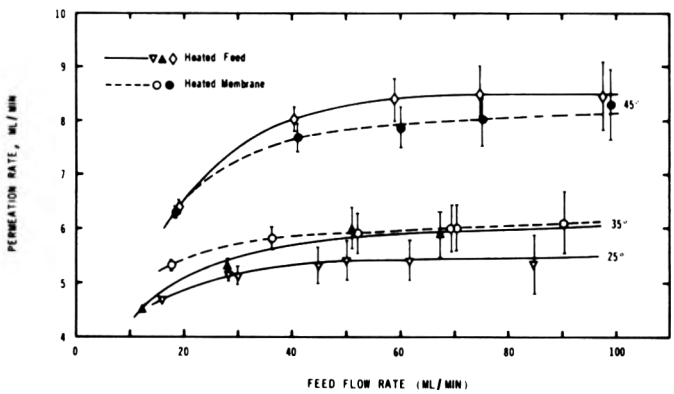

Fig. 4—Experimental permeation rates (membrane II) calculated from mass balance. To convert permeation rates in ml/min in Fig. 3 and 4 to flux, multiply by 1.71 × 10^{-5} (flux in g/cm²-sec) or 0.363 (flux in gal/ft²-day).

Differences between the two methods of determining permeation rate (e.g., comparing the 25°C data in Fig. 3 and 4) were always less than half of the maximum error ranges in Figure 4.

In deciding whether or not to use elevated temperatures, processors must balance the merits of improved performance, greater membrane plant capacity and lower product cost against the disadvantages of possible shortened membrane life, possible increased microbial growth, heat stability of the product and increases or decreases in membrane fouling at elevated temperatures. Theoretically, higher temperatures will shorten membrane life (Vos et al., 1966). For cellulose acetate membranes life time is determined by the rate of hydrolysis of the ester and the rate of compaction of the porous membrane structure. Considering only hydrolysis, Lonsdale (1970) has predicted membrane life to be about 310 days at 45°C compared to 1200 days at 25°C. One year is considered acceptable membrane life for commercial applications with foodstuffs and other biological substances. Other membrane materials are less sensitive to temperature.

CONCLUSION

REVERSE OSMOSIS experiments at low flow rates confirm theoretical predictions that heating increases the membrane coefficient and decreases concentration polarization. The net result is that significant increases in the permeation flux may be achieved by heating within a practical temperature range.

LIST OF SYMBOLS

A	membrane coefficient, cm/(min psi)
A_o	constant in Eq. (2), cm/(min psi)
$\bar{c}$	average solute concentration, g/ml
c_b	solute concentration in the bulk, g/ml
c_w	solute concentration at the wall, g/ml
d	tube diameter, cm
D	diffusion coefficient, cm^2/sec
H_o	energy term in Arrhenius expression, Eq. (2), cal
k	mass transfer coefficient, cm/min
L	length of tube, cm
P	pressure, psi
$Pe = \bar{u}d/D$	Peclet number
R	gas constant, 1.987 cal/(K mole)
$Sh = kd/\rho D$	Sherwood number
T	temperature, K
$\bar{u}$	average bulk velocity, cm/min
v_w	permeation velocity, cm/min
$\alpha = 2D/v_w d$	parameter in Eq. (5)
$\delta = v_w L/\bar{u}d$	parameter in Eq. (5)
$\xi = \delta/8\alpha^2$	parameter in Eq. (5)
μ	viscosity, cp
μ_b	viscosity of fluid in bulk, cp
μ_w	viscosity of fluid at the wall, cp
π_w	osmotic pressure at the wall, psi
ρ	concentration of water, g/ml
ΔP	imposed pressure difference across membrane, psig

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JFS BASIC SCIENCE

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COMPARISON OF THE PROTEIN NUTRITIONAL VALUE OF TVP, METHIONINE-ENRICHED TVP AND BEEF FOR ADOLESCENT BOYS

INTRODUCTION

TEXTURED vegetable protein foods may be used increasingly as an economical source of protein. USDA approval has been granted to partially substitute meat analogs for meat for federally approved school lunch programs. Thus, the investigation of human utilization of textured vegetable protein foods is necessary, particularly for children since they constitute a population group who will be likely to receive these foods. A study was designed to compare the adequacy of a soybean textured vegetable protein product (TVP) (supplied gratis by Archer Daniels Midland Co., Decatur, Ill.), the identical product supplemented with methionine and beef for supporting nitrogen equilibrium in 12–16-yr-old boys.

PROCEDURE

THE EXPERIMENTAL plan is shown in Table 1. The 23-day study consisted of a 5-day adjustment period and three 6-day experimental periods. Mean nitrogen balances were calculated from the last 5 days of each experimental period.

Subjects were nine adolescent boys recruited from a group participating in a nutritional status survey. All were white Americans. Vital statistics of the subjects are shown in Table 2. Approval for participating in the balance study was obtained from the individual, the family physician and parents. During the study subjects carried on their usual activities except for the exclusive consumption of the food provided in the experimental diets and the collection of excreta.

The basal diet (described in Kies and Fox, 1971) consisted of a low nitrogen starch bread and a few low nitrogen fruits and vegetables. Carbonated beverages, jelly and hard candy were added as needed to maintain weight. Tea (1-1/2 teaspoons daily, instant, dry weight basis) was allowed if the subject desired. Fat in an amount to supply 20% of the total calories was provided from either beef tallow or safflower oil (Table 1.) The selection of fat was based on the objectives of another research project (Kies et al., 1972).

The test foods, soybean textured vegetable protein food, 1% DL-methionine supplemented soybean textured vegetable protein food, or beef provided 4.0g nitrogen daily. The test products were combined with vegetables and fat from the basal diet and served as a casserole dish at the noon and evening meals. The test products were not included in the breakfast meal. The basal diet provided approximately 1g nitrogen daily. Vitamin and mineral supplements were added to meet or exceed the NRC recommended levels.

Complete urine and fecal excretions were collected throughout the balance study. Urine was composited in 24-hr aliquots and feces were composited to correspond with the time intervals of the adjustment and experimental periods. Creatinine excretion in the urine was determined as a measure of accuracy of the 24-hr collection by the method of Folin (1914). Nitrogen was determined in the test products, basal diet, daily urine collections and fecal composites by the Kjeldahl method of AOAC (1970).

RESULTS & DISCUSSION

MEAN NITROGEN balances for subjects fed TVP, methionine-enriched TVP or beef as the primary source of dietary protein were −0.08, +0.48 and +0.32g per day, respectively (Table 3). Nitrogen retention was significantly higher in re-

Table 1—Experimental plan

Period[a]	No. of days	N intake from test product[b] Kind	Amount (g N/day)	N intake-basal diet[cd] (g N/day)	Total N intake (g N/day)
Adjustment	5	Varied	4.00	0.96	4.96
Expt. 1	6	TVP	4.00	0.96	4.96
Expt. 2	6	TVP + Methionine	4.00	0.96	4.96
Expt. 3	6	Beef	4.00	0.96	4.96

[a] Each subject was given all experimental treatments in random order. Hence, order of experimental treatment varied for each individual. Subjects 390, 394, 397, 408 and 409 received safflower oil and subjects 392, 393, 406 and 410 received beef tallow as the dietary fat source in all periods.

[b] TVP = textured vegetable protein, extruded soy products simulating beef. TVP + methionine = TVP fortified with 1% DL-methionine. Beef = Lean ground beef.

[c] Nitrogen intake from basal diet was 0.98g/day for subjects who drank tea and 0.96g/day for those who did not.

[d] Details concerning composition of basal diet and vitamin and mineral supplements given in an earlier paper (Kies and Fox, 1971).

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Table 2—Vital statistics of subjects

Subject	Age (mo)	Height (cm)	Body weight Initial (kg)	Body weight Final (kg)
390	185	164.2	52.6	51.6
392	184	178.4	58.2	56.9
393	200	172.1	56.1	54.5
394	190	169.9	64.1	62.0
397	176	174.3	64.1	63.2
406	153	152.4	40.0	39.2
408	185	164.2	48.2	48.8
409	177	161.6	44.2	43.5
410	168	170.2	67.7	66.4

Table 3—Nitrogen balances of adolescent boys fed TVP, methionine-enriched TVP and ground beef[a]

Subject no.	Nitrogen balance TVP	Methionine-enriched TVP (g/day)	Beef
390	0.52	0.89	0.36
392	0.14	0.39	0.08
393	−0.37	−0.21	0.19
394	−0.78	0.25	0.19
397	−1.31	−0.63	−0.55
406	−0.04	0.53	0.78
408	0.20	0.89	0.90
409	1.25	1.36	1.43
410	−0.34	0.82	−0.48
Mean N balance	−0.08	0.48	0.32

[a] Fed to provide 4.0g N per day

sponse to methionine-enriched TVP or beef than to TVP alone ($P < 0.05$, Duncan's Multiple Range Test). There was no statistical difference in nitrogen balances of subjects fed beef or methionine-enriched TVP. The biological significance of these results is suggested by the observation that all subjects showed a more positive nitrogen balance when methionine-enriched TVP was fed than when TVP alone was fed. Similarly, six of nine subjects retained more nitrogen from beef than from TVP. On the other hand, methionine-enriched TVP and beef were indistinguishable biologically since five subjects retained more nitrogen on beef and four subjects retained more on enriched TVP. The lower protein quality of the TVP was apparently not attributable to a difference in digestibility. Mean apparent digestibility values for TVP, methionine-enriched TVP and beef were 79, 80 and 82%, respectively.

The results of this study agree with the findings of Kies and Fox (1971) for adult men and with those of Bressani et al. (1967) for children. In the latter studies, soybean products were lower in protein nutritional value than beef or milk when fed at critical levels of intake. Differences in protein quality of test products can be demonstrated only when products are fed in insufficient amounts to meet the needs of the animal or human being studied since at higher levels of intake quantity compensates for quality differences.

In contrast to the findings at low total protein intake levels the two groups of investigators (Kies and Fox, 1971, and Bressani et al., 1967) showed that when larger amounts of soybean products were fed they did not differ in protein value from animal products according to the evaluation criteria used. It should be noted that in one study (Kies and Fox, 1971) the same TVP as used in the present study was evaluated while in the other study (Bressani et al., 1967) another soybean product containing some egg albumin and wheat gluten was tested.

Additional data supplied by the Archer Daniels Midland Company (Kies and Fox, 1971) also confirmed that TVP differed in protein quality from beef. PER values obtained in growing rats on the basis of casein (2.50) were 2.12, 2.82 and 2.37 for TVP, methionine-supplemented TVP, and beef, respectively.

While these results indicate that the protein quality of soybean-containing products is somewhat lower than that of high quality animal products, the practical significance of this difference depends upon the situation in which the product is used. The composition of the remainder of the diet, both with regard to protein and other nutrients, as well as the level of dietary protein might be expected to affect utilization of the soybean product (NAS-NRC, 1959). Thus partial replacement of meat with soybean products may or may not detract from the protein nutritional value of the diet depending upon the total protein content and the quality of the protein in the diet in question (NAS-NRC, 1959).

One way of further improving the soybean product which might be important, especially if the product is used alone, is methionine fortification. The near equivalence of methionine-fortified TVP and beef argues for the use of the enriched product.

Since TVP might be expected to be combined with meat rather than used alone, the value of the meat-soybean combination should be considered. Studies to determine the protein value of various combinations of meat and TVP are underway in this laboratory. These studies will provide information on the quantity of meat that can be replaced with TVP without impairment in protein nutritional value.

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INHIBITION OF RIPENING AND INDOLE-3-ACETIC ACID OXIDASE OF BANANA FRUIT BY p-2,4-CHLOROPHENOXY-ISOBUTYRIC ACID

INTRODUCTION

THE GROWTH INHIBITOR p-2,4-dichlorophenoxy-isobutyric acid (p-CPIB) has been referred to as an "anti-auxin" because it interferes with auxin induced growth of juvenile plant tissues. The growth response of plant tissue cultures to auxin is a linear function of $(auxin)^{-1}$ such that growth inhibition may be subjected to Lineweaver-Burk analysis. p-CPIB was shown to competitively inhibit the auxin growth response in various tissue culture bioassays (Mcrae and Bonner, 1953). This finding led to the suggestion that p-CPIB competes with auxins for the same active site. Mcrae and Bonner have suggested that the site of auxin and p-CPIB interaction is a regulator protein of gene transcription.

It is known that auxin catabolism is closely associated with senescence phenomena including fruit ripening. Application of indole-3-acetic acid or auxin analoques to mature plant tissue results in delayed fruit ripening (Vendrell, 1969) and endogenous auxins appear to disappear concomitantly with the onset of senescence (Lewis et al., 1965). In addition, indole-3-acetic acid oxidase (IAA oxidase) activity is more pronounced in ripening fruit than in mature fruit (Frenkel, 1972; Haard, 1972).

If, indeed, auxin catabolism is requisite for the onset of normal fruit ripening one might expect the "anti-auxin," studied by Mcrae and Bonner (1953) with juvenile tissue, to affect the time course of ripening by interacting with the active site of endogenous auxins. The studies reported in this paper show that p-CPIB represses the onset of ripening in banana fruit and, furthermore, support the theory that the site of anti-auxin interaction is IAA oxidase.

EXPERIMENTAL

Materials

Banana fruit were generously donated by the United Fruit Co. The fruit were approximately 8 days postharvest on receipt from Honduras and classified as No. 2 according to the peel color scale of Loesecke (1950). After treatment, as described below, fruit were stored at 18°C, 90% relative humidity in a ventilated environmental room.

Application of growth substances

The growth regulators indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D) (Sigma Chemical Co., St. Louis, Mo.) and p-2,4-dichlorophenoxy-isobutyric acid (K & K Chemicals, Plainview, N.Y.) were applied to the peel-pulp junction of green banana fruit by injection with sterile syringe. A 1-ml aliquot containing either 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} or 10^{-2} mM of the growth substance was applied at a point approximately one-third the banana length from the stem end and at a point equidistant from the blossom end. The respective auxins were dissolved in 0.4M mannitol which was adjusted to pH 6.0 with HCl. Mannitol was employed as a carrier because it is known to minimize stress to infiltrated plant tissues (Frenkel et al., 1969).

Isolation of IAA oxidase

Cell free extracts of green banana pulp were prepared by a method previously described for the isolation of "soluble" peroxidase from banana (Haard and Tobin, 1971). We had earlier reported that peroxidase isozymes which are most active in oxidatic degradation of indole-3-acetic acid are localized in the "soluble" pool of peroxidase and not the "wall bound" or "intercellular pools" which are isolated by different extraction techniques (Haard, 1972). The cell-free extract was passed through a column containing Sephadex G-50 (500 ml bed volume). The protein fraction eluted with distilled water was dialyzed against four liters of tris-Cl, 0.01M, pH 8.0. The dialyzed material was directly assayed for IAA oxidase.

Assay of IAA oxidase

The formation of auxin degradation products was followed by reaction with dimethylaminocinnamaldehyde at pH 6.0 as described by Meudt and Gaines (1967). In preliminary experiments the disappearance of IAA was monitored by the Salkowski reaction (Julian et al., 1952). The pH optimum of IAA oxidase from banana was previously identified as 6.0 in 0.01M acetic acid-sodium acetate buffer. Stock solutions of p-CPIB were prepared in distilled water and adjusted to pH 6.0 with HCl for study of its influence on the oxidatic degradation of IAA by the enzyme preparation.

RESULTS & DISCUSSION

APPLICATION of the growth regulators IAA and 2,4-D to the peel-pulp juncture of green (No. 2) banana fruit resulted in a transient delay of ripening at the local site of injection. These results, summarized in Table 1, are consistent with those reported by Vendrell (1969) with infil-

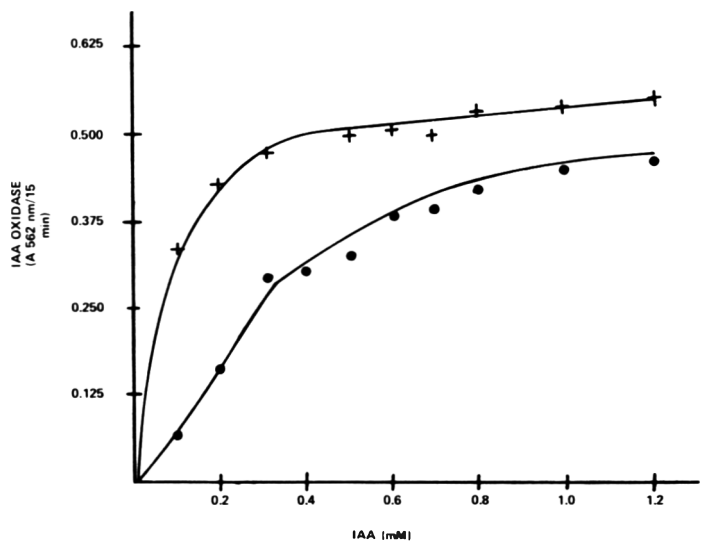

Fig. 1—Substrate velocity plot for IAA oxidase in the absence (+—+) and presence (o—o) of 0.5 mM p-CPIB. Assay time was 15 min at 20° C, pH 6.0.

Table 1—Influence of IAA and 2,4-D on the time course of ripening

Application	Delay in ripening at site of application	
	Mean (days)[a]	Range (days)
Mannitol, 0.4 mM	0	–
Mannitol, 0.4 mM + C_2H_4	0	–
Distilled H_2O	0.5	–
Distilled H_2O + C_2H_4	1.3	0.5–2
IAA (10^{-5} mM), mannitol (0.4 mM)	1.5	1–2
IAA (10^{-5} mM), mannitol + C_2H_4	2.5	2–4
IAA (10^{-4} mM), mannitol (0.4 mM)	7.5	5–8
IAA (10^{-4} mM), mannitol + C_2H_4	6.5	5–7
IAA (10^{-3} mM), mannitol (0.4 mM)	15.5	15–17
IAA (10^{-3} mM), mannitol + C_2H_4	14.5	13–17
IAA (10^{-2} mM), mannitol (0.4 mM)	14.5[b]	13–17
IAA (10^{-2} mM), mannitol + C_2H_4	15.0[b]	14–17
2,4-D (10^{-5} mM), mannitol (0.4 mM)	3.0	1–5
2,4-D (10^{-5} mM), mannitol + C_2H_4	3.5	1–5
2,4-D (10^{-4} mM), mannitol (0.4 mM)	5.5	5–7
2,4-D (10^{-4} mM), mannitol + C_2H_4	8.0	7–9
2,4-D (10^{-3} mM), mannitol (0.4 mM)	9.0	8–10
2,4-D (10^{-3} mM), mannitol + C_2H_4	9.0	8–10
2,4-D (10^{-2} mM), mannitol (0.4 mM)	11.5	10–13
2,4-D (10^{-2} mM), mannitol + C_2H_4	15.0	13–16

[a]Ripening indices judged were peel color, pulp texture and starch hydrolysis at the site of injection. Temporal comparisons were with air controls. Data are the average of 20-lb lots of fruit. Air control fruit not gassed with exogenous C_2H_4 ripened in 21 days and C_2H_4-treated controls ripened in 7 days.
[b]Partial necrosis at site of injection

Table 2—Repression of fruit ripening by p-2,4-dichlorophenoxy-isobutyric acid

Application	Repression of Ripening[a]
p-CPIB (10^{-5} mM), mannitol, (0.4 mM) ± C_2H_4	60%
p-CPIB (10^{-4} or 10^{-3} mM), mannitol (0.4 mM) ± C_2H_4	100%
p-CPIB (10^{-2} mM), mannitol (0.4 mM) ± C_2H_4	Peel and pulp became necrotic within 6 hr after application. Pulp remained firm and failed to ripen.

[a]Repression was judged as a complete failure of the fruit to ripen. After 25 days (C_2H_4 treated) distal sites had ripened and turned completely black while treated areas had greenish-black peel and firm pulp. This was preceded by a direct progression into necrosis (no normal ripening) at the site of application.

trated slices of banana fruit. The delay in ripening elicited by IAA and 2,4-D was consistently observed when 10^{-4}, 10^{-3} or 10^{-2} mM was applied to fruit which were subsequently gassed with ethylene prior to storage. Fruit which were not gassed with ethylene generally ripened faster (than untreated fruit) at loci distant from the point of application; however, there was a clear delay in ripening at the site of application (Table 1). It was previously reported that dipping bananas in 2,4-D accelerated ripening of banana fruit; this effect appears to be caused by stimulating ethylene formation without exposing tissue cells to the growth regulator (Vendrell, 1969). We might reason that a similar effect occurred at distal sites of auxin application in fruit which were not gassed with exogenous ethylene. The data in Table 1, indicate the temporal delay in ripening at the site of injection. Texture softening, flavor development and starch hydrolysis of the pulp were delayed concomitantly with peel degreening. The depth of pulp affected was approximately 10–20 cm. The auxin treated areas ripened normally except for some occasional brown streaking in the turning peel.

Application of p-CPIB also affected ripening of the peel-pulp tissue; however, the "antiauxin" repressed ripening such that normal ripening was never observed (Table 2). Repression of ripening was striking when 10^{-3} or 10^{-4} mM of p-CPIB were applied and somewhat less pronounced in the fruit treated with 10^{-5} mM of the auxin analoque. The local peel-pulp region remained green and firm to the point when distal regions were completely blackened and infested with fungi. During the final stages of senescence the treated areas became necrotic without going through the ripening phase of senescence. Areas of pulp and peel treated with 10^{-2} mM of p-CPIB became necrotic within approximately 6 hr of application. Loci of banana distal from the site of application (10^{-2} mM) ripened some 1–3 days faster than control fruit. It is difficult to explain the latter observation as a ethylene wound response since the fruit were gassed with approximately 100 ppm ethylene for 4 hr immediately after treatment. The important distinguishing difference between the data presented in Tables 1 and 2 is that the auxins delayed ripening while the "anti-auxin" repressed ripening.

IAA oxidase

Pulp extracts which were assayed without subsequent purification as outlined in the experimental section, exhibited sigmoidal velocity-substrate plots and progressive inhibition when increased extract volumes were assayed. The anomalous behavior of this preparation has been reported elsewhere (Haard, 1972). However, isolates obtained by the technique described in this study exhibited Michaelis-Menton kinetics and a linear relation between extract volume and activity. The presence of 0.5 mM p-CPIB in the assay media was observed to inhibit the oxidatic function of this preparation (Fig. 1). The velocity of IAA oxidation was depressed at all substrate concentrations although it was most pronounced at the lower substrate concentrations studied. Note from Figure 1 that the maximum velocity of the inhibited preparation appears to be approaching that of the control at high substrate concentration. Lineweaver-Burk analysis of this data did not yield straight line plots. This is a reflection of the sigmoidal nature of Figure 1. While the data are suggestive that p-CPIB is a competitive inhibitor of IAA oxidase it may well be that mixed inhibition is occurring. It is not surprising to observe anomalous inhibition kinetics since several isozyme species contribute to banana IAA oxidase activity (Haard, 1972). Similar effects were observed with 0.1, 1.0 and 5.0 mM p-CPIB. The resulting increase in K_m for IAA by p-CPIB may explain the repressive effect of this substance on fruit ripening. No attempt has been made to calculate the magnitude of this shift in K_m since the kinetics may differ with respect to enzyme concentration and as-

say time. We have found no evidence that p-CPIB is metabolized by IAA oxidase. That is, prolonged incubation of the inhibitor with our enzyme preparation resulted in no change in the UV absorption spectrum of p-CPIB. In addition, similar data were obtained when IAA oxidase activity was monitored by the Salkowski method indicating that the inhibitor acted to prevent the catabolism of IAA rather than to accelerate further metabolism of the product(s) (presumably oxindole derivatives) to metabolites not reactive with dimethylaminocinnamaldehyde. The auxin analoque 2,4-D did not influence the kinetics of IAA oxidase when included in the assay reaction at 0.5 mM.

CONCLUSION

THE DATA PRESENTED in this communication support the view that the oxidatic metabolism of endogenous auxins is an essential element of normal fruit ripening. It was previously suggested that p-CPIB exhibits "anti-auxin" properties in juvenile tissues by virtue of its affinity for a site involved in gene expression. The finding that p-CPIB significantly increases the Michaelis constant of banana IAA oxidase in vitro at concentrations which repress ripening in normal fruit in situ leads to the conclusion that the inhibitor prevents the oxidative metabolism of endogenous auxins. The bulky isobutyrate group on this phenoxy auxin may facilitate binding to the active site of IAA oxidase without subsequent catalysis. Indole-3-acetic acid is active as a hormone at $10^{-6}-10^{-9}$ M levels in juvenile tissue (Price, 1970) and it is accordingly reasonable to surmise that IAA oxidase activity (in situ) was extensively, if not completely, blocked by application of millimolar levels of p-CPIB.

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CHILLING INJURY IN GREEN BANANA FRUIT: CHANGES IN PEROXIDASE ISOZYMES IN SOLUBLE AND PARTICULATE POOLS

INTRODUCTION

CHILLING INJURY of green banana fruit is characterized by delayed ripening or, when severe, by complete failure of pulp ripening. Symptoms of low temperature strain include darkening of the central placenta and subepidermal of the peel and closing of the stomata. With increasing time-temperature dependent severity of the chill, there is a discoordination of the biochemical events characteristic of normal ripening. For example, moderately chilled fruit have reduced starch hydrolysis and associated sugar accumulation on ripening and lack the normal development of flavor, color and texture (Loesecke, 1950). There is mounting evidence that the "plastic strain" (Levitt, 1972) arising from low temperature stress is caused by phase changes in the membrane lipids and proteins of sensitive cells (Lyons and Asmundson, 1965; Lyons and Raison, 1970; Raison et al., 1971). Chilling injury of banana fruit results in the accumulation of acetaldehyde and ethanol in the peel and pulp and the build up of α-keto acids in the peel (Murata, 1969). These observations are consistent with the view that chilling injury relates to the disjunction of mitochondrial integrity.

Several lines of evidence suggest the cold adaptation in hardy plant tissues is a function of peroxidase catalyzed reactions. Early evidence indicated that plants which are susceptible to chilling injury may harden, or become more resistant, and therefore are capable of surviving chilling temperatures (Sellschop and Salmon, 1928). Hardening of plant tissues may be accomplished by alternating a gradual drop in temperature with a gradual rise back to optimum temperature (Al'tergot and Bukhol'tsev, 1967). The formation of lipid peroxides from unsaturated fatty acids of membranes has been suggested to be the direct locus of low temperature injury (Levitt, 1972). Glutathione peroxidase can catalyze the reduction of all the polyunsaturated fatty acids that occur in subcellular membranes of rat liver (Christophersen, 1969), and peroxidase activity has been observed to increase during the hardening of cold resistant species (Gerloff et al., 1967; McGown et al., 1969). Accordingly, a hardening treatment would induce chilling resistance in plant tissues by preventing peroxidation of membrane lipids through peroxidase catalyzed reduction of hydroperoxides. There is some evidence that NADPH is the reductant in chilling adaptation (Kuraishi et al., 1968) although this has not been linked to a peroxidase catalyzed reaction in plant tissues.

We had previously reported that changes in one of three cellular pools of peroxidase occur during the onset of normal ripening in banana fruit (Haard and Tobin, 1971; Haard, 1972, 1973; Haard and Nagle, 1972). A "soluble" or cytoplasmic pool and a "cell wall" bound peroxidase were invarient in peroxidatic function during normal fruit ripening. A third cellular loci of peroxidase, identified extra-cellularly in the middle lamella, was observed to increase approximately 12-fold concomitantly with climacteric respiration. We have suggested that the extra-cellular pool of peroxidase functions in the coordination and amplification of biochemical events during normal fruit ripening. Alternatively, peroxidase associated with the soluble and wall bound fractions of plant tissues are presumably involved in lignin biosynthesis (Lipetz and Garro, 1965; Ranadive and Haard, 1972) and the reduction of membrane proteins and lipids (McGown et al., 1969). According to these views, one would predict that severe chilling of banana fruit would delimit the emergence of isozyme species involved in peroxidatic stress adaptation (soluble and wall bound fractions) as well as the appearance of peroxidase associated with the coordination of normal ripening. The present paper describes the effect of low temperature storage of banana fruit on the activity of peroxidase recovered in the above mentioned cellular pools.

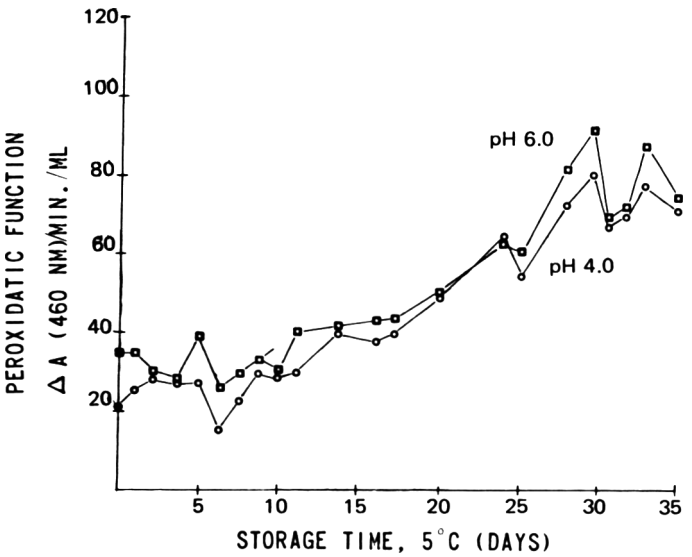

Fig. 1—"Soluble peroxidase" in low temperature stored banana fruit. Activity recovered from pulp homogenates on extraction with medium devoid of $CaCl_2$. Data are for one experiment and are typical of two other experiments.

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EXPERIMENTAL

Source of fruit

Preclimacteric banana fruit, No. 2 according to the scheme of Loesecke (1950), were generously donated by the United Fruit Co. The fruit were grown in Honduras and were approximately 8 days post-harvest (16°C) on receipt. The green banana fruit were stored at 5°C, 80% relative humidity with adequate ventilation for up to 30 days with the resulting severe chilling. It was important to adequately control relative humidity and the ventilation of air since conditions of water vapor deficit or anaerobiosis can cause other physiological disorders in banana fruit.

Isolation of peroxidase

Differential extraction of pulp tissue (25g) with 50 ml of each of the following media was used to isolate peroxidase pools designated as the "soluble," "wall bound" and "intercellular" fractions, respectively:

(1) sodium maleate buffer (0.02M, pH 6.0), casein disperions (1g) and insolubilized polyvinylpyrrolidone (0.5g);

(2) as (1) with 0.2M $CaCl_2$; and

(3) as (1) with 0.8M $CaCl_2$.

Liquid nitrogen powders of banana pulp were mixed with an appropriate isolation medium and centrifuged at 60,000 × G for 60 min at −5°C. The resulting supernatant fractions, which exhibited negligible oxidative browning, were assayed within 15–30 min after preparation. The rationale of these procedures has been described in detail elsewhere (Haard and Tobin, 1971; Haard, 1973). Briefly, the "soluble" peroxidase fraction is released from cell homogenates in extraction media of low ionic strength (less than 0.05) and is also released from whole slices (no solvent) centrifuged at 60,000 × G for 12 hr. The "wall bound" fraction remains associated with cell wall fragments and membrane debris of homogenates after repeated washing with low ionic strength buffer (independent of pH). The "wall bound" pool of peroxidase is released from cell particulates when the wash contains 0.1–0.2M $CaCl_2$. This fraction exhibits similar solubilization properties as the "wall bound" peroxidase identified in Crown-Gall tissue cultures (Lipetz and Garro, 1965). The third cellular pool is released from cell particulates on washing with 0.4–0.8M $CaCl_2$ and may be observed histochemically in the intercellular space. This appears to be an insoluble enzyme complex or extracellular body.

Table 1–Changes in Peroxidase and Visual Manifestations Associated with Chilled Banana Fruit.

Time (hr)	Visual manifestation of chilling	Peroxidatic function[a] Soluble	Wall bound	Extra cellular
6	None	Constant	Declining	Constant
24	Slight darkening of vascular tissue	Constant	Declining	Constant
72	Grayish-black mottled peel	Constant	Declining	Constant
120	Discoordination to ripening on return to 20°C, 85% RH	Constant	Minimal	Constant
144	Severe chilling, failure to ripen	Rising	Rising	Constant
168	Severe chilling, failure to ripen	Rising	Rising	Constant
600	Severe chilling, failure to ripen	Maximal	Maximal	Constant
600–720	Severe chilling, failure to ripen	Erratic	Declining	Constant

[a]Oxidation of o-dianisidine, pH 6.0.

Peroxidase assay

Tissue extracts were assayed for peroxidatic activity using 3,3'-dimethoxybenzidine dihydrochloride as H-donor and hydrogen peroxide as oxidant (Guilbault, 1968). The assay solutions were buffered at either pH 4.0 or 6.0 with sodium acetate-acetic acid at a final concentration of 0.01M. Extract volumes ranging from 10–200 µl in 3.0 ml of total cuvette volume were assayed with a Beckman DBG recording spectrophotometer at 460 nm. Activity was expressed as ΔA 460 nm/min for 1.0 ml of extract at 20°C.

Polyacrylamide gel electrophoresis

Extracts for analysis of isozyme profiles were prepared by the following procedure. A

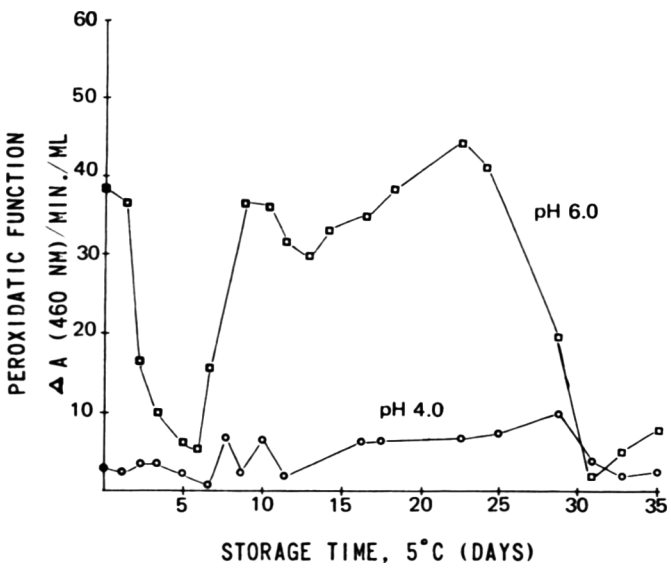

Fig. 2–Peroxidase bound to cell particulates in low temperature stored banana fruit. Activity recovered from pulp homogenates on extraction with medium containing 0.2M $CaCl_2$ less that recovered in "soluble peroxidase" fraction.

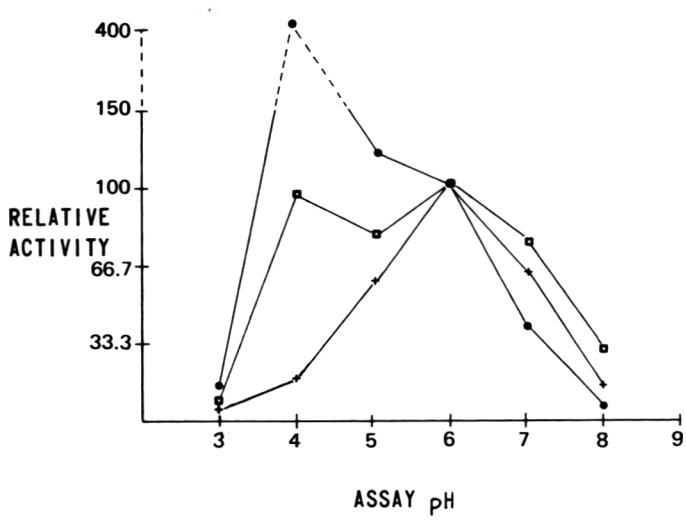

Fig. 3–pH optima of total peroxidase recovered from the homogenates of pulp from normal ripe (●–●), normal green (□–□) and severely chilled (+–+) banana fruit. Each set of data is expressed as relative to the activity at pH 6.0. Data are for one experiment and are typical of a duplicate trial.

25-g sample of liquid N_2 powder was extracted with a basic medium containing sucrose (17%), sodium ascorbate (0.1%), L-cysteine hydrochloride (0.1%), Tris-Cl (0.1M, pH 8.0) and appropriate levels of $CaCl_2$. The homogenate was centrifuged at 60,000 × G for 30 min and the supernatant was passed through a column containing Sephadex G-25 coarse (500 ml bed volume). The protein eluted in the void volume was dialyzed against 100 vol of 0.0M Tris-Cl, pH 8.0 made 10% in sucrose and applied to the surface of polyacrylamide gel tubes in aliquots ranging from 10–50 µl. Gels were prepared according to Seevers et al. (1971) and electrophoresed at a constant current of 3 milliamps per tube for 40 min. Gels were stained for peroxidase zones by incubation in benzidine-H_2O_2 according to Seevers et al. (1971).

RESULTS

Soluble peroxidase

Banana fruit stored at 5°C exhibited a gradual increase in the peroxidase pool solubilized from pulp at relatively low ionic strength (extraction medium 1) after plastic strain had occurred (Fig. 1). The relative activity at pH 6 to 4, initially at approximately 1:1.7, increased at about the same rate such that the ratio in severely chilled fruit was close to unity (e.g., at 15 days 1:1.1). The initiation of the rise in soluble peroxidase occurring at 5–10 days, coincided with the appearance of severe peel blackening, placenta darkening and failure of the fruit to ripen when transferred to conditions conducive to ripening (Table 1). The soluble peroxidase fraction was previously shown to be invarient during normal ripening at 20°C, 85% relative humidity (Haard and Tobin, 1971).

Wall bound peroxidase

The particulate bound pool of peroxidase (solubilized from pulp with extraction medium 2, less that solubilized with extraction medium 1) exhibited a rapid initial decline during the first few days of storage at 5°C followed by a progressive increase in activity at assay pH 6.0 (Fig. 2). The extracts exhibited little change in activity at pH 4.0 suggesting the appearance of specific isozyme species. The somewhat erratic increase in wall bound enzyme peaked at approximately 25 days and declined through the final stages of storage to 35 days. The wall bound fraction of peroxidase did not significantly change when fruit were normally ripened at 20°C, 85% relative humidity (Haard, 1973). The narrow pH optima of this fraction (predominant in total fraction) (Fig. 3) has been related to its isozyme content (Haard and Sedgewick, 1973).

Intercellular bound peroxidase

The pool of peroxidase extractable with isolation medium 3, less that solubilized with isolation medium 2, exhibited negligible change in green fruit subjected to the chilling environment. During normal ripening this fraction exhibited a 12-fold increase at assay pH 4.0 and a 4-fold increase at assay pH 6.0. The fraction, identified in the middle lamella region of ripening fruit (Haard, 1972, 1973) was not observed to emerge during low temperature storage. The influence of $CaCl_2$, in the isolation medium, on peroxidase solubilization from normal ripe, normal green and chilled fruit (15 day), shown in Figure 4, illustrates the different conditions required to release the fractions from the pulp of ripe fruit, green fruit and chilled fruit.

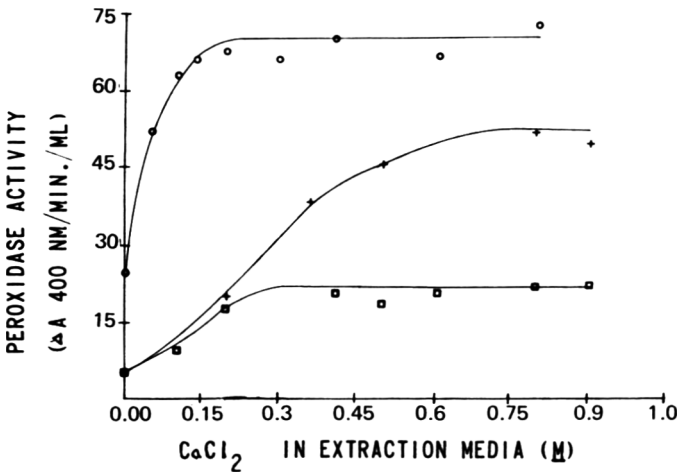

Fig. 4—Release of peroxidase from cell particulates as a function of $CaCl_2$ concentration in the extraction medium. Normal green (□—□), normal ripe (+—+) and severely chilled (○—○). Data are the average of duplicate experiments.

Polyacrylamide gel electrophoresis

Enzyme isolates prepared by the methods outlined above exhibited inconsistent isoenzyme profiles on polyacrylamide gels. This was apparently the result of peroxidase polymerization or aggregation occurring under these conditions (Haard and Nagle, 1972). However, when extracts were prepared in isolation medium buffered to pH 8.0 which contained cysteine and ascorbate and were rapidly passed through Sephadex G-50 (coarse) to remove low molecular weight substances, reproducible isozymic profiles were observed in the protein fraction. The distribution of isoenzymes from normal green severely chilled (15 days, 5°C) and normally ripened fruit, in the total recoverable peroxidase fractions are shown in Figure 5. The assay method employed was specific for peroxidase heme such that the pH optimum of isozyme species did not affect the observed distribution. These profiles are representative of the dominant pools in the respective tissues: wall bound fraction of the normal green and chilled fruit and the intracellular fraction of the ripening fruit. The increase in peroxidase activity associated with the onset of chilling injury appears to relate to the emergence of unique isozyme species and changes in the qualitative distribution of isozymes. The activity of extracts prepared by this technique was qualitatively (pH optima) and quantitatively similar to those prepared by extraction in maleate buffer. The isolation technique employed for electrophoretic analyses was not used for quantitative comparisons of peroxidase activity because of residual traces of antioxidant carried over during molecular sieve chromatography which made quantitation laborious and less accurate.

DISCUSSION

OUR PREVIOUS FINDINGS that the extraction techniques employed here yield peroxidase from the cytoplasm and cell wall fractions lead us to suggest that the peroxidase changes observed in chilled fruit reflect changes, or lack thereof, in these respective pools. This interpretation is also supported by histochemical analysis of chilled fruit. That is, one observes negligible peroxidatic function in the extracellular region of chilled fruit. Our view is that the observed increases in particulate bound and soluble peroxidase represents an attempt at cold adaptation or "hardening" which falls short because of the time lag. Note from our data that the "wall bound" peroxidase initially declines over the first 5–7 days of storage at 5°C and that increases of recovered enzyme did not ensue until after severe chilling injury or "plastic strain" was manifest. One line of evidence consistent with our interpretation is that increased peroxidase is related to a hardening

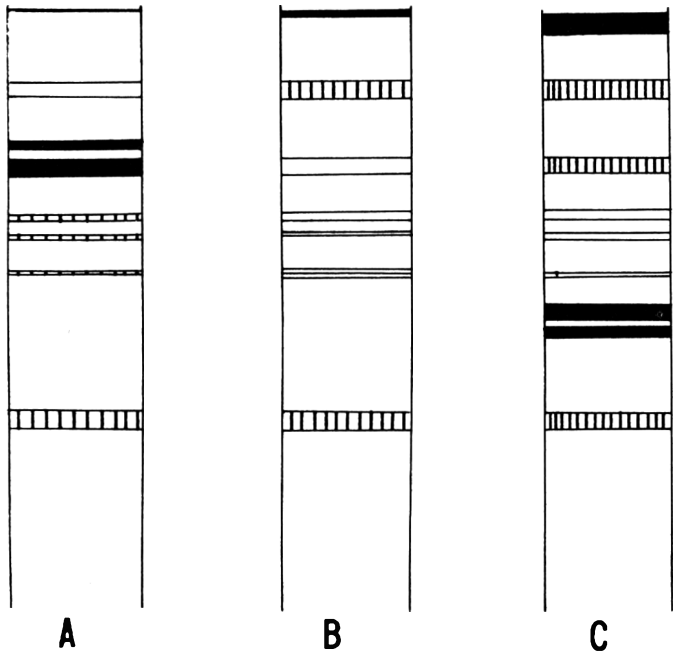

Fig. 5—Electrophoretic separation of peroxidase isozymes or polyacrylamide gels. Anode and origin at upper portion of gel diagram. Extracts (50 µl) recovered from severely chilled (A), normal green (B) and normal ripe fruit (C) by extraction method outlined in Experimental. Relative activity of zones: (● > ◍ > ○).

mechanism in that low molecular weight phenolic compounds decline with chilling while the highly polymerized phenols (polyleuco-anthocyanins and lignin) increase during this period (Haard and Ranadive, 1973). The synthesis of polymeric phenols has been associated with the cold hardening process and is indeed a reflection of the term "hardening" which describes the woody development of succulent tissues. Currently we are attempting to acclimate fruit to low temperature by gradually exposing the tissue to chilling conditions intermittently with nonchilling temperatures. If our interpretation of the data is correct it will be possible to store fruit at low temperatures by gradually acclimating the tissue to low temperature stress prior to the development of plastic strain. These interpretations, of course, hinge on the assumption that peroxidatic function estimated with o-dianisidine as H-donor relates to the type of reductive events we visualize occurring in situ. Further studies in our laboratory will be directed towards this supposition.

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CHARACTERISTICS OF PECTINS ISOLATED FROM SOFT AND FIRM FLESHED PEACH VARIETIES

INTRODUCTION

THE NATURALLY OCCURRING pectic materials present in most fruits and vegetables often have a marked effect on the texture of both the fresh and the processed product. This is one reason why numerous studies have been made to determine the quantity and characteristics of pectins in plant products. In the case of peaches, pectin is important for two reasons. Studies during the last 40–50 yr have clearly shown that texture is closely associated with the amount and nature of pectins in the fruit during the final stages of ripening. The typical melting fleshed varieties usually have a relatively high proportion of their pectins in a water soluble form when ripe. In contrast, firm fleshed varieties show a higher proportion of their pectins to be present in an insoluble form when the optimum stage of maturity is reached (Postlmayr et al., 1956; Shewfelt et al., 1971). Secondly, the texture and consistency of jam, nectar or syrup made from peaches, and the viscosity of the syrup in canned peaches are markedly influenced by the pectins that are originally present in the fruit because of their thickening and gelling properties.

No information is available on the gel forming characteristics of peach pectins and the factors influencing their gelation. The work reported here was done in an attempt to determine whether there are detectable differences in the composition and gelling characteristics of extracted pectins from green and ripe fruit of both melting flesh and firm fleshed peaches.

EXPERIMENTAL

Samples and sample preparation

Both hard-green and firm-yellow Elberta peaches were picked in a commercial orchard near Athens, Ga. The sample of firm-yellow peaches was left at room temperature for 3 days to become soft-ripe. Fruits which could not be prepared immediately for freezing were stored at 3°C. The fruit from both samples was cut into quarters with an electric saw and immediately dipped for 5 min in cold water containing 3% citric acid. The quarters were then frozen in a blast freezer on trays at a temperature of −30°C before packing in polyethylene bags and storing at −34°C.

Babygold Number 6 peaches (called Babygold peaches in the remainder of the text) which were picked in a commercial orchard in South Carolina were sorted by hand into green and ripe lots and then treated in the same way as the Elberta peaches except that the ripe fruit was not left at room temperature for further ripening.

The frozen quartered peaches were dried in a Virtis freeze dryer at a shelf temperature of −20°C and a vacuum of 100-250 microns to give a weight loss of approximately 80–85%. When necessary, the dried material was stored in thick-walled polyethylene bags at a temperature of −34°C.

After removal of pieces of seed and seedcoat, the dried material from each peach sample was blended with 95% boiling alcohol (dried material:alcohol::1:3) in a Waring Blendor for 5 min. The blended material was then brought to a boil and simmered for 5 min before filtering under vacuum. The filter cake was suspended in two volumes of 80% alcohol and left for 30 min at room temperature. It was filtered again and washed two more times. After the third washing the alcohol insoluble solids (AIS) were washed with 95% alcohol, followed by acetone. The AIS were then dried at 45°C for 48 hr.

A 100-g portion of peach AIS was suspended in 1,500 ml distilled water and thoroughly mixed. The pH was adjusted to 2.0 with concentrated HCl and the mixture was left for 30 min at room temperature. Thereafter, it was heated to 80°C and kept at that temperature for 60 min. After cooling, the extract was recovered by centrifugation in a Sorvall SS-3 Automatic Superspeed Centrifuge at 15,000 rpm for 10 min. The cake was suspended in another 600 ml distilled water, acidified to pH 2.0 with HCl, heated at 80°C for 10 min and centrifuged again. The liquid recovered was added to that of the first extraction before filtering under vacuum through a very rapid, qualitative filter paper. The clear filtrate was added to two volumes of 95% ethanol with stirring and left for 12 hr to allow precipitation and hardening of the pectin.

The precipitated pectin was recovered on cheesecloth and then washed twice by suspending it in 1,000 ml 80% ethanol. These suspensions were left for 1 hr before recovery of the precipitate on cheesecloth. Two final washings were carried out using 1,000 ml 95% ethanol and then 1,000 ml acetone. The recovered crude pectin was left at room temperature for 48 hr before drying for 48 hr at 45°C in an oven.

Dried pectins were ground in a Wiley Mill fitted with a 60 mesh screen before acid washing with 80% ethanol containing 5% HCl (by volume). The ratio of pectin to acid-alcohol was 1g:10 ml and acid washing was carried out three successive times by suspending the pectin in the acid-alcohol and waiting 5 min before filtration. Acid-alcohol washing was followed by 80% ethanol washing until the filtrate was free of chlorides. The washed pectin samples were buffered by suspending them in 80% ethanol and adjusting the pH to 3.8 with concentrated ammonia. After filtration the pectin was rinsed with 95% ethanol, followed by acetone. The pectin was then dried and ground as described earlier, packed in closed jars and stored at 5°C until used.

Analysis of fruit samples

Total solids were determined by drying under vacuum (Owens et al., 1952). Soluble solids were determined with a Bausch and Lomb Refractometer and readings were corrected to 20°C. AIS were determined by weighing 100-g portions of the freshly macerated sample into 600 ml beakers and adding 300 ml 95% ethanol. The suspension was brought to a boil and boiling was continued for 5 min. After standing for 12 hr the AIS were recovered and washed three times with 100 ml 80% ethanol, once with 100 ml 95% ethanol and finally with 100 ml acetone. Recovered precipitates were dried for 48 hr at 45°C in an oven before cooling in a desiccator and weighing.

Analysis of isolated pectins

Moisture and ash content of the isolated pectins were determined as described by Owens et al. (1952).

Acetyl content was determined by a modification of the distillation procedure described by Schultz (1965). The equipment used consisted of a steam generator, a distilling flask and two water-cooled condensers. The distillation rate was controlled by using a variable voltage transformer. A 0.5-g sample was weighed into a 250 ml Erlenmeyer flask and moistened with 2 ml 95% ethanol before addition of 25 ml 0.1N NaOH. The flask was stoppered and shaken to

Table 1—Analyses of fresh peaches

	Elberta		Babygold	
Analysis	Unripe	Ripe	Unripe	Ripe
Moisture (%)	88.2	87.5	87.3	87.6
Total solids (%)	11.8	12.6	12.7	12.4
AIS (%)	2.3	2.8	2.3	2.8
Soluble solids (%)	9.6	11.0	9.8	10.4

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dissolve the sample. After standing at room temperature for 1 hr it was diluted to 50 ml with distilled water. A 20 ml aliquot was transferred to the distilling flask and a 20 ml portion of Clark's magnesium sulfate-sulfuric acid solution (100g of crystalline magnesium sulfate and 1.5g concentrated sulfuric acid, made up with distilled water to 180 ml) was added. Two drops of 2-octanol were added as a defoaming agent. Steam distillation was then started and 150 ml of distillate were collected. This was titrated with 0.1N NaOH to a phenolphthalein endpoint. A blank test was made by distilling a mixture of 20 ml magnesium sulfate-sulfuric acid solution and 20 ml of distilled water and titrating the distillate in the same manner.

Percent methoxyl, degree of esterification, percent anhydrogalacturonic acid and equivalent weight were determined by titration after acid washing and drying (Smit and Bryant, 1967).

Intrinsic viscosity was obtained by measuring flow times in 1% sodium hexametaphosphate solution at pH 4.5 (Christensen, 1954). Apparent molecular weight was then calculated by dividing intrinsic viscosity with 4.7×10^{-5} as suggested by Christensen (1954).

Jellies were prepared and their grades were determined according to the procedure developed by the IFT Committee on Pectin Standardization (1959). After the determination of sag, jellies were cut lengthwise into slices of approximately 7 mm thick and a Marine Colloids Gel Tester was used to measure the breaking pressure in g. For each jelly, readings were obtained on three center slices and the values were averaged.

RESULTS & DISCUSSION

THE COMPOSITION of the four peach samples used is given in Table 1. As expected the ripe fruit from both varieties had a higher soluble solids content than the green fruit. Further, the percent AIS was higher in the more mature samples.

In Table 2 the yield and composition of the isolated pectins are presented. Recovery of the pectin was obviously not quantitative but the data show a markedly lower yield in the case of unripe Babygold peaches. This may have been due to a less efficient extraction of the AIS obtained from the green firm fleshed fruit. Methoxyl levels, degrees of esterification and equivalent weights were relatively high. In the case of the Elberta fruits, pectin obtained from the ripe fruit had a higher methoxyl content, a higher galacturonic acid content and a lower equivalent weight than the pectin obtained from the unripe fruit. This suggests that some accompanying nonuronide residues were removed from the pectin during ripening. Further, a large decrease in intrinsic viscosity and apparent molecular weight occurred during the ripening and softening of Elberta peaches. This decrease was much smaller in ripe firm fleshed peaches. Recently Pressey et al. (1971) reported the presence of polygalacturonase in ripe melting fleshed peaches and the presence of this enzyme was probably responsible for the data obtained with the ripe Elberta fruits.

Acetyl levels of all four samples ranged between 0.68–0.89%. Acetyl levels as high as 3% have been found in the pectin of peaches (McCready and McComb, 1954) but it is unlikely that the samples evaluated in the present study could have had acetyl levels as high as 3% because it was possible to prepare jellies with these samples. This would not have been the case at such high levels (Pippen et al., 1950).

Data obtained on evaluation of jellies prepared with the isolated pectin samples are given in Table 3. Jelly grades were quite low when compared with citrus pectins having fairly similar ester levels and molecular weights (Smit and Bryant, 1969). In contrast with citrus pectin, the isolated peach pectins had acetyl levels ranging between 0.68 and 0.89% and this may have been responsible for the poor gelling characteristics observed (Pippen et al., 1950). It should be stressed though that pectins from different sources often differ with respect to their gel forming ability and a comparison of the pectin from two different peach varieties or between peach pectin and citrus pectin is therefore questionable.

It is generally true that within certain limits, an increase in methoxyl level or degree of esterification and a decrease in molecular weight will decrease jelly grade and strength of high solids pectin jellies. Further, acetyl content also influences gel characteristics (Doesburg, 1965). In the case of the Elberta fruits, ripening and softening of the fruit was accompanied by a large decrease in apparent molecular weight of the pectin. This did not affect grade seriously, but breaking pressure was markedly decreased. It is not clear how a small difference in acetyl level below 1% affects grade and breaking pressure, but its possible effect, as well as the small difference in methoxyl content, were apparently far outweighed by the large difference in molecular weight. In the case of the Babygold peaches it is probably reasonable to speculate that the somewhat lower grade and breaking pressure of the pectin from the ripe fruit was due to a lower apparent molecular weight and a slightly higher acetyl content in spite of the slightly lower methoxyl content. Pectin from the green Elberta fruit and the ripe Babygold fruit had very similar methoxyl levels, but the apparent molecular weight of the pectin from the ripe Babygold fruit was considerably lower. In spite of this, grades and breaking pressures were almost identical. The only explanation which can be offered is that the lower acetyl level of the pectin in the ripe Babygold peaches helped to increase grade and breaking pressure. Pectin from

Table 2–Properties of isolated peach pectins

	Elberta		Babygold	
Analysis	Unripe	Ripe	Unripe	Ripe
Moisture (%)	10.8	10.7	10.9	10.5
Ash (%)	0.53	0.58	0.51	0.54
Acetyl[a] (%)	0.89	0.81	0.68	0.73
Methoxyl[a] (%)	11.5	12.1	11.8	11.4
Anhydrogalacturonic acid[a] (%)	84.2	89.9	85.1	85.0
Esterification (%)	79.9	79.1	80.8	77.7
Equiv wt[a]	1,306	1,164	1,341	1,171
Intrinsic viscosity	8.91	3.68	6.65	5.71
Apparent mol wt $\times 10^{-3}$	210	78	141	121
Pectin yield (%)[b]	0.44	0.44	0.24	0.40

[a] On ash- and moisture-free basis
[b] On fresh fruit basis

Table 3–Jellies obtained with peach pectins

	Elberta		Babygold	
Nature of Jelly[a]	Unripe	Ripe	Unripe	Ripe
Sag (%)	21.3	22.4	17.7	20.5
pH	2.2	2.2	2.2	2.2
Soluble solids (%)	65.0	65.7	65.5	65.5
Jelly grade	110	102	123	111
Breaking pressure (g)	112	55	132	111

[a] % of pectin in jelly mixture was 0.64%. Jelly aging time was 24 hr at room temperature.

the green Babygold fruit had a considerably lower apparent molecular weight and a slightly higher methoxyl content than the pectin from the green Elberta fruit. In spite of this its grade and breaking pressure were higher. Again, a lower acetyl level may have been the reason for this. Pectin from the ripe Elberta peaches had a low molecular weight compared to the pectins from the two Babygold samples and this was probably the major reason why its jelly grade and breaking pressure were lower. In addition, its methoxyl content and acetyl content were higher.

Although the extraction and purification of pectins result in considerable depolymerization and deesterification, the data presented here suggest that peach pectins are generally poor gel formers when compared with pectins derived from such fruits as citrus or apples. This is apparently due to the presence of acetyl groups, high methoxyl levels and low molecular weights. Further, the softening of melting flesh peaches is accompanied by a marked decrease in the molecular weight of the pectin resulting in poor gel characteristics.

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ANTHOCYANIN PIGMENTS OF SOUR CHERRIES

INTRODUCTION

THE TWO MAJOR pigments of sour cherries (Prunus cerasus L., var Montmorency) were reported as antirrhinin and mecocyanin by Li and Wagenknecht (1956). Later, these pigments were shown to be structurally similar to cyanidin-3-rutinoside and cyanidin-3-sophoroside by Harborne (1963). Harborne and Hall (1964) also studied seven varieties of sour cherries, excluding Montmorency, and found cyanidin-3-rutinoside in all varieties, along with cyanidin-3-sophoroside and branched cyanidin-3-glucosylrutinoside in six out of seven varieties. Branched cyanidin-3-glucosylrutinoside in Montmorency cherries was confirmed simultaneously by Dekazos (1970) and Fisher and Von Elbe (1970) as a major pigment. Dekazos (1970) also reported the occurence of peonidin-3-rutinoside, peonidin and cyanidin, along with cyanidin-3-sophoroside, cyanidin-3-rutinoside and cyanidin-3-glucoside.

The simultaneous presence of two branched trisaccharides of cyanidin, e.g., cyanidin-3-glucosylrutinoside and cyanidin-3-xylosylrutinoside were reported in the fruits of Ribes and in the flowers and leaves of Begonia. However, cyanidin-3-xylosylrutinoside was uniformly absent from the fruits of Prunus (Harborne and Hall, 1964).

Evidence is presented here to show that cyanidin-3-xyloxylrutinoside is a minor pigment component of Prunus cerasus L., var Montmorency. It was discovered by the use of the improved solvent system, BFW, developed by Fuleki (1969). This pigment was also found in three other varieties of sour cherries, i.e., English Morello, Early Richmond and Meteor.

MATERIALS & METHODS

Extraction

Sour cherries (Prunus cerasus L., var Montmorency) obtained from a local grower near Amherst were pitted and frozen. About 1 kg of frozen cherries were blended with 1.5 liters of 1% HCl in methanol and stored overnight at 4°C. The extract was filtered and evaporated under vacuum at 30°C to a final volume of 150 ml. The pigment extract was partially purified by using CG 50 ion exchange resin by the method of Fuleki and Francis (1968).

Chromatographic methods

All separations and purifications were made with Whatman No. 3 filter paper, but Whatman No. 1 paper was used for R_f and R_g data. In all cases, descending chromatography was used at 20°C in the dark. The following solvents were used for anthocyanins, aglycones and sugars:

1. BAW. 1-butanol, glacial acetic acid, water (4:1:5). Upper phase. Made up fresh for R_f data.
2. BFW. 1-butanol, formic acid, water (100:25:60). Upper phase. Aged 3 days (Fuleki, 1969).
3. BBPW. 1-butanol, benzene, pyridine, water (5:1:3:3).
4. Forestal. Glacial acetic acid, conc hydrochloric acid, water (30:3:10).
5. Formic. Formic acid, conc hydrochloric acid, water (5:3:3).
6. HAC-HCl. Water, glacial acetic acid, conc hydrochloric acid (82:15:3).
7. 15% HOAC. Water, glacial acetic acid (85:15).
8. Phenol. Phenol, water (4:1).
9. 1% HCl. Conc hydrochloric acid, water (3:97).
10. MAW. Methanol, glacial acetic acid, water (90:5:5).

Chromatographic separations and purifications

The partially purified anthocyanins from the ion exchange resin were concentrated under vacuum, streaked on 10 sheets of Whatman No. 3 filter paper and developed in BFW till the fastest moving band reached the end of paper. The separated bands were cut and eluted with MAW, concentrated, reapplied on No. 3 paper and developed in 1% HCl. Similarly, the third and fourth purifications were achieved by using BAW and 15% HOAC solvents respectively.

Paper chromatography of anthocyanins

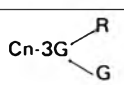
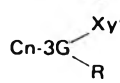
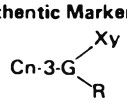
The concentrated purified pigments were applied as small dots on No. 1 paper and developed in 1% HCl, HAC-HCl, 15% HOAC and BAW. The average R_f value of each pigment from two or more papers was reported.

Identification of aglycones and sugars

The aglycones were obtained by heating approximately 1 mg of purified pigment with 2N HCl in a water bath for 30 min. The aglycones were extracted with amyl alcohol, evaporated to dryness and spotted on No. 1 paper together with authentic markers. The papers were developed in forestal and formic solvents.

The solution remaining after the extraction of aglycones was treated with di-n-octyl methyl amine (Harborne, 1958) to remove the mineral acids, washed with chloroform, evaporated to dryness and spotted on No. 1 paper together with authentic markers. The papers were run in phenol and BBPW solvents. After development, the papers were dried, sprayed with aniline hydrogen phthalate (Partridge, 1949), heated in an oven at 105°C for 2–5 min and the spots were viewed under a UV illuminator. The re-

Table 1—R_f data for cherry anthocyanins

		Solvents			
		1% HCl	HAC-HCl	15% HOAC	BAW
1		0.61	0.75	0.79	0.24
2b		0.49	0.71	0.76	0.26
2c	Cn-3GG	0.34	0.60	0.71	0.29
3a	Cn-3RG	0.14	0.43	0.58	0.31
3b	Cn-3G	0.045	0.25	0.37	0.39
4a	Pn-3RG	0.19	0.49	0.63	0.33
4b	Isomer of 3b	0.055	0.26	0.39	0.40
Authentic Markers					
		0.49	0.71	0.76	0.26
	Cn-3-GG	0.36	0.60	0.71	0.30
	Cn-3-GR	0.14	0.44	0.60	0.31
	Cn-3-XyG	0.24	0.54	0.65	–

sults were expressed as the R_g value, i.e., the R_f related to that of glucose as unity.

Controlled hydrolysis

Acid hydrolysis for determination of intermediate pigments was performed on 3–5 mg of pigment in 2N HCl by heating in a boiling water bath. Portions were removed at 0, 1, 2, 4 and 6 min, etc. and spotted directly on No. 1 paper. The papers were run in 1% HCl, HAC-HCl, 15% HOAC and BAW.

Spectral data

All spectral data was determined with a Perkin-Elmer 450 recording spectrophotometer. The $AlCl_3$ shift was determined by dissolving the purified pigment in 0.01% HCl methanol and adding 1–2 drops of 5% ethanolic $AlCl_3$ solution.

General

The separation, purification and identification of anthocyanins was confirmed on Montmorency cherries. The pigment identification of other varieties of sour cherries was done by comparison of the chromatograms of separated pigments with the chromatograph of Montmorency cherries under identical conditions. Also controlled hydrolysis data for cyanidin-3-(2G-xylosylrutinoside) were reported with cyanidin-3-(2G-glucosylrutinoside) and cyanidin-3-sophoroside for easy comparison for similarities and dissimilarities in the intermediate products of acid hydrolysis.

Authentic pigments

Branched cyanidin-3-xylosylrutinoside was isolated and purified from red currants. This compound was reported to be absent in Red Lake currants in England by Harborne and Hall (1964), but it was found in this variety grown in Amherst, Mass. (unpublished data). Cyanidin-3-sophoroside and cyanidin-3-rutinoside were purified from red raspberries (Francis, 1972). Cyanidin-3-sambubioside was supplied by Du and Francis (1972). Cyanidin and peonidin aglycones were obtained and purified from cranberries (Zapalis and Francis, 1965).

RESULTS & DISCUSSION

Separation and purification

The resin purified concentrated pigment solution when streaked on No. 3 paper and developed in BFW for 72–80 hr, separated into four bands. The separated bands were numbered 1–4 in order of increasing R_f value. Clear separation of the No. 2 band into two bands could be achieved by a second purification in 1% HCl. Similarly, band No. 3 and 4 from BFW each separated into two more bands in 1% HCl. The pigments were eluted and final purification was done in successive steps by running the chromatograms in BAW and 15% HOAC.

The R_f values of seven purified pigments in four solvent systems indicated the presence of two trisaccharides of cyanidin, two disaccharides of cyanidin, one disaccharide of peonidin and two monoglucosides of cyanidin (Table 1).

Acid hydrolysis of the pigments for aglycones and sugars confirmed this finding. The comparisons of R_f data of the pigments and the R_f of aglycones and R_g values of sugars indicated that pigment 1 is cyanidin-3-(2G-glucosylrutinoside), pigment 2b is cyanidin-3-(2G-xylosylrutinoside), pigments 3a and 4a are the 3-rutinosides of cyanidin and peonidin and 3b and 4b are both cyanidin-3-monoglucoside.

Controlled hydrolysis was performed on pigments 1, 2b and 2c (Table 2). Pigment 1 produced 3 intermediate bands which in order of appearance were cyanidin-3-sophoroside, cyanidin-3-rutinoside, cyanidin-3-monoglucoside. Pigment 2b produced cyanidin-3-sambubioside, cyanidin-3-rutinoside and cyanidin-3-monoglucoside. Pigment 2c gave only cyanidin-3-monoglucoside as an intermediate. Therefore, pigment 2b must be cyanidin-3-(2G-xylosylrutinoside).

Spectral data for A_{440}/A_{max} (Table 3) indicated that all the pigments have the five position free and three position blocked by sugars (Harborne, 1967). The $AlCl_3$ shift was positive in all cases, except pigment 4a in which the negative shift suggested peonidin as the aglycone (Table 4). The absence of peaks in the region 310–350 nm suggested no acylation of the pigments.

In this study, cyanidin-3-(2G-xylosylrutinoside) is reported for the first time in the fruits of sour cherries. This pigment (as well as the other five anthocyanins listed in Table 1) is also present in other varieties of sour cherries studied, e.g., English Morello, Early Richmond and Meteor. The presence of this pigment in sour cherries in not surprising, because its occurence was already confirmed in the fruits of Rubus and Ribes.

Table 2—Chromatographic data on products of controlled hydrolysis of cyanidin triglycosides in sour cherries

Original pigment	Intermediate pigments	Solvent 1% HCl R_f value	Solvent HAC-HCl (× 100)
Cn-3G(R,G)		57	81
	Cn-3GG	36	69
	Cn-3GR	12	47
	Cn-3G	5.0	31
	Cn	1.4	12
Cn-3G(R,X)		50	72
	Cn-3GX	24	52
	Cn-3GR	14	42
	Cn-3G	5.0	28
	Cn	1.0	9.0

Table 3—Spectral data for anthocyanins in 0.01% HCl in MeOH

Pigments		Abs_{max} nm (Vis)	UV_{max} nm	A_{440}/A_{max}	$AlCl_3$ shift
1	Cn-3G(R,G)	523	278	31	+12
2b	Cn-3G(Xyl,R)	525	–	27	+15
2c	Cn-3GG	523	278	32	+15
3a	Cn-3RG	525	290	20	+10
3b	Cn-3G	528	–	35	+10
4a	Pn-3RG	524	280	26	no shift

Table 4—Products of acid hydrolysis of anthocyanins

Pigment	Aglycone	Sugars
1	cn	glucose, rhamnose
2b	cn	glucose, xylose, rhamnose
2c	cn	glucose
3a	cn	glucose, rhamnose
3b	cn	glucose
4a	pn	glucose, rhamnose
4b	cn	glucose

It is possible that the presence of this pigment was not noticed previously, due to the inefficient solvent systems used for the separations. It is our experience that if aqueous solvents are used for the first purifications, the separation of cyanidin-3-glucosylrutinoside and cyanidin-3-xylosylrutinoside is very difficult, especially when the latter is a minor pigment. In this study, BAW also failed to separate the two pigments. The only solvent suitable for this work was BFW. Even after 72–80 hr of development time, both pigments were not clearly separated. However, in the second purification with 1% HCl, both separated clearly. With red currants, when cyanidin-3-xylosylrutinoside is the major pigment, both separated easily in BFW.

The other pigments observed in this study have already been reported by Dekazos (1970). His study had shown cyanidin and peonidin in the free states. In the course of this study, with four varieties of sour cherries, free aglycones were never observed. It is possible that these aglycones are artifacts due to chemical degradation of anthocyanins (Von Elbe and Shaller, 1968) or enzymatic hydrolysis of the pigments which have been reported from time to time (Van Buren et al., 1960; Siegel et al., 1970).

The presence of 4b which has a higher R_f than cyanidin-3-glucoside (3b) in all four solvent systems may be an isomer of pigment 3b. Unfortunately, not enough pigment was available for identification studies.

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GINGER RHIZOME: A NEW SOURCE OF PROTEOLYTIC ENZYME

INTRODUCTION

PROTEOLYTIC ENZYMES are sometimes used in meat cooking and curing, and have been shown to increase the tenderness of meat as a result of proteolysis of the various meat protein fractions (Gottschall and Kies, 1942; Miyada and Tappel, 1956; Tappel et al., 1956; Wang et al., 1958a, b). Among the proteolytic enzymes of plant origin, papain, ficin and bromelain have been most extensively studied (Glazer and Smith, 1971) and are commonly used for tenderization of meat. This study with ginger rhizome investigated a new source of plant proteolytic enzymes that are of importance to food products.

Ginger is the washed and dried rhizome of a root stock of *Zingiber officinale roscoe.* Ginger grows in tropical Asia and is cultivated in the West Indies, Africa, China, Japan and the East Indies. The chief commercial varieties of ginger are Jamaican, Indian, African and Japanese. Fresh ginger sliced, minced, crushed or as juice is used extensively in Chinese foods. Ginger is used to a lesser extent in Japanese and Filipino cuisine. In the U.S., ginger is used primarily in a powdered form as a flavoring for bakery products, sausage seasoning, mince meat and in curry powder (Merory, 1968).

From personal observations, a tenderizing effect was noted when meat was cooked with fresh ginger slices. The authors are not acquainted with any previous work on the proteolytic activity of ginger rhizome. Investigations of the proteolytic effects of ginger rhizome were therefore undertaken, and the proteolytically active principle isolated was named Zingibain, according to the suggestion of Greenberg, Winnick and Lineweaver for naming plant proteolytic enzymes (Greenberg and Winnick, 1940a).

EXPERIMENTAL

Extraction of protease activity

Fresh unprocessed ginger rhizome was obtained from a local supermarket. An acetone powder was prepared by dicing the rhizomes and homogenizing in five parts (w/v) of acetone using a Polytron homogenizer. The homogenate was suction filtered and the filter cake was rinsed with an additional five parts of acetone. The filter cake was dried in a forced air oven at 40°C until no acetone odor was detectable and the dry powder was further pulverized with a Polytron homogenizer.

The acetone powder was gently homogenized for 5 min in 20 parts (w/v) of 0.1M phosphate buffer (pH 6.0) containing 0.6 mM dithiothreitol (DTT). The extract was suction filtered and the filtrate was centrifuged at 48,000 × G for 30 min at 0°C. The supernatant, which contained all of the proteolytic activity, was adjusted to pH 5.0 and the protein was precipitated from solution by slowly adding 1.1 part (v/v) of cold acetone with gentle stirring. Stirring was continued for 5 min and the precipitate was sedimented by centrifugation at 13,000 × G for 10 min at 0°C. The supernatant was decanted and the precipitate was rinsed with acetone and dried in a forced air oven at 40°C until no acetone odor was detectable.

Estimation of proteolytic activity

Bovine serum albumin. Proteolytic activity of the above acetone precipitate was measured by the release of 3% trichloroacetic acid (TCA) soluble peptides from 3% bovine serum albumin (BSA). 10 mg of the enzyme preparation was solubilized per ml of 0.1M phosphate buffer (pH 5.0) containing 0.3 mM DTT. 2 ml of 3% BSA solution in 0.1M phosphate buffer were pre-incubated for 5 min and 100 μl of enzyme solution were added. Reaction was terminated after 10 min by adding 3 ml of 5% TCA and cooling in a 12°C water bath. The reaction mixture was filtered through Whatman No. 1 filter paper and the TCA soluble peptides in the filtrate were quantitated by measuring extinction at 280 mμ. A standard 280 mμ extinction curve was prepared with BSA in phosphate buffer. Blanks were prepared by incubating BSA in buffer for 15 min, then adding the enzyme solution and followed immediately by the addition of 5% TCA. Enzyme activities are expressed as mg TCA soluble peptides/min/mg enzyme protein.

Preparation of collagen and actomyosin.

Hide powder (Calbiochem) was used as a collagen source after extracting with 50 parts (w/v) of 0.1M phosphate buffer (pH 6.0) for 20 min at 60°C, followed by suction filtration. The filter cake was rinsed with distilled water followed by acetone, dried in a forced air oven at 40°C and pulverized with a Polytron homogenizer. This procedure removed the readily soluble protein (about 20%) from the hide powder preparation. A repeated extraction of the buffer-washed hide powder at 60°C for 15 min in 0.1M phosphate buffer followed by addition of 5% TCA resulted in the solubilization of only about 5% additional protein (collagen).

Actomyosin was extracted from beef muscle as outlined by Briskey and Fukazawa (1971). The purified actomyosin thus obtained was repeatedly washed with distilled water and centrifuged to reduce the KCl concentration to a negligible level (0.07 mM/g dry actomyosin). The final actomyosin precipitate was freeze dried and pulverized with a Polytron homogenizer.

Proteolysis of collagen and actomyosin. Collagen and actomyosin were rehydrated in 0.1M phosphate buffer (pH 5.0) at 5°C for 20 hr and incubated with the ginger protease in the same manner as with BSA. Soluble peptide content of the TCA filtrates was determined with Folin-phenol reagent (Lowry et al., 1951). TCA-buffer filtrates were neutralized with 0.05 parts (v/v) of 4.2N NaOH prior to assay. An actomyosin standard curve was prepared by dissolving actomyosin in 1.25N NaOH and using the appropriate reagents for protein determination with this modification (Lowry et al., 1951). A standard curve determined with gelatin was used for collagen quantitation. Terminal amino groups of the TCA-soluble peptides were quantitated by use of trinitrobenzenesulfonic acid (Satake et al., 1960; Habeeb, 1966). Average length of soluble peptides was estimated from the ratio μM of total amino acids/μM of terminal amino acids.

Tenderization of meat

The ginger protease preparation was dissolved in 0.1M phosphate buffer (pH 5.0) containing 0.3 mM DTT at a concentration of 0.2 mg/ml. Sixty, 100-g samples of ovine biceps femoris muscle from six carcasses were injected with 0.05 ml of the enzyme preparation per g meat and stored at 5°C for 20 hr. Muscle samples taken adjacent to these treatment samples were similarly injected with buffer to serve as controls. Samples were placed individually in plastic cooking bags and baked at 163°C to an internal temperature of 70°C. The cooked samples were cooled to 5°C. Six shear values were determined on each of two adjacent 1 cm × 1 cm × 7 cm strips from each sample using a Warner-Bratzler shear apparatus. An F-test (Steel and Torrie, 1960) was used to test for statistical differences in shear values between the control and enzyme-treated samples.

RESULTS & DISCUSSION

IT WAS FOUND advantageous to extract fresh ginger rhizome with acetone to remove pigments which interfered with spectrophotometric analysis of proteolytic activity. This also eliminated problems of sprouting or molding of the rhizomes since the dry powder was stable at room temperature. In addition, this technique allowed a number of rhizomes to be combined and used as a standard source of ginger protease throughout these experiments.

Table 1–Proteolysis of collagen and actomyosin by acetone precipitated ginger rhizome protease

Temp °C	Soluble peptide amino acid			Terminal amino acid			Average peptide length[c]	
	Collagen[a]	Actomyosin[a]	Collagen / Actomyosin	Collagen[b]	Actomyosin[b]	Collagen / Actomyosin	Collagen	Actomyosin
30	9.303	0.306	30.402	0.333	0.038	8.763	27.9	8.1
40	19.589	0.664	29.502	0.693	0.089	7.787	28.3	7.5
50	29.603	0.936	31.627	0.927	0.147	6.306	31.9	6.4
60	33.652	0.741	45.414	1.318	0.083	15.880	25.5	8.9
70	25.525	0.124	205.847	0.990	0.019	52.105	25.8	6.5
							X = 27.9	7.5

[a] μM of peptide amino acid solubilized/mg enzyme protein/min

[b] μM of terminal amino acid in soluble peptide amino acid/mg enzyme protein/min

[c] (μM of peptide amino acid solubilized/mg enzyme protein/min)/(μM of terminal amino acid in soluble peptide amino acid/mg enzyme protein/min)

Optimum conditions for extraction and precipitation of enzyme

The pH of fresh ginger rhizome was found to be about 6.0. Extraction of the acetone powder with buffer over a pH range of 4.0–8.5 showed that maximum proteolytic activity was extracted at pH 6.0 (Fig. 1). A preparation with a greater specific activity (proteolytic activity/unit protein) was obtained by extracting at pH 5.3, but at this pH, only about 65% of the total proteolytic activity was extracted.

The supernatant of the buffer extract (pH 6.0) was adjusted to pH values ranging from 3.0–8.0 before precipitation of enzyme protein with acetone. Maximum proteolytic activity was precipitated at pH 5.0 (Fig. 2).

Upon slow addition of acetone, precipitation first occurred at 0.8 parts of acetone:1.0 part of buffer extract (Fig. 3). This initial precipitate had a relatively high specific activity, but less than half of the total proteolytic activity was precipitated. At 1.1 part acetone:1.0 part buffer, nearly all of the proteolytic activity was precipitated and specific activity was reasonably high. The decrease in specific proteolytic activity of precipitated enzyme material between 1.1 and 1.5 parts acetone:1.0 part buffer was due to precipitation of nonenzyme material.

The specific activity of the acetone powder of ginger rhizome was 0.032 mg peptide/min/mg powder, whereas that of the acetone precipitated proteolytic principle under optimum conditions of extraction and precipitation was 0.736 mg peptide/min/mg powder. Thus, a 23-fold increase in protease activity was obtained by the extraction-precipitation treatment.

The enzyme preparation used for the remainder of this study was prepared under optimum conditions of extraction and precipitation. Rhizomes with a light blue ring visible on cross-sectional slices were used in preparing the acetone powder, since these rhizomes generally contained more protease activity than those without a blue ring. 60g of a typical rhizome with the blue ring present yielded 1.356g of dried enzyme preparation (acetone precipitate), which is 2.26% of the fresh rhizome. As a comparison, it has been reported that approximately 8000 lb of fruit of the papaya plant are required to produce 1 lb of commercial papain (Kimmel and Smith, 1957).

Effect of pH on proteolytic activity

The effect of pH on proteolytic activity with 3% BSA as substrate is shown in Figure 4. This plot represents the means of eight replicates at each of the indicated pH values. The data indicate that there may be at least two enzymes (or iso-enzymes) present in this preparation, resulting in pH optima of 5.0 and 5.6. No attempt has been made to isolate individual enzymes from this crude preparation. The well-known plant proteases

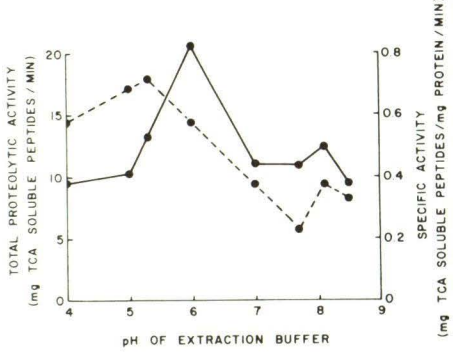

Fig. 1–Effect of pH of buffer used for extraction of acetone powder on total proteolytic activity extracted from 1g acetone powder and on specific activity of the acetone precipitate with BSA as substrate. (——, total activity extracted; ----, specific activity of acetone precipitate.)

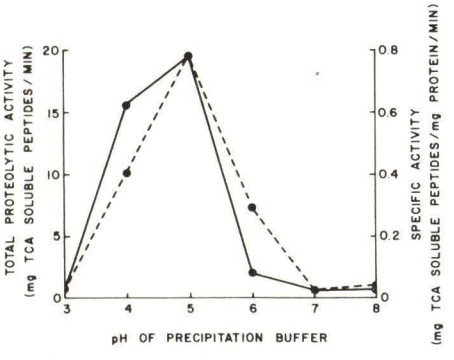

Fig. 2–Effect of pH at which acetone precipitation is carried out on total proteolytic activity precipitated from 1g acetone powder and on specific activity of the acetone precipitate with BSA as substrate. (——, total activity precipitated; ----, specific activity of acetone precipitate.)

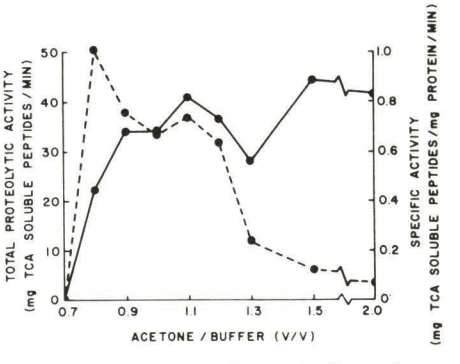

Fig. 3–Effect of the acetone:buffer ratio on total proteolytic activity precipitated from 1g acetone powder and on specific activity of the acetone precipitate with BSA as substrate. (——, total activity precipitated; ----, specific activity of acetone precipitate.)

consist of a mixture of two or more enzymes with different pH optima (Whitaker, 1957b; Heinicke and Gortner, 1957; Glazer and Smith, 1971). The pH curve is affected somewhat by the type of substrate and buffer (Heinicke and Gortner, 1957), and this must be considered in comparing different plant proteases. BSA was selected for this experiment because of its high solubility over a broad pH range.

Effect of substrate concentration on proteolytic activity

The effect of BSA substrate concentration was studied at 60°C and pH 5.0. Reaction velocity (mg peptide/min/mg powder) was independent of substrate concentration from 2–3% BSA, and was directly proportional to enzyme concentration with these conditions. The rate of BSA hydrolysis was linear at 3% substrate for at least 10 min indicating that a true measure of initial velocity was obtained under these experimental conditions.

The Michaelis constant (Km), as determined by the graphic method of Lineweaver and Burk (1934), was found to be 0.37. This value is in the same general range as that reported for ficin hydrolysis of casein (Whitaker, 1957b) and for papain and bromelain hydrolysis of hemoglobin (Greenberg and Winnick, 1940b).

Effect of temperature on proteolytic activity

The effect of temperature on initial reaction velocity is shown in Figure 5. Maximum velocity was achieved at 60°C, and apparent rapid denaturation of the enzyme occurred at 70°C.

Temperature optima of other proteolytic enzymes of plant origin have been reported, but under varying conditions of time and substrate (Tsen and Tappel, 1959; Grover, 1965; Whitaker, 1957a); therefore it is difficult to obtain an accurate comparison. However, the optimum temperature ranges of ficin and papain are generally higher and of bromelain is generally lower than that of the ginger rhizome protease.

Thiol group

Dithiothreitol (DTT) is a thiol group protector similar to cysteine and glutathione. Maximum proteolytic activity was achieved at DTT concentrations greater than 0.2 mM (0.4 μM/mg enzyme protein) and was approximately double that obtained without DTT in the reaction system. It was also found that by including DTT (50 μM/g powder) in the phosphate buffer used for extraction of the acetone-dried powder, the specific activity of the acetone precipitated proteolytic principle was considerably greater than that extracted without DTT. These findings indicate the probable involvement of thiol groups at the active sites of the enzyme. This is of interest since papain, ficin and bromelain are cysteine proteases with very similar amino acid sequences at the active sites of the enzymes (Light et al., 1964; Wong and Liener, 1964; Chao and Liener, 1967).

Proteolysis of collagen and actomyosin

Preliminary studies showed that fresh ginger rhizome prevented gel formation when suspended in a solution of gelatin, whereas ginger rhizome that had been boiled previously did not prevent gel formation. The same phenomenon was observed with fresh and boiled acetone precipitated ginger protease.

The proteolysis of collagen and actomyosin by ginger protease was studied over a temperature range of 30–70°C. There was a linear increase in proteolysis rate between 30 and 50°C for both protein fractions (Table 1). At temperatures greater than 50°C, proteolysis rate of actomyosin was reduced more rapidly than that of collagen. Proteolysis at temperatures between 30 and 50°C resulted in the solubilization of about 30 times more collagen than actomyosin. On the basis of the terminal amino acid analysis, 6–9 times more peptide bonds of collagen were hydrolyzed than of actomyosin. Average peptide length of solubilized collagen was 27.9 amino acid residues compared to 7.5 for actomyosin, with little variation over the temperature range studied.

Additional experiments involving the proteolysis of collagen and actomyosin by ginger protease were carried out for 20 hr at 5°C followed by 10 min at 60°C to simulate meat marination and cooking conditions. A somewhat different pattern was developed as compared to incubations for 10 min at 60°C only. This treatment resulted in the solubilization of about three times as much actomyosin as when incubations were carried out at 60°C only. The total collagen solubilized was almost identical for the two treatments. Therefore, with these conditions, the amount of collagen solubilized was about 12 times greater that that of actomyosin.

Kang and Rice (1970) reported that the major meat protein fractions hydrolyzed by ficin, bromelain and papain were, respectively, salt soluble, insoluble and salt soluble. Tsen and Tappel (1959) reported that papain was more active on actomyosin than on collagen. Average peptide lengths reported in this study are in the same general range as those reported for peptides solubilized by bromelain, ficin and papain with similar substrates (Kang and Rice, 1970).

Effect of salt on proteolytic activity

The effect of salt on proteolytic activity was studied since proteolytic enzymes are sometimes used for tenderization of cured meat products such as corned beef brisket. Sodium chloride had an inhibitory effect on proteolysis of BSA and collagen (Fig. 6). Proteolysis of BSA was

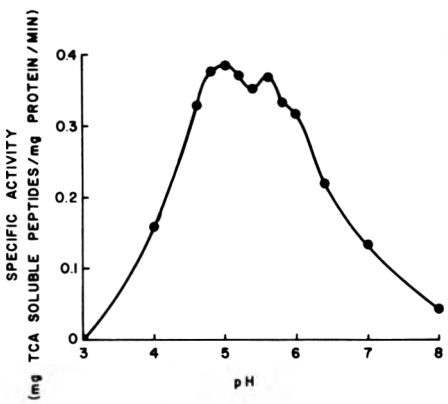

Fig. 4—Effect of pH on proteolytic activity with BSA as substrate.

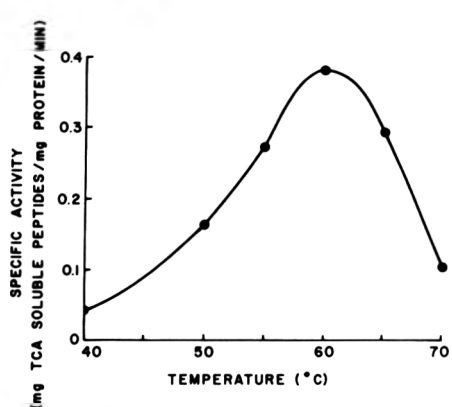

Fig. 5—Effect of temperature on proteolytic activity with BSA as substrate.

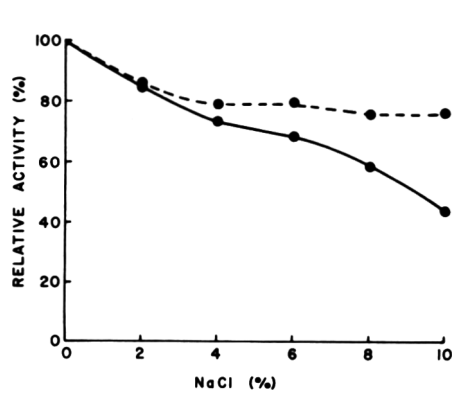

Fig. 6—Effect of salt on proteolytic activity (——, BSA; ····, collagen.)

inhibited to a greater extent than that of collagen at salt concentrations greater than 2%.

Enzyme protein did not appear to be precipitated by salt at the concentrations studied, but the structure of the enzyme as well as that of the substrate may have been altered sufficiently to produce a loss in proteolytic activity. Since the pI of BSA is about 4.6, the difference between the collagen and BSA curves in Figure 6 may be explained as a salt induced pI effect on BSA. The degree of proteolytic inhibition at salt concentrations normally found in cured meat products is low enough so that there would still be considerable proteolysis by this enzyme preparation.

Tenderization of meat

On the basis of the pH activity curve with BSA as substrate, the range of optimum activity from about pH 4.5–6.0 would be ideal for use in meat marinades or for treatment of fresh meat since these would normally fall within this pH range.

The effective temperature range would also be suitable for meat tenderization since proteolysis would occur mainly during the cooking process, with denaturation of the enzyme occurring toward completion of cooking.

Analysis of variance of shear values of cooked meat showed that the enzyme treated samples were significantly ($P < 0.05$) more tender than the untreated controls. The overall mean of shear values for controls was 4.27 kg, and that for enzyme-treated samples was 2.8 kg. Shear values from the centers of treated strips were less than those from the end of the strips, sometimes resulting in mushiness at the center. This was probably due to uneven enzyme distribution as a result of the injection technique used since there was little variation in shear values over the entire length of control strips. Using the conditions of this study, 1g of the acetone precipitated proteolytic fraction (equivalent to about 40g fresh rhizome) would significantly tenderize 10 kg of meat. Further characterization of this proteolytic activity is underway.

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MEASUREMENT OF CHLOROGENIC ACID AND FLAVONOL GLYCOSIDES IN APPLE JUICE BY A CHROMATOGRAPHIC-FLUOROMETRIC METHOD

INTRODUCTION

THERE HAS BEEN considerable interest in the phenolic compounds of apples because of the influence of these compounds on the color and clarity of the juice. Knowledge of the amounts of specific phenolics is worthwhile because of differences in reactivity between the various types. For example, flavans and chlorogenic acid are good substrates for apple polyphenol oxidase (Stelzig et al., 1972) while quercetin glycosides are very poor substitutes. Flavans and quercetin glycosides are lost during juice storage while chlorogenic acid is quite stable in pasteurized juice (Tanner and Rentschler, 1956). In connection with a general study of the characteristics of apple juice prepared from enzyme treated pulp (Pilnik and de Vos, 1970; Verspuy et al., 1970; de Vos, 1970) a quantitative description of the phenolic material was desirable. In the course of that work relatively simple procedures were developed for the measurement of chlorogenic acid and flavonol glycosides.

Measurement of specific phenolic compounds in fruit juices is usually quite difficult. Widely used chemical methods, such as those involving the Folin–Ciocalteu reagent (Singleton and Rossi, 1965) or the vanillin–sulfuric acid reagent (Swain and Hillis, 1959) give positive results with a wide variety of substances. The same is true for direct spectrophotometric determinations. When these methods are used to estimate particular phenolic compounds a preliminary separation is necessary involving problems of sample handling and corrections for incomplete recoveries.

Chlorogenic acid has been measured by a number of different methods. After paper chromatographic separation and elution of the chlorogenic acid containing areas the amount of chlorogenic acid was estimated by Walker (1963) and Pomenta and Burns (1971) using the Hoepfner reaction (Zucker and Aherns, 1958), while Macheix (1967) estimated the chlorogenic acid by means of its absorbance at a wavelength of 328 nm. Sondheimer (1958) used a silica gel column to remove interfering compounds before direct measurement at 330 nm. A spectrophotometric method based on spectral shifts between different solvents has been proposed by Tissut (1970).

Flavonol glycosides have been measured by means of their absorbance in solutions and extracts. Some investigators measured the absorbance in the presence of aluminum ions (Dame et al., 1958) while others did not use aluminum (Workman, 1963). The amount of clean-up needed before spectrophotometric measurement varies, but most workers have carried out a chromatographic separation or a lead acetate precipitation.

A decrease in the time needed and an avoidance of handling correction could result if the compounds were measured directly on the chromatograms. Chlorogenic acid has a natural fluorescence and this property has been used to locate its presence on chromatograms and to provide a visual estimation of its presence in apple juice (Tanner and Rentschler, 1956). Flavonol glycosides are made fluorescent through the addition of aluminum (Harborne, 1959). Ibrahim (1969) has shown that the fluorescence of many compounds can be used for their quantitative measurement directly on thin layer chromatograms. His method has been adapted to apple juice for the measurement of chlorogenic acid and flavonol glycosides.

EXPERIMENTAL

A VARIETY of commercial cellulose thin-layer chromatography plates and solvents were tested for suitability with regard to the compounds under consideration. Criteria used were compactness of developed spots, degree of separation from possible interfering substances and intensity of fluorescence.

Fluorescence measurements were obtained directly from the thin layer plates through the use of a Vitatron TLD100 (flying spot) densitometer. The light beam from the mercury lamp was passed through a UVB filter before it struck the thin layer plate. The scan speed of the beam across the plate was 3 cm/min. The intensity of light reaching the photo-multiplier

Table 1—Recovery of chlorogenic acid added to apple juice

Sample	Chlorogenic acid measured, mg/100 ml	% recovery	Std. error of mean
Juice A	14.5		
Juice A + 5 mg chlorogenic acid per 100 ml	19.6	102	± 8
Juice A + 10 mg chlorogenic acid per 100 ml	23.6	91	± 6
Juice B	3.2		
Juice B + 5 mg chlorogenic acid per 100 ml	8.1	98	± 7
Juice B + 10 mg chlorogenic acid per 100 ml	12.5	93	± 4

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Table 2—Effects of dilution[a] on measurement of chlorogenic acid in apple juice

Sample	Chlorogenic acid measured, mg/100 ml	Std. error of mean	Chlorogenic acid expected mg/100 ml
Juice C	19.1	± .62	
Diluted to give 50% juice C	10.2	± .38	9.5
Diluted to give 25% juice C	5.84	± .33	4.8

[a] Dilutions were made using a 10% sucrose solution.

Table 3—Apple juice flavonol glycoside groups separated on chromatograms

R_{fn}	Probable flavonol glycoside
0.29	Quercetin-3-arabinoside (avicularin) Quercetin-3-xyloside (reynoutrin)
0.38	Quercetin-3-galactoside (hyperin) Quercetin-3-glucoside (isoquercitrin)
0.49	Quercetin-3-rhamnoside (quercitrin)

n = 3

was recorded on a strip chart (Vitatron UR 402/M) equipped with an integrator. The integrator readings were used for standard curves and the quantitative measurements on juice.

It was found desirable to treat the juice with a pectolytic enzyme preparation, Ultrazym Spezial (Dr. Schubert AG) at a concentration of 0.04g/liter, in order to obtain clean running chromatograms. Incubation was continued at room temperature until the juice no longer showed gel formation when 2 volumes of ethanol were added. This method did not give rise to detectable quantities of caffeic acid or quercetin.

Chlorogenic acid

The 20 × 20 cm thin-layer plates used were DC Fertigplatten Cellulose, without fluorescence indicator, 0.1 mm thick (Merck, Darmstadt), a crystalline cellulose layer. Before chromatography they were washed with a 1:1 mixture of water and ethanol (v/v) to remove material that might cause a variation in background fluorescence. The plates were dried and a thick line of cellulose was removed 5 cm from the top of the plate to provide an upper boundary for the ascending chromatographic solvent.

Samples were applied 3.5 cm apart on a line 3 cm from the bottom edge of the plate. Volumes of sample per spot ranged from 5–20 μl, depending on the expected concentration of chlorogenic acid. The amount of chlorogenic acid per spot ranged from 0.15–3.0 μg. Chlorogenic acid (Fluka) was used as the standard.

The spots were allowed to dry and then the plates were placed in a chromatography tank containing the developing solvent, a mixture of 200 ml of n-butanol and 55 ml of 20% acetic acid (v/v) in water. With this sort of solvent the chlorogenic acid does not separate into its cis and trans isomers (Williams, 1955), thus making quantitative measurements easier. After the solvent had moved to the upper boundary lines the plates were removed from the tanks and allowed to air dry overnight. The chlorogenic acid spots visible under UV light were lightly marked with pencil, and the fluorescence at these locations was measured with the densitometer. A U5 filter and a 1.0 mm diameter slit opening were used between the fluorescing spot and the photo-multiplier. The output voltage level was c, the zeroing switch at 5, and the span potentiometer setting was 5.0.

The sample holder was set to oscillate 2.2 cm. The direction of scanning was perpendicular to the direction of chromatography since there was a tendency for the background fluorescence to increase at higher R_f values.

This procedure was tested in apple juices to which a known amount of chlorogenic acid had been added, Table 1. The recovery of chlorogenic acid was between 102% and 91%. A further test was conducted on the effect of juice dilution on the amount of chlorogenic acid indicated by the method, Table 2. These results showed slightly higher values than had been expected from calculation based on the concentration in the original juice.

Flavonol glycosides

The 20 × 20 cm thin-layer plates used were MN-Polygram CEL 300 (Machery-Nagel, Düren) without prior washing. Samples were applied 3.5 cm apart on a line 3 cm from the bottom end of the plate. Volumes of sample per spot varied from 5–20 μl, and amounts of flavonol glycosides ranged between 0.05 and 2.0 μg. The standard compound was rutin (Fluka) and amounts of flavonol glycosides were expressed as weight of rutin.

Before the spots were completely dry the plates were placed in the chromatography tank containing the developing solvent, an aqueous solution containing 5% (v/v) n-butanol and 0.2% (w/v) HCl. After the solvent had moved to the upper edge the plate was removed, allowed to air dry for 90 min and replaced in the chromatography tank. Development was carried out a total of three times.

Multiple development was used because the rather low mobilities of the flavonol monoglycosides in the aqueous solvent resulted, after one development, in a compact flavonol area where the concentrations of flavonols, and the corresponding fluorescence, were often higher than could be accommodated on a good standard curve. Multiple development separated individual flavonol glycosides, reducing the total concentration at any one location. Furthermore, multiple development made apparent that chromatography with the aqueous solvent separated the apple flavonols into three groups with central R_{fn} values as listed in Table 3. Included here are the probable specific flavonol glycosides present in each group as deduced from reports on the flavonol glycosides of apple

Table 4—Effects of diluting[a] or mixing on the measured amounts of flavonol glycosides[b] in apple juice

Sample	Flavonol glycoside measured, mg/100 ml	Std. error of mean	Flavonol glycoside expected, mg/100 ml
Juice D	8.5	± .18	
Juice D diluted to give 50% D	4.4	± .23	4.2
Juice D diluted to give 25% D	2.1	± .13	2.1
Juice D diluted to give 10% D	.82	± .04	.85
Juice E	.7	± .03	
Equal volumes of juice D and juice E mixed together	4.9	± .16	4.6

[a] Dilutions were made using a 10% sucrose solution.
[b] Expressed as weight of rutin

Table 5—Chlorogenic acid in juice from different varieties of apples[a]

Variety	Chlorogenic acid (mg/100 ml)
Golden Delicious	12
Lombard	18
Carrel	31
Goudrenet	29

[a] Juices prepared by conventional procedure: mature apples were pulped, pressed, the juice was enzymatically clarified, pasteurized and filtered.

Table 6—Flavonol glycoside content of Golden Delicious apple juice

Type of juice	Flavonol glycoside[a] (mg/100 ml)
Conventional, freshly made	0.4
From pulp that had been oxidized 3 hr, freshly made	6.5[b]
From pulp that had been oxidized 3 hr. Pasteurized juice stored 3 months at 35°C	2.5

[a] Expressed as weight of rutin

[b] More flavonol glycoside extracted from skin portion of pulp

(Siegelman, 1955) and the R_f values of these glycosides on paper or cellulose using aqueous solvents (Harborne, 1959; Fisher, 1965).

R_f was calculated from the total distance moved by the flavonols during the multiple development. The conversion formula was:

$$(1 - R_f)^n = 1 - R_{fn}$$

where n = number of developments and R_{fn} = total distance moved by the material divided by the distance from the spot origin to the upper limit of the solvent front.

The multiple development also gave a better separation between the fastest moving flavonol glycoside and the only slightly faster moving caffeic acid present in some samples of juice.

After the third development the plate was allowed to air dry overnight. A 4% solution of $Al_2(SO_4)_3 \cdot 18\ H_2O$ was applied to the plate with a paint roller and the plate allowed to air dry until the next day. The paint roller allowed uniform saturation of the plates with the Al_2SO_4 solution without worry of excess liquid running off the plate.

The intensity of the yellow fluorescing areas was measured with the densitometer utilizing a U11 filter and a 2 × 0.25 mm slit before the photo-multiplier. The voltage output level was c, the zeroing switch at 5 and the span potentiometer at 5.0 The direction of scan was the same as for the chromatography and the sample holder had an oscillation of 1.2 cm.

The integrator values for the amount of fluorescence between R_{fn} 0.16 and 0.56 were used as a measure for the flavonol glycosides of the juice. For the rutin standards the fluorescence between R_{fn} 0.58 and 0.79 was used. In the case of the apple juice rutin was a very minor part of the total flavonol glycosides and was not quantitatively measured.

The validity of the use of rutin as a standard was checked by a chemical determination of phenolic compounds. An apple juice prepared from oxidized pulp was analyzed by both the chromatographic-fluorometric method and the Folin-Ciocalteu method. This juice was known to have flavonol glycosides as the only phenolic material present in significant amounts. The chromatographic-fluorometric method indicated a content of 6.0 mg/100 ml, and the chemical method gave 8.2 mg/100 ml, in both cases expressed as mg of rutin.

The method for flavonol glycosides was tested in diluted and mixed juices, Table 4. The results corresponded closely to those expected.

RESULTS

WHEN THE chlorogenic acid procedure was applied to juices prepared from different varieties of apples the results shown in Table 5 were obtained.

The flavonol glycoside procedure was used to estimate these compounds in apple juices prepared by different methods of pulp handling and also in a juice stored under adverse temperature conditions, Table 6.

DISCUSSION

THE TWO METHODS described here have been adapted for use with apple juice. Their simplicity is due, in large part, to the fact that apple juice is free of materials, such as anthocyanins and flavones, that might interfere with the fluorescence measurements. It might be expected that the procedures could also be applied to pear juice, white grape juice and white wines.

The advantages of the methods are that they avoid laborious separations, eliminate losses of materials during elution, and permit a rough visual estimate of concentrations before the densitometric readings.

The results obtained can be compared to those found by earlier workers. For chlorogenic acid in mature apples there has been reported 25 mg/100g fresh fruit (Macheix, 1967), 89 mg/100g fresh fruit (Walker, 1963) and 84 mg/100g dry fruit (Sondheimer, 1958). In crab apples Kuusi and Pajunen (1971) reported levels of 46–205 mg/100g of fresh fruit and 39–135 mg/100 ml of juice. Thus the described method for chlorogenic acid yields results of the same magnitude as indicated by earlier research. The same might be said of the flavonol glycoside method. The amounts found in juice derived from oxidized pulp were similar to those reported by Workman (1963) and Walker (1964) for whole apples.

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SPECTRAL CHARACTERISTICS OF THREE VARIETIES OF FLORIDA ORANGE JUICE

INTRODUCTION

SOME RESEARCH has been published relating the ultraviolet absorption spectra of solutions of citrus juices to extractor pressure and degree of finishing during processing. Both of these variables influence the quality and yield of commercial citrus juices and concentrates. Hendrickson et al. (1958) investigated the effect of processing variables on the ultraviolet absorption of grapefruit juice. They observed an increase in naringin absorption at 285 nm with increased extractor pressure, or with the addition of rag and pulp. They also observed increased absorption when they applied heat to the raw juice. Subsequently, Hendrickson et al. (1959) developed an ultraviolet absorption method for determining the hesperidin content in orange juice and peel extracts.

Vandercook and Rolle (1963) investigated the total polyphenolic content of lemon juices and measured ultraviolet absorption maxima at 326–332 nm and 273–277 nm. They reported that the ratio of the absorbance at 273 nm to the absorbance at 326 nm was essentially constant for lemon juice. From analyses of the ultraviolet absorption spectra of solutions of grapefruit, orange and apple fruit juices they indicated that, if one were added to lemon juice, its presence could be detected by a displacement of this ratio.

The investigations of carotenoid pigments in citrus fruits and juices are many and complex. Most of the methods, such as those of Higby (1962) and Benk (1961), require extraction and separation of the carotenoids and involve elaborate procedures entailing a series of operations. Ting (1961) reported spectral curves of carotenoids and carotenes extracted from commercial samples of early, mid- and late season packs of Florida frozen orange juice concentrate. The results revealed a much higher total carotenoid and carotene concentration in late season samples. Absorption peaks or shoulders were indicated at 400, 423, 445 and 470 nm. Miller et al. (1941) found that the carotenoid content in the juice of Hamlin and Pineapple oranges gradually increased during the varietal season, but that Valencia increased to a maximum and then decreased. Bernath and Swisher (1969) reported on a method for determining color adulteration of orange juice with carotenes or carotene derivatives.

In the above mentioned work the investigators were concerned with either the visible absorption (carotenoids) or with the ultraviolet absorption (polyphenols, flavonoids) portion of the spectrum. It is our opinion that much useful information related to citrus products and processing may be gained by obtaining the visible and ultraviolet absorption concurrently on the same sample. The work presented herein was undertaken to

Table 1—Absorption characteristics for Hamlin orange juice obtained at three extraction pressures

Date	443 nm	325 nm	280 nm	245 nm	443/325 nm	280/325 nm	Sum 443 + 325 + 280 nm
				State Test Squeeze			
11/ 3/71	.040	.487	.898	1.580	.082	1.844	1.425
11/17/71	.040	.526	1.002	1.828	.076	1.905	1.568
12/ 1/71	.050	.574	1.100	1.980	.087	1.916	1.724
12/29/71	.061	.625	1.310	2.100	.098	2.096	1.996
1/12/72	.071	.598	1.318	1.928	.119	2.204	1.987
2/ 2/72	.081	.668	1.567	2.060	.121	2.346	2.316
				Light Squeeze			
11/ 3/71	.038	.444	.842	1.528	.086	1.896	1.324
11/17/71	.043	.475	.932	1.740	.091	1.962	1.450
12/ 1/71	.057	.523	1.090	1.980	.109	2.084	1.670
12/29/71	.051	.503	1.078	1.960	.101	2.143	1.632
1/12/72	.070	.529	1.270	1.920	.132	2.401	1.869
2/ 2/72	.071	.539	1.300	1.950	.132	2.412	1.910
				Hard Squeeze			
11/ 3/71	.039	.687	1.272	1.692	.057	1.852	1.998
11/17/71	.048	.740	1.382	1.960	.065	1.868	2.170
12/ 2/71	.053	.709	1.382	2.100	.075	1.949	2.144
12/29/71	.057	.727	1.498	2.140	.078	2.061	2.282
1/13/72	.073	.724	1.600	2.040	.101	2.210	2.397
2/ 3/72	.078	.715	1.580	2.120	.109	2.210	2.373

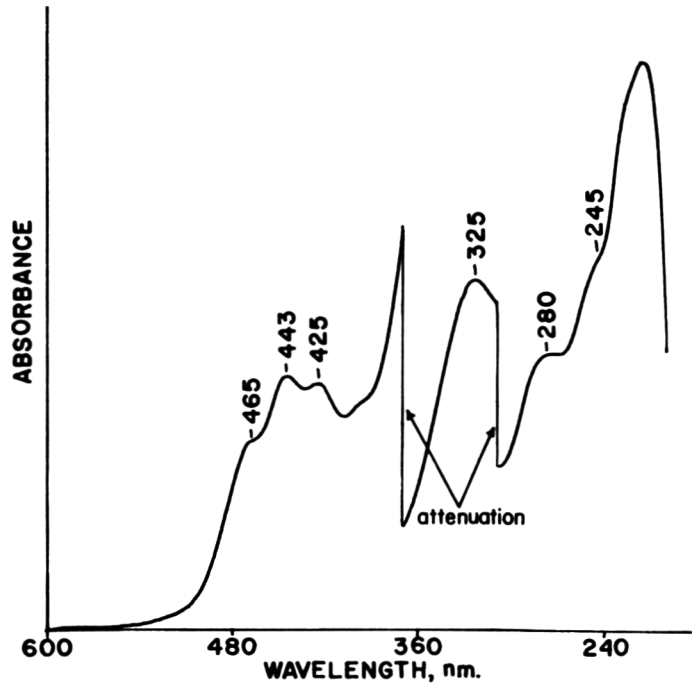

Fig. 1—Absorption spectrum of Florida Valencia orange juice.

investigate the combined visible and ultraviolet absorption characteristics of Florida Hamlin, Pineapple and Valencia orange juices and show how these characteristics may be used to show changes in the juice during fruit maturation or processing.

EXPERIMENTAL

Juice sample preparation

A 1800–2200-lb sample of each variety (Florida Hamlin, Pineapple and Valencia) of fruit was harvested at 2-wk intervals during the 1971–72 seasons. Each sample was washed, graded and divided into three lots for extraction at three different pressures on an FMC In-Line extractor set to give a light squeeze, a hard squeeze and one corresponding to the State official test (1970). All juices were immediately heat stabilized (195°F for approximately 28 sec), rapidly cooled to 43–46°F, canned and stored at 32°F. On two occasions during each varietal season a portion of the light squeeze and hard squeeze juices were concentrated to approximately 60° Brix and stored at −8°F. For the purposes of analysis, the chilled juices were analyzed as they were when canned, but the concentrated juices were reconstituted to 12.8° Brix which is the standard procedure for commercial Florida frozen orange concentrate.

Preparation of alcoholic solution for spectrophotometric analysis

The juice sample (single strength or reconstituted concentrate) was diluted with an equal volume of water. 5 ml of the diluted juice were made to 50 ml with absolute alcohol. The alcoholic solution was then placed in a dark place until the flocculent precipitate formed and the sample could be centrifuged to remove the precipitate. Care was taken to avoid exposing the sample to light which was found to cause significant decreases in absorption intensities in some areas of the spectrum. As a matter of convenience the samples were prepared in the afternoon placed in the dark and the following morning centrifuged to remove the flocculent precipitate. The spectrophotometric determinations were then conducted on the centrifugates.

Spectra of the orange components (juice, rag, albedo and flavedo) were also obtained periodically for each variety. The components were separated very carefully by hand. The juice component was obtained by first cold peeling and sectionizing the fruit. The juice was

Table 2—Absorption characteristics for Pineapple orange juice obtained at three extraction pressures

Date	443 nm	325 nm	280 nm	245 nm	443/325 nm	280/325 nm	Sum 443 + 325 + 280 nm
				State Test Squeeze			
12/15/71	.080	.643	1.225	2.120	.124	1.905	1.948
1/ 5/72	.091	.730	1.408	2.260	.125	1.929	2.229
1/25/72	.098	.726	1.399	2.220	.135	1.927	2.223
2/16/72	.110	.780	1.542	2.220	.141	1.977	2.432
2/23/72	.122	.829	1.613	2.220	.147	1.946	2.564
3/ 1/72	.127	.783	1.505	2.120	.162	1.922	2.415
				Light Squeeze			
12/15/71	.073	.591	1.163	2.080	.124	1.968	1.827
1/ 5/72	.085	.670	1.349	2.260	.127	2.013	2.104
1/25/72	.088	.682	1.325	2.200	.129	1.943	2.095
2/16/72	.102	.722	1.498	2.184	.141	2.075	2.322
2/23/72	.105	.734	1.520	2.160	.143	2.071	2.359
3/ 1/72	.119	.720	1.448	2.100	.165	2.011	2.287
				Hard Squeeze			
12/15/71	.075	.771	1.480	2.280	.097	1.920	2.326
1/ 6/72	.089	.870	1.619	2.460	.102	1.861	2.578
1/25/72	.093	.874	1.619	2.340	.106	1.852	2.586
2/17/72	.105	.889	1.665	2.360	.118	1.873	2.659
2/23/72	.113	.923	1.758	2.340	.122	1.905	2.794
3/ 1/72	.122	.927	1.768	2.280	.132	1.907	2.817

then removed from the juice sacs by gently pressing the sections on a 20 mesh, stainless steel screen until enough juice was obtained for analysis. The alcoholic solution of the juice sample was prepared as stated above. Samples of the other components were obtained by mixing 10g with 90g of distilled water and blending the mixture in a Sorvall Omni-Mixer. After blending, the samples were deaerated and the alcoholic solution prepared in the same manner as juice.

A Coleman Model 124 Recording Spectrophotometer employing an R-136 photomultiplier detector and 1.000-cm cells was used. The alcoholic juice solutions were scanned from 600 to 200 nm using 90% ethanol as a reference.

RESULTS & DISCUSSION

A TYPICAL SPECTRUM of heat stabilized juices obtained for mature Valencia oranges is shown in Figure 1. The visible portion of the spectrum is due mainly to the carotenoids present. The ultraviolet portion is due mainly to the total polyphenols (325 nm), total flavonoids (280 nm) and ascorbic acid (245 nm). This does not imply that individual polyphenols or flavonoids all have the peak absorbances at the wavelengths indicated. The spectral shape of the curves for the three varieties studied was quite similar except for differences in the absorption intensities. A comparison of the spectra of the fresh and corresponding heat stabilized juices revealed that they were superimposable indicating little or no change in absorption characteristics due to heat stabilization.

A direct comparison of the spectrum of a heat stabilized juice with that of its corresponding reconstituted concentrate was not attempted because the reconstituted concentrate was adjusted to 12.8° Brix and not the °Brix of the original single strength juice from which the concentrate was made. These differences in juice concentration did not change the spectral shape of the curves but did change the absorption intensities. The important observation was that the 443/325 nm and 280/325 nm absorption ratios were changed little or not at all by concentrating. The same observation was evident when serial dilutions of orange juices were analyzed. All observed absorption maxima obeyed Beer's law for known concentrations of orange juice solutions prepared as described.

Tables 1, 2 and 3 present the absorption data, at the various wavelengths of interest, for the three varieties and three extractor pressures. Hamlin and Pineapple orange juices, Tables 1 and 2, revealed a general increase in absorption with maturity from the visible through 280 nm. The absorption attributed to ascorbic acid (245 nm) increased to a maximum and leveled off or slowly decreased with maturity. Visible absorption for Valencia orange juice, Table 3, revealed only small changes with maturity. Absorption at 325 and 280 nm appeared mixed but with a tendency to increase as the season progressed. Absorption at 245 nm varied only slightly with maturity.

Tables 1, 2 and 3 also show the effects of extractor pressures on the absorption within a variety. There appear to be only slight changes in the visible absorption with increased extractor pressure. However, ultraviolet absorption for all three varieties increased with increasing extractor pressure. Spectra of the alcoholic solutions of the orange juice component revealed it to be the major contributor to the visible absorption and having also strong ultraviolet absorption. Spectra of alcoholic solutions of the rag and albedo components of the orange varieties showed very little, if any, visible absorption. However, these components showed very strong ultraviolet absorption. Higher extractor pressures would tend to incorporate more of these components into the juice causing a higher ultraviolet absorption. Therefore, the lower ultraviolet absorption indicated the light squeeze to be the most mild treatment.

The tables also show that the absorbance values at 443 nm increased from early to mid- to late season varieties. Pineapple orange juice was highest in both the 280 and 245 nm absorption, but differed little from Valencia juice in the 325 nm absorption region. Hamlin juice was lowest in absorption at 325 nm. Early in

Table 3—Absorption characteristics for Valencia orange juice obtained at three extraction pressures

Date	443 nm	325 nm	280 nm	245 nm	443/325 nm	280/325 nm	Sum 443 + 325 + 280 nm
				State Test Squeeze			
3/15/72	.149	.707	1.200	1.940	.210	1.697	2.056
4/13/72	.146	.662	1.170	1.800	.221	1.767	1.978
4/26/72	.154	.688	1.199	1.690	.224	1.743	2.033
5/10/72	.152	.729	1.247	1.778	.209	1.711	2.128
5/24/72	.139	.709	1.165	1.645	.196	1.643	2.013
6/ 7/72	.138	.759	1.270	1.585	.182	1.673	2.167
6/21/72	.152	.813	1.430	1.688	.187	1.759	2.395
				Light Squeeze			
3/15/72	.139	.640	1.090	1.859	.217	1.703	1.869
4/13/72	.140	.639	1.130	1.755	.219	1.768	1.909
4/26/72	.140	.627	1.137	1.678	.223	1.813	1.904
5/10/72	.137	.679	1.170	1.739	.202	1.723	1.986
5/24/72	.130	.689	1.113	1.580	.189	1.615	1.932
6/ 8/72	.132	.653	1.115	1.470	.202	1.708	1.900
6/21/72	.129	.682	1.170	1.518	.189	1.716	1.981
				Hard Squeeze			
3/16/72	.148	.855	1.338	2.040	.173	1.565	2.341
4/13/72	.146	.873	1.550	1.965	.167	1.775	2.569
4/27/72	.143	.858	1.488	1.840	.167	1.734	2.489
5/10/72	.143	.921	1.490	1.975	.155	1.618	2.554
5/24/72	.137	.922	1.413	1.820	.149	1.533	2.472
6/ 8/72	.130	.930	1.603	1.760	.140	1.724	2.663
6/22/72	.130	.931	1.605	1.708	.140	1.724	2.666

their varietal seasons Hamlin was lower than Valencia in absorption at 280 and 245 nm but as maturity progressed it surpassed Valencia.

The absorbance ratios and sums for Hamlin increased with maturity. Pineapple increased with maturity in the 443/325 nm absorption ratio and in the sum. However, only small changes were observed for the 280/325 nm ratio. Valencia generally decreased in the 443/325 nm absorption ratio and increased slightly in absorption sum with maturity. Slight changes were observed for the 280/325 nm absorption ratio as the Valencia season progressed.

In all varieties the hard extraction decreased the 443/325 nm absorption ratio and increased the sum. However, the 280/325 nm ratio remained fairly constant. A comparison of the State Test with the light extraction indicated similar ratios within a variety. The sum, however, was always lower for the light extraction.

Varietal comparisons indicated increasing 443/325 nm absorption ratios in the order Hamlin, Pineapple and Valencia. The 280/325 nm absorption ratio was lowest for Valencia and highest for Hamlin. Hamlin also showed the greatest change in this ratio with maturity. The absorption sum appeared to be highest for Pineapple and lowest for Hamlin. Hamlin showed the greatest change in the sum with maturity while Pineapple changed only slightly and Valencia remained almost constant.

CONCLUSIONS

IT HAS BEEN SHOWN that the combined visible and ultraviolet absorption of alcoholic solutions of orange juices can be useful in characterizing these juices. The absorption curves for the three varieties are similar, except for absorption intensities, and a shift in wavelength or unusual change in intensity may be indicative of some type of additive. Heat of stabilization did not appear to affect the spectral characteristics. Maturity influenced the spectra of Hamlin and Pineapple orange juices, causing a general increase in the visible and ultraviolet absorption with increasing maturity. Valencia absorption remained fairly constant as maturity increased. Extractor pressure also affected the spectra, the hard squeeze juices having a higher ultraviolet absorption than either the light or State test squeezes. The absorption characteristics (spectral shapes, absorption wavelengths, intensities, ratios and sums) may be helpful in analyzing and determining the quality of juice products. The relationship of these spectral characteristics to the determination of juice content and adulteration of orange juices and related products is currently being investigated.

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A STUDY ON SURVIVAL OF Staphylococcus aureus IN DARK AND MILK CHOCOLATE

INTRODUCTION

IT IS WELL ESTABLISHED that foods which are usually involved in staphylococcal food poisoning are those with high moisture and protein content. Minor and Marth (1972) in their recent review papers have extensively discussed such food items. However, the occurrence of *Staphylococcus aureus* in dried food products has been rather uncommon.

Although chocolate with its low moisture (1–2%) and high fat and sugar content is considered to be an unlikely medium for the growth of microorganisms, isolation of salmonellae from milk chocolate was reported by the Food & Drug Administration (Lennington, 1968). However, studies on survival of *Salmonella* in milk chocolate performed at our laboratory revealed that not only did milk chocolate fail to promote the growth of *Salmonella*, but that the initial population decreased during the storage period (Barrile et al. 1970).

Thus far, to the knowledge of the author there have been no reports on the isolation of staphylococci from chocolate or any other cocoa products. However, the possibility of staphylococci occurrence in the final product due to the contaminated ingredients and lack of personnel sanitation should not be overlooked. This study was therefore designed to investigate the fate of *S. aureus* in milk and dark chocolate during the storage period.

EXPERIMENTAL

Sample preparation

Dark and milk chocolate used in this study were provided by one of the chocolate manufacturers in Pennsylvania in 5 kg blocks. To temper the chocolate, each block was broken into small pieces, placed in sterile stainless steel trays and melted at 40°C for 3–4 hr. The melted chocolate was poured into sterile beakers and the temperature was lowered to 26°C by placing the beakers in containers of cold water. However, prior to the moulding, the temperature was raised to 30°C by placing the beakers in warm water.

Inoculation of the samples

S. aureus ATCC-12600 was rehydrated and stocked on brain heart infusion (Difco) agar slants. Prior to the inoculation, the culture was transferred twice into brain heart infusion broth and incubated at 37°C for 18 hr. To establish cell population of 10^3, 10^5 and 10^7 organisms per gram, tempered chocolated was inoculated with 0.5 ml of the young culture, mixed well and moulded into 50-g capacity sterile metal containers. The moulded chocolate bars were placed in a cooler (6°C) for approximately 30 min to solidify. After this period, the bars were aseptically removed, placed in sterile containers and stored at room temperature.

Analysis of the samples

The chocolate bars were examined for the presence of *S. aureus* at 2-day intervals for the first 6 days and every 8 days thereafter for a period of 110 days. At each sampling time, three bars from each level of inoculation were examined by blending them with sterile 0.1% peptone water for 3 min. Appropriate serial dilutions were prepared and 0.1 ml of each dilution was streaked on Mannitol Salt and Vogel-Johnson (Difco) agar plates using a sterile bent glass rod. All plates were incubated at 37°C for 48 hr. Typical staphylococci colonies which appeared on both selective media were isolated and confirmed according to the procedure outlined by Thatcher and Clark (1968).

RESULTS AND DISCUSSION

THE FATE OF *S. aureus* in dark and milk chocolate during storage is presented in Figures 1 and 2. Counts of less than 100 cells per gram were obtained from the samples inoculated with approximately 10^3 cells per gram after 2 days of storage in dark chocolate. However, in milk chocolate, similar counts were observed after 14 days of storage.

Reduction of staphylococci was again more pronounced in dark than milk chocolate when the bars were inoculated with approximately 10^5 cells per gram. A 2-fold reduction in dark chocolate was noticed after 22 days of storage, whereas 70 days were required for a similar reduction in milk chocolate. Both dark and milk chocolate bar samples showed staphylococci counts of less than 100 cells per gram after 38 and 86 days respectively.

Storage of the samples inoculated with 10^7 cells per gram resulted in a gradual reduction of cells during the first 22 days of storage in both dark and milk chocolate bars. However, in dark chocolate, a sharp decrease occurred between the 22nd and 38th day. From this period on, the reduction was not as pronounced and a population of 7.8×10^3 cells per gram was shown after 70 days of storage. The sharp reduction in staphylococci population of milk chocolate was noticed at 54 and 70 days which leveled-off until the 94th day of storage. Staphylococci counts of less than 100 cells per gram were obtained in dark chocolate after 86

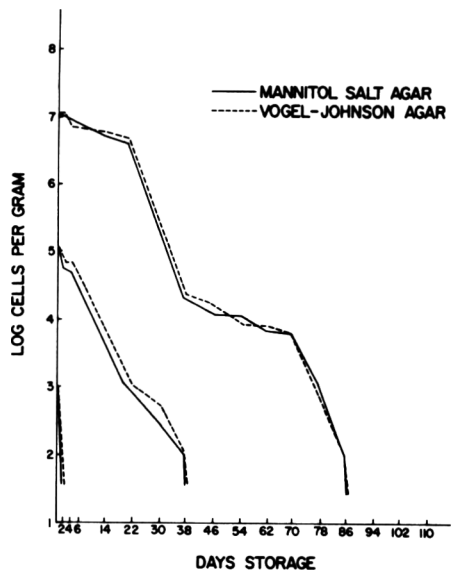

Fig. 1—Survival of S. aureus in dark chocolate stored at room temperature.

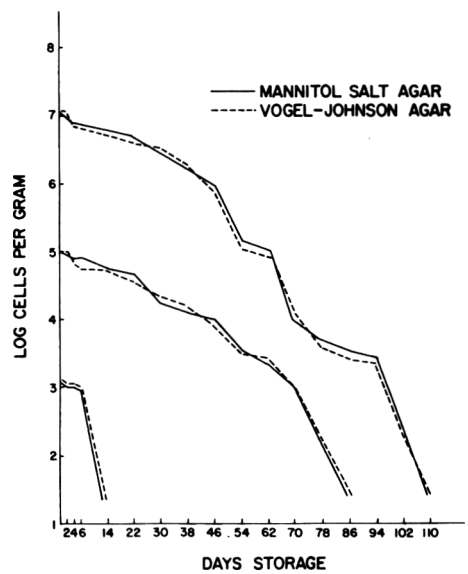

Fig. 2—Survival of S. aureus in milk chocolate stored at room temperature.

days of storage and in milk chocolate after 110 days of storage.

It is safe to assume that the reduction of *S. aureus* population was mainly due to the low moisture content of the samples, which was 2.1% for both types of chocolate used in this study. However, the presence of anthocyanin compounds in cocoa beans (Forsyth and Quesnel, 1963) and their inhibitory effect on microorganisms as reported by Power (1964) could also have been responsible for the decreased survival of the staphylococci. This is supported by the findings of Gabis and Langlois (1967) and Busta and Speck (1968) who reported the antimicrobial effect of cocoa powder on certain species of microorganisms.

Regardless of the level of inoculation, the inactivation of *S. aureus* was greater in dark than milk chocolate throughout the storage period. This can be attributed primarily to the presence of more nutrients in milk chocolate than in dark chocolate. At the same time, it should be noted that milk used in the production of milk chocolate can be considered as one of the major mechanisms by which staphylococci are introduced into chocolate products.

Results clearly indicate that chocolate does not support the growth of *S. aureus*. The time required for reduction of staphylococci depend upon the initial level of contamination; high levels of contamination may require as long as 86–110 days of storage before cell population of less than 100 cells per gram is achieved. Although the chances for enterotoxin production during extended storage periods are remote, handling of such contaminated products may lead to the cross contamination of other food items. To ensure the absence of *S. aureus* from chocolate, regular analysis of the ingredients, especially milk and milk products, should be performed by the manufacturers.

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STRUCTURAL FUNCTIONS OF TASTE IN THE SUGAR SERIES: EFFECTS OF AGLYCONES ON THE SENSORY PROPERTIES OF SIMPLE GLYCOSIDE STRUCTURES

INTRODUCTION

IN THE MALTODEXTRIN series (1→4 α-D-linked oligosaccharides) it is well known (Green, 1971; Hodge et al., 1972) that sweetness decreases with increasing molecular weight. Thus maltose has only about half the sweetness of glucose whereas maltotriose has very little sweetness and maltotetraose and higher homologues have none. This loss of sweetness with increasing molecular size is not confined to this series nor to this ring size of sugar moiety. Sucrose, which is one of the sweeter sugars known, may be glycosidically joined to galactose to form raffinose (6-o-galactosyl sucrose) which is practically tasteless.

Previously we have tried to explain this phenomenon (Birch et al., 1970) by producing evidence in support of the notion that only one-half of a disaccharide molecule actually binds to the taste bud protein, the other half being excluded presumably for steric reasons (Shallenberger et al., 1969).

If the sensory properties of simple molecules such as sugars can be predicted from considerations of molecular dimensions and binding properties to proteins (Shallenberger, 1966; Shallenberger and Acree, 1967) it would be of interest to examine a range of molecules of well understood molecular geometry in relation to their sensory properties. In earlier work (Birch et al., 1970) we have pointed out the logical necessity of using glycosides or similar conformationally defined molecules as models for this work. We now report the sensory properties of a number of glycosides of increasing chain length and size of aglycone, all in the favored Cl conformation. Hydroxy functions which contribute to the intense sweetness of β-D-fructopyranose are also discussed.

EXPERIMENTAL

ALL PARENT SUGARS used in this study were crystalline pyranose forms obtained from British Drug Houses, Chemicals, Poole, Dorset, England. Methyl- and benzyl-glycosides were obtained by refluxing the parent sugar in methanolic HCl for up to 12 hr, cooling and neutralizing with Permutit Biodeminrolit mixed bed ion-exchange resin (CO_3^{2-}form), filtering, evaporating to a syrup and crystallising from ethanol. All other glycopyranosides were obtained by a Koenigs-Knorr type of synthesis (Koenigs and Knorr, 1901) except for the phenyl derivatives which were obtaind by an alternative technique (Helferich and Schmitz-Hillebrecht, 1933). Glucofuranosides were obtained by Phillips' (1954) technique. β-D-glycosides were converted into α forms as their acetates using titanium tetrachloride in chloroform (Lindberg, 1948). All glycosides examined had physical constants previously reported in the literature and were chromatographically pure. 1-Deoxy sugars were a kind gift from Dr. R. Barker of Iowa State University. The taste panel consisted of College personnel who were selected and trained following the recommendations of Spencer (1971). The final panel (averaging 10 members) was then asked to place a few milligrams of each substance on the tongue and to comment whether it tasted trace-sweet (tr S), sweet (S) or intensely sweet (SS), or trace-bitter (tr B), bitter (B) or intensely bitter (BB). Zero (0) is no response either way. Majority deci-

Table 1—Taste properties of 1-deoxy sugars and glycosides[a]

	1-Deoxy derivative		Methyl glycoside		Ethyl glycoside		Propyl glycoside		Butyl glycoside		Phenyl glycoside		Benzyl glycoside	
Sugar	S	B	S	B	S	B	S	B	S	B	S	B	S	B
α-D-Glucopyranose	S	0	S	0	S	B	0	BB	0	BB	0	B	0	BB
β-D-Glucopyranose	S	0	S	B	tr	B	0	BB	0	BB	0	B	0	BB
β-D-Fructopyranose			S	0	0	tr	0	B	–	–	0	B	0	BB
α-D-Galactopyranose	S	0	S	0	–	–	–	–	–	–	0	B	–	–
β-D-Galactopyranose	S	0	S	0	–	–	–	–	–	–	–	–	0	BB
α-D-Mannopyranose	S	0	S	B	–	–	–	–	–	–	–	–	0	BB
β-D-Mannopyranose	S	0	–	–	0	B	–	–	–	–	–	–	–	–
α-L-Arabinopyranose	–	–	S	B	–	–	–	–	–	–	–	–	–	–
β-L-Arabinopyranose	–	–	S	B	tr	B	–	–	–	–	–	–	0	BB
β-D-Arabinopyranose	–	–	tr	tr	–	–	–	–	–	–	–	–	0	BB
α-L-Sorbopyranose	–	–	S	0	tr	B	–	–	–	–	–	–	–	–
α-D-Xylopyranose	–	–	S	B	–	–	–	–	–	–	–	–	–	–
β-O-Xylopyranose	–	–	S	B	–	–	–	–	–	–	–	–	–	–
α-D-Glucofuranose	S	0	–	–	–	–	–	–	–	–	–	–	–	–
β-D-Glucofuranose	S	0	S	0	tr	B	–	–	–	–	–	–	–	–
α-D-Xylofuranose	S	0	–	–	–	–	–	–	–	–	–	–	–	–
β-D-Xylofuranose	S	0	–	–	–	–	–	–	–	–	–	–	–	–
α-D-Ribofuranose	S	0	–	–	–	–	–	–	–	–	–	–	–	–

[a](S = sweet; B = bitter; tr = trace; 0 = no response either way; BB = intensely bitter.

sions are given in Table 1. Each panelist tasted all 18 substances listed in Table 1, once each at one session, rinsing with distilled water between substances, and pausing 1 min before passing on to the following substance.

RESULTS & DISCUSSION

IT IS CLEAR from the results listed in Table 1 that the likelihood of glycosides being sweet depends on the nature of the substituent R depicted in Figure 1. If R is H (the 1-deoxy sugars) all the molecules are sweet and devoid of bitterness but if R is OH (the free sugars), only in the case of β-D-mannose does bitterness arise. If R is OCH_3 the molecules may be either sweet or bitter/sweet depending on the anomeric configuration of hydroxyl groups around the ring. If R is OC_3H_7, OC_4H_9, OPh or OBz, the molecules are entirely bitter and devoid of sweetness. Furthermore bitterness appears to increase in intensity as molecular weight of the aglycone increases. These results prove that increasing the size of these types of aglycone does not sterically exclude the molecule from the taste bud. Possibly fresh binding sites are made available due to the increasing lipophilicity of the molecules passing from left to right across the Table, bitterness ensuing as the new sites are bound.

We have also examined a number of inositols and other cyclohexane polyols which will be the subject of another paper. Although many of these substances are sweet, none so far has shown bitterness. Myoinositol, as shown in Figure 2, is an analogue of β-Dmannose, which is unique among the free sugars in being bitter. Since 1-deoxy mannose is not bitter this provides conclusive evidence that the bitterness of β-D-mannose is due to the interrelationships of the hydroxyl groups on carbon atoms 1 and 2, the ring oxygen atom and possibly also the primary alcohol group. The latter possibility was tested by examining a solution of D-lyxose, which differs from D-mannose only in the absence of a primary alcohol group. Since D-lyxose is devoid of bitterness even after allowing mutarotation to occur, the primary alcohol group of β-D-mannose must participate in eliciting the bitter response.

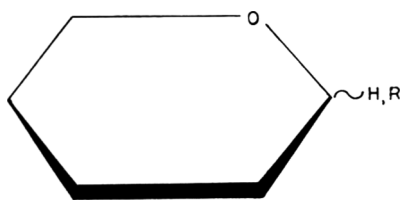

Fig. 1—Types of glycopyranoside structure.

R = H	*Deoxy sugars*
R = OH	*Free sugars*
R = OCH_3	*Methyl glycosides*
R = OC_2H_5	*Ethyl glycosides*
R = OC_3H_7	*Propyl glycosides*
R = OC_4H_9	*Butyl glycosides*
R = OPh	*Phenyl glycosides*
R = OBz	*Benzyl glycosides*

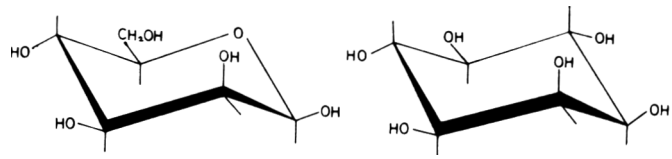

Fig. 2—β-D-Mannose (Left, bitter) and myoinositol (right, not bitter).

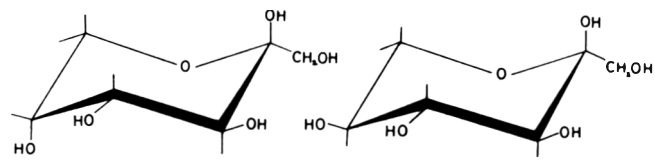

Fig. 3—β-D-Fructopyranose (left, intensely sweet) and α-L-sorbopyranose (right, sweet).

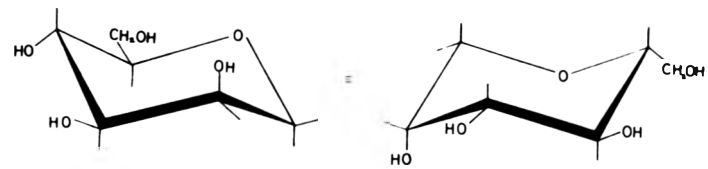

Fig. 4—Identity of 1-deoxy-D-mannopyranose (left) and 2-deoxy-D-fructopyranose (right).

Although most of the substances listed in Table 1 are sugars and glycosides which exist in the favored Cl conformation (e.g., see Fig. 2 for β-D-mannose), β-D-fructopyranose is unusual in that it exists in the alternative 1C form. Previously we have suggested (Birch et al., 1971) that the fourth hydroxyl group of glucopyranoside structures is of unique importance in eliciting the sweet response, possibly acting as the AH of Shallenberger's (1966) AH, B system. We now observe that although β-D-fructopyranose (Fig. 3) is the sweetest known sugar, it differs from α-L-sorbopyranose only in the configuration of the hydroxyl group on carbon atom no. 5, which corresponds to carbon atom no. 4 in the favored Cl conformation of the other sugars listed in the Table. Furthermore, 1-deoxy mannopyranose is identical with 2-deoxy fructopyranose (Fig. 4).

Although 1-deoxy mannopyranose and α-L-sorbopyranose are both sweet, our tests reveal that each molecule has only about one-quarter of the sweetness of β-D-fructopyranose. The methyl glycosides of β-D-fructopyranose and α-L-sorbopyranose have also been examined (Table). Although both are devoid of bitterness the sorboside is again considerably less sweet than the fructoside. This gives overriding proof that the hydroxyl groups on carbon atoms 1, 2 and 5 govern the sweetness characteristics of these molecules. If these substances had been tasted as solutions rather than crystals we would anticipate no qualitative differences due to the intrinsic stability of the glycoside structures themselves. Some differences in intensity would possibly have occurred due to absence of the hydrogen bonding which exists in the crystal lattice.

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POLYSACCHARIDE 13140: A NEW THERMO-GELABLE POLYSACCHARIDE

INTRODUCTION

A THERMALLY GELABLE β-1,3-glucan was found to be produced by cultivation of microbial mutant strain K of *Alcaligenes faecalis* var. myxogenes 10C3 and the chemical properties and structure of the polysaccharide 10C3K were reported (Harada et al., 1968).

In our laboratories, *Alcaligenes faecalis* var. myxogenes strain NTK-u was obtained by treating strain K with N-methyl-N-nitro-N-nitrosoguanidine and a new thermo-gelable polysaccharide 13140 was obtained from a culture broth of the strain NTK-u using glucose as the substrate. Outline of the production and the chemical properties and structure of the polysaccharide 13140 were explained by Harada at the 4th International Fermentation Symposium at Kyoto, Japan in March, 1972 (Harada, 1972). Recently, Nakanishi et al. (1972a, b) reported in details about the production, chemical properties, structure and some rheological properties of polysaccharide 13140.

The present paper deals with potential applications of the polysaccharide 13140 in food and food processing.

EXPERIMENTAL

Procedure for study of the principal properties of polysaccharide 13140

Viscosity measurement procedure. An aqueous suspension of polysaccharide 13140 was homogenized in a teflon homogenizer (potter type) for 5 min at 35°C. The viscosity of a 1% aqueous suspension of polysaccharide 13140 was measured in a temperature range of 25–85°C at the rate of shear of 1046.7 sec^{-1} using a Rotovisco viscometer (Haake), a coaxial rotating cylinder viscometer. The specific viscosity, η_{sp}, is defined as follows:

$$\eta_{sp} = (\eta - \eta_s)/\eta_s$$

where η and η_s indicate the solution and the solvent viscosity, respectively.

Measurement of gel strength with a Curd Meter. An aqueous suspension of polysaccharide 13140 was homogenized with a teflon homogenizer for 5 min and degassed under 5–10 mmHg for 5 min. The suspension was poured into a test tube (15 mm diam) and heated at various temperatures for optional durations. The various samples of gel thus obtained were sliced into cylindrically shaped pieces 10 mm thick.

Gel strength was measured (Iio, 1969) with a Curd Meter Model 301 (Iio Electric Co.). This instrument was initially developed for evaluating the texture of soft milk curd. A weight is hung on a spring with a known instrumental constant, a plunger is set at the bottom of the weight and the sample is placed just under the plunger on a moving plate. The latter is moved upward at a constant rate (21 sec/in., in this study), subjecting the sample to an increasing load. The sample is deformed and broken at the breaking point. The load on the sample and the deforming rate can be read from the position of the plunger. The sample thickness was 10 mm, and a plunger with a cross-sectional area of 0.25 cm^2 was used in this study. Gel strength was the quantity corresponding to the force at the breaking point.

Measurement of gel properties with an Autograph. Various samples of gel were prepared by the method previously described, but a casing tube (30 mm diam) was used in place of a test tube. Samples were sliced into cylindrically shaped pieces 23 mm thick. The mechanical properties of the gel were measured with an Autograph Model IM-100 (Shimazu Seisakusho Ltd.). This instrument is one of the most versatile for rheological testing of solid-like systems. It consists of two units: a cross-head loading assembly and control console.

The sample (30 mm diam × 23 mm thick) was placed on a compression plate, which was in contact with a compression cell, and compressed with a flat circular plate. A typical stress-strain curve obtained from the Autograph is illustrated in Figure 1.

Two parameters, breaking stress and breaking strain, are defined as the stress and the strain at the breaking point, as shown in Figure 1. Young's Modulus is defined from the initial slope and is given by the following equation:

$$E = (F/A_0)/(\Delta L/L)$$

where F is the applied force, A_0 is the original cross-sectional area, ΔL is the compression and L is the initial height of the sample.

Application studies in food products

Application in boiled noodles. A mixture of 1.5 kg of wheat flour and 4.5g of polysaccharide 13140 was mechanically blended for 5 min. Then it was mechanically mixed with 480 ml of an aqueous solution of sodium chloride (containing 30g sodium chloride) for 20 min. After the dough thus obtained was allowed to stand for 30 min at room temperature, it was formed into noodles by sheeting five times and cutting the dough sheets into strips approximately 0.20 by 0.25 cm in cross-section.

The dissolved-out solid was the solid material remaining in broth when 10g of the noodles were placed in 100 ml of boiling water for 15 min. The weight gain was the weight increase percent when 400g of noodles were placed in boiling water for 15 min and cooled in cold water for 5 min.

The breaking strength and elongation at the breaking point of the boiled noodles were measured with a Food Rheometer (Tabai Seisakusho Co., Ltd.) (Shimizu and Shimizu, 1953). This instrument was developed originally for texture evaluation of boiled fish paste (Kamaboko). The stress and strain of a sample was measured when it was ruptured by stretching at a constant rate of load.

The dough (2.5 mm thick) was heated for 15 min in boiling water and cooled for 5 min in cold water. Then it was cut into strips 40 mm in length and 10 mm in width, parallel and transverse respectively to the direction in which the dough was rolled. The breaking stress and elongation of the sample was measured.

Application in hamburger. 500g of ground beef, 200g of butter-roasted minced onion, 50g of egg, 100g of water, 50g of rusk, 0.25g of pepper, 0.25g of nutmeg, 7.5g of salt and optional amounts of polysaccharide 13140 were

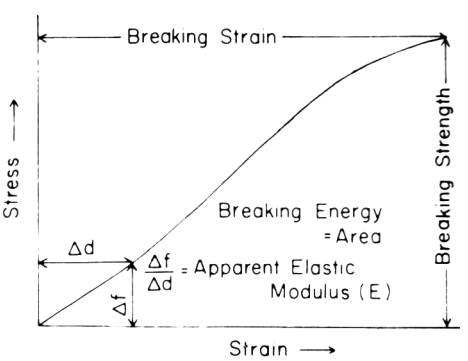

Fig. 1—Autograph measurement.

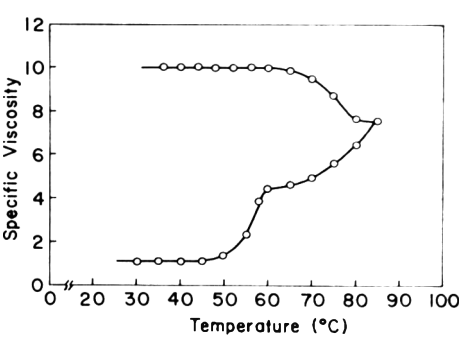

Fig. 2—Relation of temperature to the specific viscosity of water suspension of polysaccharide 13140.

mixed and molded into hamburger patties 5 cm in diam and 2 cm thick and cooked in a frying pan.

The firmness of the patties was measured with a Texturometer Model GTX-2 (Zenken Co.) (Friedman et al., 1963). In a sensory test, each of the trained panels rated the samples from 1st to 6th according to binding quality, firmness and palatability. The total sum of the scores for each property was statistically analyzed according to Kramer's table (Kramer, 1960).

Application in sausage. In the conventional procedure, 5 kg of mutton, 3 kg of pork, 2 kg of beef and 3 kg of lard were mechanically ground and then fed into a Silent Cutter, in which the ground meat was blended with 500g of potato starch, 300g of sodium chloride, 30g of monosodium glutamate, 200g of sugar, 30g of spice, 250g of curing agent, 3 kg of ice water and optional amounts of polysaccharide 13140.

The firmness and product yield of the samples were determined. The firmness was measured with a Texturometer Model GTX-2.

RESULTS & DISCUSSION

Principal properties of polysaccharide 13140

The relationship between specific viscosity and heating temperature is shown in Figure 2. Specific viscosity showed a rapid increase at a temperature of about 54°C and continued to increase sharply up to 60°C. Then specific viscosity increased only slightly between 60°C and 70°C. Finally, specific viscosity was observed to increase even more as the temperature was raised to 85°C. When the temperature was decreased from above 54°C, specific viscosity was observed to increase as shown in Figure 2, which showed an increase of specific viscosity as the temperature was decreased from 85°C. These results suggest that this polysaccharide gel is thermally irreversible.

The effect of heating temperature and heating time on the gel strength of polysaccharide 13140 gel is shown in Figure 3. Gel strength depended upon heating temperature. Namely, gel strength increased as the heating temperature increased. Also, the effect of heating time on gel strength correlated with heating temperature. Namely, the gel strength of the gel, heated at higher than 85°C, increased as heating time increased, but that of the gel heated at 65°C and 75°C was almost independent of heating time. These data are compatible with the change of specific viscosity shown in Figure 2.

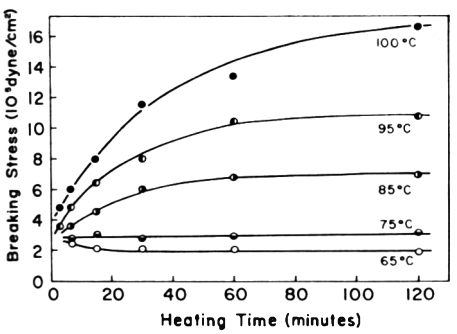

Fig. 3–Effect of heating temperature and heating time on gel strength of polysaccharide 13140 gel.

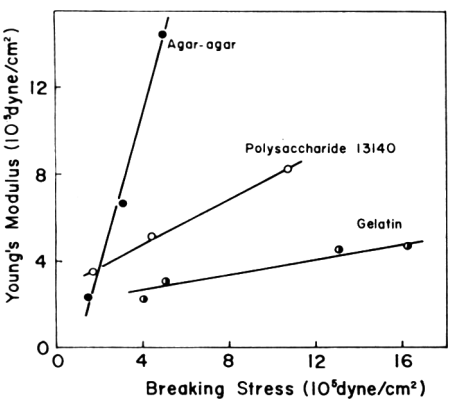

Fig. 4–Comparison of the texture property of agar-agar gel, gelatin gel and polysaccharide 13140 gel.

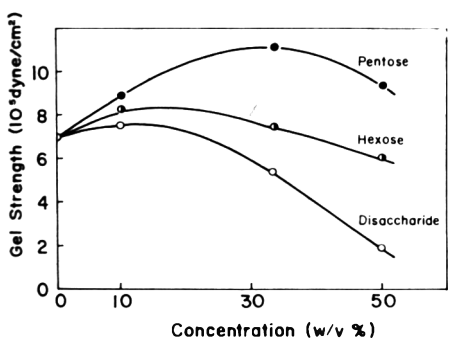

Fig. 5–Relation of pH to the texture property of polysaccharide 13140 gel.

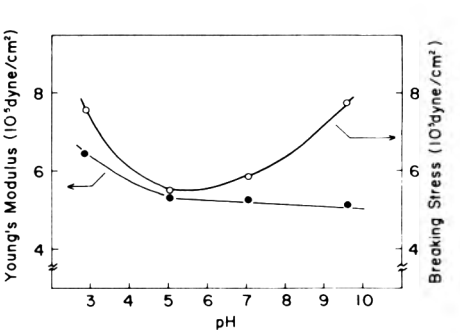

Fig. 6–Effect of sugars on gel strength of polysaccharide 13140 gel.

Harada et al. (1968) also reported that the gel strength of polysaccharide 10C3K gel was almost the same at temperatures between 60° and 70°C.

Figure 4 shows the relationship between the breaking stress and Young's modulus of various gels at various concentrations prepared from agar-agar, gelatin and polysaccharide 13140. The slope of the agar-agar gel curve was steep, that of gelatin gel curve was slight and that of polysaccharide 13140 gel curve was in between. In other words, the breaking stress of a 2% agar-agar gel, a 3% polysaccharide 13140 gel and a 5–6% gelatin gel were almost the same, but Young's modulus for each were quite different. Young's modulus for a 2% agar-agar gel was very high and that of a 3% polysaccharide 13140 gel was in between that of a 2% agar-agar gel and a 5–6% gelatin gel. These results indicate that the texture of agar-agar gel is hard and brittle, that of gelatin gel is soft and elastic and that of polysaccharide 13140 gel is in between.

The effect of pH on the mechanical properties of polysaccharide 13140 gel was studied between pH 2.9 and 9.6. Harada et al. (1968) reported that the gel strength of polysaccharide 10C3K gel was essentially constant between pH 2.5 and 10. Regarding polysaccharide 13140, breaking stress was minimal at about neutral pH and increased gradually on both the acid and alkaline pH sides as reported previously (Nakanishi et al., 1972a, b). On the other hand, Young's modulus was the highest at pH 2.9 and gradually decreased as pH increased (Fig. 5). This result suggests that the texture of polysaccharide 13140 gel is hard and brittle on the acid pH side, but soft and elastic on the alkaline pH side.

As shown in Figure 6, gel strength of polysaccharide 13140 gel varies with the type and concentration of sugars present in the system. Increase of gel strength was observed in the presence of 5–50% pentose (arabinose, ribose and xylose), 5–30% hexose (fructose, glucose and galactose) and 5–15% disaccharide (maltose and sucrose), respectively. These results indicate that polysaccharide 13140 has good compatibility with various sugars.

Applications of polysaccharide 13140 in food products

Food products in which polysaccharide 11340 can be used as an additive or as a raw material include any and all types of products which involve heating in the presence of water during production or in the course of cooking.

Among these are noodles, spagetti, cereal flour-based products, comminuted meats, hamburger, soy bean products, peanut butter, jellies, puddings, freeze-thaw foods, dressing, mayonnaise and so on. From the view point of thermal gel-

Table 1—Effect of polysaccharide 13140 on boiled noodles

	Control	Added
Weight gain (%)	252	280
Dissolved-out solids (%)	7.6	5.2
Parallel breaking strength (dyne/cm²)	1.14×10^5	1.24×10^5
Elongation at break (%)	113	126
Transverse breaking strength (dyne/cm²)	0.83×10^5	0.95×10^5
Elongation at breaking (%)	79	94

Table 2—Effect of polysaccharide 13140 on hamburger

Amount of polysaccharide 13140 (wt %)	Sensory tests			Firmness (T.U.)
	Binding quality	Firmness	Palatability	
None	94**	97**	90**	3.5
0.3	85	85	68	3.6
0.5	66	67	46**	3.7
1.0	77	75	54	3.9
1.5	51*	50*	70	5.1
2.0	47**	46**	92*	6.0

* significant at 5% level
** significant at 1% level

ability of polysaccharide 13140, care should be taken to protect it from exposure to heat prior to its incorporation in food products.

In this paper, some examples of applications of polysaccharide 13140 as food additives are shown as follows.

Application in boiled noodles. Table 1 shows the effect of polysaccharide 13140 in boiled noodles. Noodles containing polysaccharide 13140 had superior elasticity and body, produced less dissolved-out solid and consequently, reduced turbidity in a broth and an increased cooking weight. Similar results were obtained with the concomitant use of such conventional additives as sodium polyphosphates, monoglycerides of fatty acids, sucrose fatty acid esters, gluten and the like. Namely, use of these additives produced no negative effects on the properties of noodles containing polysaccharide 13140. It was also found that improvement could be demonstrated on similar products such as soba noodles, Chinese noodles, fried noodles, pregelatinized noodles and the like.

Application in hamburger. Table 2 shows the effect of polysaccharide 13140 in hamburger. In a sensory test, the polysaccharide 13140-free control sample resulted in unfavorable scores. Hamburger produced with the addition of polysaccharide 13140 had favorable scores. However, addition of 1.5% or more of polysaccharide 13140 resulted in unfavorable scores for palatability but favorable scores for binding quality and firmness. Namely, binding quality and firmness increased with the amount of polysaccharide 13140, and increase in firmness was clearly substantiated by a Texturometer measurement. Consequently, the effective level of addition in hamburger was approximately 0.3–1.0%.

Application in sausage. Table 3 shows an increase in product yield in proportion to the amount of polysaccharide 13140.

Table 3—Effect of polysaccharide 13140 on sausage

Amount of polysaccharide 13140 (wt %)	Firmness (T.U.)	Product yield (wt %)
None	5.0	91.8
0.5	5.2	92.5
1.0	5.3	92.6
1.5	5.6	92.9
2.0	6.1	92.8

This result indicates that there is a reduced loss of water from the sausage during smoking due to the water-holding capacity of polysaccharide 13140. Also, firmness of product increased in proportion to the amount of polysaccharide 13140. These results suggest that polysaccharide 13140 can be applied very effectively in sausage.

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STUDIES ON MECHANISMS OF RETENTION OF VOLATILE IN FREEZE-DRIED FOOD MODELS: THE SYSTEM PVP-n-PROPANOL

INTRODUCTION

FOOD MATERIALS are generally freeze dehydrated by placing the frozen food in a high vacuum environment. Contrary to what one might expect, volatile compounds of different vapor pressures have similar retentions. The retention of the organic volatiles is based largely on the properties of the solute which forms the amorphous matrix of the freeze-dried solid.

It is believed that the retention of organic volatiles results from surface adsorption of the volatile on the dry layer of the freeze-drying sample (Rey and Bastien, 1962) or from an entrapment mechanism which immobilizes the volatile compounds within the amorphous solute matrix (Flink and Karel, 1970a; Thijssen, 1971; Thijssen and Rulkens, 1969; Chandrasekaran and King, 1971).

The physical aspects by which the volatile is entrapped within the amorphous solute matrix are only partially understood. Two mechanisms, selective diffusion (Thijssen, 1971; King, 1971, 1970a; Rulkens and Thijssen, 1972; Thijssen and Rulkens, 1968) and microregions (Flink and Karel, 1970a, 1972) perhaps represent macro- and microviews of the same basic phenomena. The selective diffusion concept utilizes diffusional analysis on the system components to develop mathematical expressions by which volatile retention behavior during freeze drying can be predicted. Phenomenologically, as noted by King (1970b): "A simple diffusion mechanism is not in itself sufficient." Structural aspects of the microregion concept allow qualitative explanation of behavior both during freeze drying and during humidification of the dried material. Flink and Karel (1970a) postulated that crystallization of water during freezing and other concentration processes result in the formation of "microregions" containing highly concentrated solutions of carbohydrates and volatile organic compounds. As the local moisture content within the microregion decreases, first due to freezing and then to freeze drying, molecular association occurs; in the case of carbohydrates, this association is caused by H-bonds. The structure of the microregion, as well as its permeability to organic compounds and water, depends upon local moisture content. As the moisture content decreases, the ease of loss of the organic compound decreases until, at some critical moisture, there is no further loss (Flink and Karel, 1970a, b; 1972). The size of the microregions is small, since grinding and evacuation of the dry material do not release any volatile. Recently the size of the volatile entrapments has been shown to vary with, among other things, the solubility of the organic volatile in the aqueous solution. Retained hexanal (about 1g hexanal per 100g maltodextrin) freeze dried from an aqueous 20% maltodextrin solution appeared in the optical microscope within the amorphous solute matrix as 2- to 6-μm droplets (Flink and Gejl-Hansen, 1972). Concurring evaluations have been made with the scanning electron microscope. The addition of sufficient water to the dry material will cause volatile loss, the extent of which depends on the amount of water added and the particular solute matrix. Water influences volatile loss according to changes in the matrix in the amorphous state.

It seems important to extend the applicability of above concepts to systems other than carbohydrates; therefore, we have initiated work on polymers, proteins and on selected foods.

In this study we are presenting results which characterize the retention of n-propanol in a model system based on polyvinylpyrrolidone (PVP), which is a polar, water-soluble polymer containing polar groups different from those of polysaccharides but similar to proteins. PVP does not, however, manifest the complexity of interactions that occurs in proteins. C^{14}-labeled n-propanol enables us to study low concentrations, both in the presence and absence of other compounds, which can potentially interfere with conventional analyses.

EXPERIMENTAL

Model system preparation

The model system consisted of a water-soluble polymer (PVP), C^{14}-labeled n-propanol and water. The model system was prepared by dissolving the desired amount of PVP in water and adding n-propanol. 5-ml aliquots of the solution were pipetted into 50-ml Erlenmeyer flasks, frozen as specified below and then freeze dried for 48 hr at room temperature and at a chamber pressure of less than 100 μm in a Virtis freeze drier (Model 10-MRTR). No partial melting or collapse was noted in the dried material following removal from the freeze drier. The volume of solution per flask and resultant sample thickness were varied in some experiments as noted under Results & Discussion. In most experiments the composition was fixed as the following initial concentration expressed in weight percent: PVP 20%, n-propanol 1%, water 79%. In several experiments, the effect of changing concentrations was studied; the changed compositions are noted under Results & Discussion.

Table 1—Effect of freezing rate on retention of propanol by model systems

Solid	Initial conc (%)	Volatile	Initial conc (%)	Retention of volatile (%) Fast freezing	Slow freezing
PVP	20.0	n-propanol	1.0	9.8	24.0
PVP	20.0	n-propanol	0.01	–	58.0
Glucose	18.8	n-propanol	0.75	47.8	–
Glucose	18.8	2-propanol	0.75	52.8	–
Maltose	18.8	2-propanol	0.75	67.6	87.5
Dextran-10	18.8	n-propanol	0.75	4.2	–
Dextran-10	18.8	2-propanol	0.75	7.5	–
Dextran-10	20.0	2-propanol	0.01	56	88

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Samples were frozen as slabs in the flasks by one of two methods: fast freezing was accomplished by immersion of flasks in liquid nitrogen; slow freezing refers to placing the stoppered flasks in still air at −40°C.

PVP

Polyvinylpyrrolidone K-30 (mol wt 40,000) was obtained from Matheson, Coleman and Bell (East Rutherford, N.J.).

n-Propanol

Reagent grade n-propanol was mixed with C^{14}-labeled n-propanol to give the desired specific radioactivity. The radioactive propanol was obtained from International Chemical and Nuclear Corporation in Irving, Calif.

Humidification experiments

In several experiments freeze-dried PVP-propanol systems were humidified by placing tared and weighed flasks in vacuum desiccators containing saturated salt solutions, which maintained the desired constant relative humidities.

n-Propanol analysis

The n-propanol content was determined by measuring the radioactivity of the samples with a liquid scintillation counter.

The dried samples of PVP were dissolved in water (to 10% solution); 1 ml of this solution was added to 10 ml of water-miscible scintillator (2,5-diphenyloxazole 1g, naphtalene 100g, dioxane to 1,000 ml vol) in the counting vial, and the resulting solution was counted with a liquid scintillation counter (Nuclear Chicago Corp., 720 series). The counting efficiency (measured with a C^{14} toluene standard) was 77.5%; no correction by quenching was found to be necessary.

Statistical evaluation of variation due to freeze drying and analysis

Ten identical samples of the model system were prepared, frozen, freeze dried and analyzed by liquid scintillation counting. The variation coefficient determined for the ten samples gives a measure of the range of significance for the overall process (freeze drying and analysis). The results were:

Fast frozen samples: n-propanol content 0.49g/100 PVP ± 3.5%

Slow frozen samples: n-propanol content 1.2g/100 PVP ± 9.0%

Water sorption isotherm

The water sorption isotherm for PVP in the absence of entrapped propanol and selected values of sorption of water in the presence of entrapped propanol were obtained by placing tared weighed samples in vacuum desiccators over constant humidity solutions and weighing periodically until equilibrium was established.

RESULTS & DISCUSSION

AS DISCUSSED previously, we conceive the retention of volatiles in freeze-dried solutions to be caused by the formation of microregions during freezing or other concentration processes preceding the final drying step. During dehydration the microregions are stabilized by formation of a matrix which becomes completely impermeable to organic volatiles but not to water when the local moisture content drops to a critical level. In the case of carbohydrates, the matrix is stabilized by hydrogen bonds and the critical moisture content tends to occur at the BET monolayer value (Flink and Karel, 1972).

Process conditions affect the formation of microregions and, consequently, the extent of retention. Freezing rate, for instance, is of considerable importance; slow rates promote volatile retention in microregions (Flink and Karel, 1970b; Flink and Labuza, 1972).

Table 1 presents a comparison between retention of propanol in freeze-dried PVP systems and retentions observed previously in freeze-dried carbohydrates. In both types of systems slow freezing resulted in higher retention than rapid freezing. The fraction retained was considerably higher at low absolute concentrations of the alcohol. Finally, the results show that both polymeric systems (PVP and Dextran) retained less alcohol than the low molecular weight carbohydrates. All of the above findings are compatible with the microregion concept: slow freezing, which allows diffusion of solute from the freezing front, results in fewer, larger, more concentrated microregions, which are less permeable than those created by rapid freezing (Flink and Karel, 1970b). Similar conclusions regarding larger regions of concentrated solute phase resulting from slow freezing were reached by King (1970b). The reduced mobility of the polymers, as compared to the low molecular weight carbohydrates, also retards formation of impermeable microregions which entrap the volatiles; this is the reason that Dextran-10 and PVP retain less alcohol under the conditions of our experiments.

We also observed a consistent effect of concentration of alcohol. When solids content is kept approximately constant, relative retention decreases with increasing alcohol concentration. The use of C^{14}-labeled n-propanol allowed for a wide range of concentrations; results obtained are shown in Figure 1. They are consistent with results obtained previously with carbohydrates, which showed that

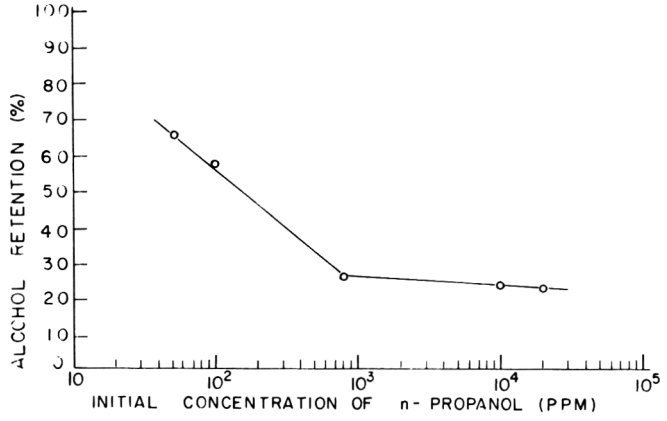

Fig. 1—Effect of initial n-propanol content on retention of PVP solutions during freeze-drying. (20% PVP)

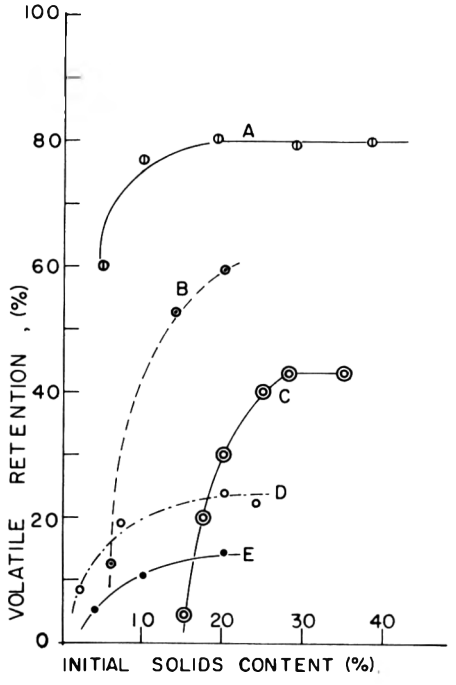

Fig. 2—Effect of concentration of solids on volatile retention during freeze drying. (A) Maltodextrin: n-propanol; initial n-propanol content: volatile-to-solid ratio constant at 1:100 (Rulkens and Thijssen, 1972). (B) Maltose: butanol; initial butanol content: 1% in initial solution (Flink and Karel, 1970b). (C) Earle salt and glucose: acetone; initial acetone content: 10% in initial solution (Rey and Bastien, 1962). (D) PVP: n-propanol; present study initial n-propanol content: 1% in initial solution. (E) Glucose: acetone; initial acetone content: 2.5% in initial solution (Flink and Karel, 1970b).

retention expressed in absolute amounts (as weight of volatile/weight of solid) increases nonlinearly with concentration, resulting in the relative retention showing the decrease noted above. This behavior reflects a saturation of microregion entrapment capacity (Flink and Karel, 1970b). The microregion theory predicts that increasing the solid concentration increases volatile retention up to a limiting concentration which depends on the type of solid and type and amount of volatile. Figure 2 shows results obtained with the PVP-n-propanol system compared with results published for other systems. There is a general agreement on shape of curves obtained.

Thickness of sample is also a factor in retention. Figure 3 presents the retention of n-propanol (initial concentration 1%) in freeze-dried PVP (initial concentration 20%). The retention decreases with increasing thickness as predicted by the theory advanced previously, primarily because the more rapid drying and steep moisture gradients in thin samples decrease the time during which the moisture content in the microregions is high enough to permit volatile escape (Flink and Karel, 1970b).

The most convincing evidence for the existence of water-sensitive volatile-entrapping microregions in carbohydrate systems was obtained through humidification experiments, in which water vapor sorption above a critical moisture level resulted in structural changes and consequent volatile release (Flink and Karel, 1972; Karel and Flink, 1972). Figure 4 presents results of humidification experiments using PVP. In the corresponding experiment, PVP solutions (20% solids, 1% n-propanol) were freeze dried under standard conditions (slow freezing, ambient freeze-drying temperature). These conditions resulted in the retention of 1.2g n-propanol per 100g of PVP. The freeze-dried systems were then exposed to different relative humidities, and water uptake and volatile loss were measured as a function of time. At 11% RH we can note a small loss of propanol from the samples. The amount of this loss is comparable to the experimental error; however, it is possible that a part of this measured loss is real, perhaps resulting from surface adsorption of the propanol or from imperfect microregions communicating with the free surface. The loss increases as the moisture contents increase with rising humidity. It should be noted that even in the case of exposure to higher humidity, sizable volatile loss does not commence until sufficient water is absorbed to begin disrupting the PVP structure. Thus, in the case of exposure to 32% relative humidity, complete humidification is achieved in about 4 hr, and the volatile loss appears to become significant at about the same time. Extensive tests have confirmed that the BET monolayer value, which for PVP is about 12.5g water/100g solids occurring at an equilibrium relative humidity of about 30%, seems to mark the initiation of volatile release due to microregion disruption.

The PVP system is similar therefore to the carbohydrate system in its sensitivity to water. At high humidities, however, there is a new constant level of retention of volatile in carbohydrate systems after an initial loss following attainment of the new moisture equilibrium, whereas with PVP we have observed a very slow, continuing loss at high humidities. These differences may be due to the reduced mobility in the polymer which results in sluggish approach to the new equilibrium value, or to the greater sensitivity of polar bonds in PVP (as compared with carbohydrates) to continuing disruption in the presence of limited amounts of water.

Water and polar volatiles can compete for polar sites in polymers and other solids (Lauer and Ayer, 1957; Bell and Breuer, 1971; Fogiel and Heller, 1966; De Boer, 1968). In the present study we have obtained indications that entrapped n-propanol competes with water for sorbing sites in PVP microregions. Figure 5 shows the sorption isotherm for propanol-free freeze-dried PVP. This isotherm was independent of the method of freezing. Table 2 compares the water sorption in the presence and absence of propanol, in which a small but significant reduction in water sorption occurs.

In studies of water sorption of PVP

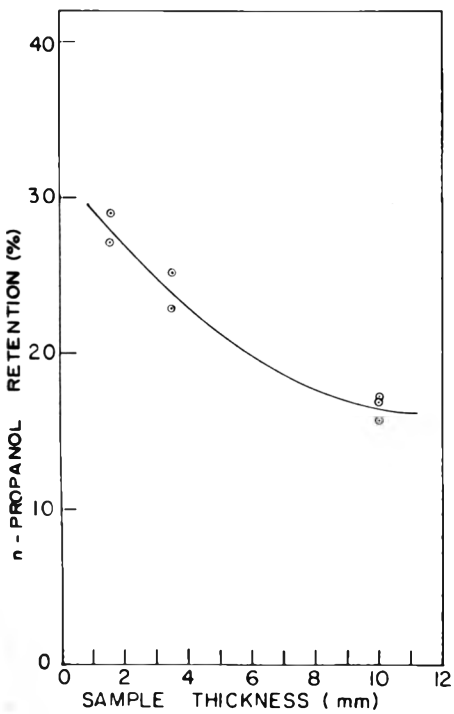

Fig. 3–Effect of sample thickness on n-propanol retention during freeze drying of PVP solutions.

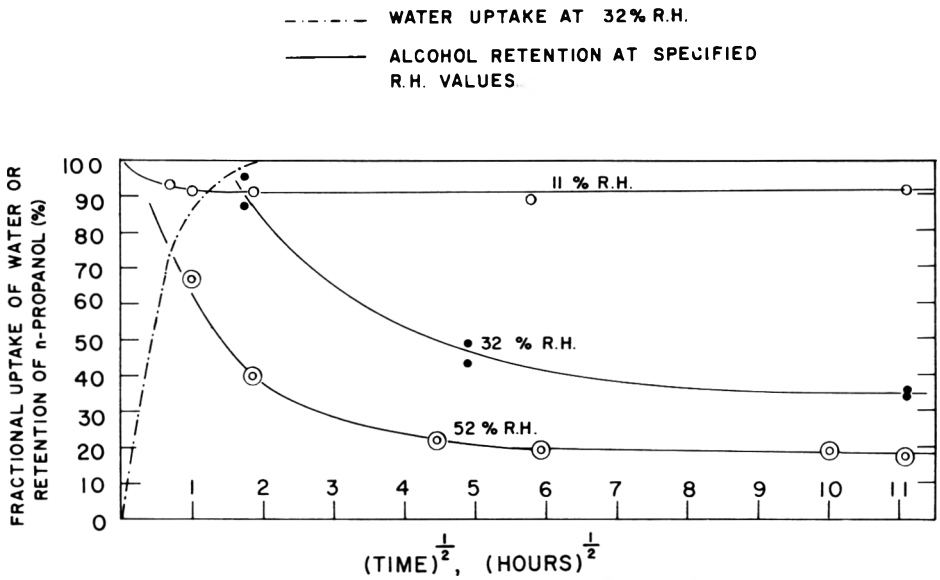

Fig. 4–Loss of n-propanol and fractional water uptake from freeze-dried PVP humidified to specified relative humidities at 25°C. (Initial n-propanol retention = 1.2g/100g PVP; Equilibrium water content at 32% RH = 100% fractional water uptake = 13g/100g PVP.)

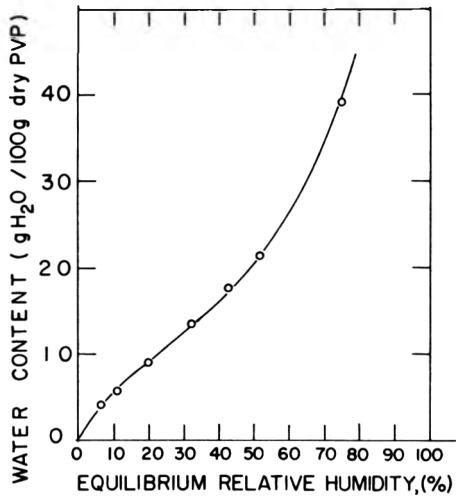

Fig. 5—Water sorption isotherm for freeze-dried PVP at 25° C.

(Dole and Fuller, 1950; Jellinek et al., 1968), it is considered that the monolayer of water is held by the carbonyl groups. We calculated the BET monolayer and found it to be 12.5% (dry basis) occurring at about 30% relative humidity. This corresponds to 0.77 moles of water per mole of CO groups, in reasonable agreement with Dole and Fuller (1950) who found that approximately 1 mole of water is bound per mole of PVP repeating units.

Some deductions can be made comparing the water sorption by dried PVP with and without n-propanol. Table 2 shows that at a relative humidity of 11%, the sorption of water is lowered for the samples with n-propanol by 11.5 moles H_2O/mole PVP. This could be considered as evidence that the n-propanol is sorbed in the PVP, thus occupying some of the available sites for water sorption.

The amount of n-propanol retained at 11% RH is about 1.1g/100 PVP, which is equal to 7.4 moles of n-propanol/mole PVP. Thus, 7.4 moles of n-propanol are apparently accomodated in the sites which held 11.5 moles of water. The preferential adsorption of the alcohol at low water contents is probably due to the alcohol's inability to be desorbed until the water content is sufficiently high to cause partial microregion disruption and alcohol release.

We are currently trying to determine how much of this effect is due to selective impermeability of the microregions, and whether chemisorption plays a part.

It is conceivable that within the microregions the organic molecules are in one of the following states:

(a) adsorbed on sites which can be competitively occupied by water;
(b) adsorbed on specifice sites, but do not reduce the total sorption capacity for water;
(c) entrapped as condensed aggregates, such as droplets, in which most of the molecules do not saturate the internal surfaces of the entrapping solids.

Flink and Gejl-Hansen (1972) showed that at least some volatiles are entrapped in maltodextrin in the form of fairly large droplets (up to several microns in size) as in "c" above.

Flink and Karel (1970a) have reported that in some freeze-dried carbohydrates, the presence of entrapped organic volatiles does not significantly affect the water sorption by these solids, indicating that the volatile was probably in a state other than "a" above.

In light of the results obtained in this study with PVP, the sorption isotherm data of Flink (1970) were reevaluated. While the maltose samples definitely show no effect of retained volatile on the water sorption, a possible effect similar to that reported here is noted for the tert-butanol retained by the polymeric carbohydrate Dextran-10. The number of samples utilized by Flink did not allow the same statistical accuracy as was achieved in this study; thus, the behavior observed in the reevaluation cannot definitely be deemed significant.

In summary, the results presented above lead us to the conclusion that the behavior of a model system based on PVP is consistent with the "microregion volatile entrapment theory," which was formulated on the basis of work with carbohydrates. The PVP system (and perhaps polymeric carbohydrates) shows differences from low molecular weight carbohydrates: an apparent reduction in water sorption below the BET monolayer due to entrapped volatile, and a lower absolute level of volatile retention.

Table 2—Effect of retained n-propanol on water sorption by PVP

Equilibrium relative humidity	Water content (% dry basis) Without propanol	With propanol	% Difference	Coefficient of variation (%)	n-propanol retained moles/mole of PVP	Apparent decrease in water sorption moles/mole PVP
11	5.0	4.98	9.5	1.2	7.4	11.5
32	13.60	13.30	2.2	0.58	4.0	6.7
52	21.40	21.14	1.2	–	1.6	5.8

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PINK DISCOLORATION IN CHEDDAR CHEESE

INTRODUCTION

PINK DISCOLORATION in aged colored Cheddar cheese is a current problem in the cheese industry. Early work by Morgan (1933), Moir (1933) and Barnicoat (1937, 1950) has shown that hydrogen sulfide was involved in the pink discoloration defect of uncoated, annatto colored, New Zealand Cheddar cheeses. Both Morgan (1933) and Moir (1933) concluded that mold growth was closely associated with the production of the pink discoloration. Moir (1933) believed that acids produced by the mold caused the pink discoloration. Today most cheeses are wrapped in flexible films, thus are rindless, and are cured in these films. As a result, mold growth is not common. But sulfide production is still an important part of the curing process (Kristoffersen, 1961).

Barnicoat (1950) concluded that the pink discoloration was due to oxidation of annatto cheese color to norbixin by sulfhydryl-type compounds. Since most American cheeses are cured in flexible films and since reducing conditions exist (Kristoffersen, 1961), this explanation for the current problem is unlikely.

Moir (1933) suggested that the insoluble pink was produced in cheese by the development of a strongly acid reaction. The change of annatto from yellow to pink or red as the pH is decreased was recently reinforced by Bridger (1967). He reported a significant change in color from yellow to red when the pH was changed from pH 5.7 to 5.0. During the manufacture and curing of Cheddar cheese, the pH changes typically from about pH 6.6 to a low of pH 5.05 at 3 days of age to pH 5.52 at 2 yr (Brown and Price, 1934). Therefore, the possibility of pink discoloration developing appears to be great.

"Pinking" also has been observed by one of us during the commercial manufacture of process and dried cheese. Identification of the pink compounds found in defective cheese and of the mechanism for their formation should help to control the defect.

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MATERIALS & METHODS

Source of samples

Many samples of commercial Cheddar cheeses exhibiting pink discoloration and normal cheeses were used in the investigation. These were obtained as needed from a process cheese manufacturer and a company specializing in the curing of cheese. The manufacturing history of the cheeses was unknown. The cheese samples were waxed and maintained at 10°C until analyzed.

Extraction of pink color

Various steps involved in the isolation of the pink color material (pink residue) are shown in Figure 1.

Fractionation of the pink residue

The pink residue was exhaustively extracted with methanol (technical grade) in a Soxhlet apparatus at room temperature for 2–3 hr until all color was removed. The methanol extract of the pink residue, yellow in color, was further purified by thin-layer chromatography. Glass plates (20 × 20 cm) were used in making silica thin layers and these were activated by heating at 100°C for 20 min. The solvent system used was petroleum ether, diethyl ether and acetic acid in the ratio of 90:10:1, respectively. After development, the spots were scraped and extracted with methanol. Silica was removed by centrifugation.

Solubility studies on pink residue

The solubility of the pink residue was tested in water, hydrochloric acid, sodium hydroxide, 6.6M ammonium sulfate, urea, ethanol, methanol, petroleum ether and chloroform.

Thin layer chromatography

The yellow extract obtained from the pink residue was identified by thin layer chromatography. Gelman's instant thin layer silica medium activated by heating to 105°C for 20 min was used. A 10-ml quantity of sample was applied to the medium. For the identification of norbixin, the solvent system used was petroleum ether (30–60°C), diethyl ether and acetic acid, 70:30:1.

Absorption spectrum analysis

For the determination of visible spectra of the pigment or ultraviolet spectra of the protein fraction, a Beckman DB Spectrophotometer was used in conjunction with a Sargent model SRLG recorder.

Protein and amino acid analysis

The nitrogen content of the pink residue was determined by a micro-Kjeldahl method (Larson and Jenness, 1950), and percent protein calculated as Nx6.38. Amino acid analysis was done in a Beckman Spinco model 120 amino acid analyzer.

Polyacrylamide vertical gel electrophoresis

The white residue obtained after the removal of color from the pink residue by solvents was dispersed in 7.0M urea and subjected to electrophoresis. An E–C vertical electrophoresis unit was used and the experiments were conducted in accordance with the manufacturer's recommended procedure. Acrylamide gel (7%) was used for the electrophoresis. To each 1 ml of sample, a drop of dye and two drops of mercaptoethanol were added. A constant voltage of 250v was applied for 3 hr at room temperature.

Moisture analysis

Cheese water content was determined by a method recommended by the U.S. Department of Agriculture (1965).

Phosphorous analysis

The method of Summer (1944) was used for analyzing phosphorous after ashing.

Lipid analysis

Lipid content was determined by the Mojonnier method (1925). Polar lipids were fractionated and identified by solvent fractionation technique of Jenness and Patton (1959). The separated compounds were identified by spray reagents for phosphate and amino groups.

Model systems

In studying the origin and development of pink discoloration, two kinds of model systems were used:

(a) The first model system was designed to test the stability of annatto cheese color towards precipitation or color change. Commercial annatto cheese color (0.5 ml) was diluted to 500 ml with distilled water. The solution was divided into 100 ml aliquots and 1 ml of 5% pink cheese extract was added to each aliquot except one which served as the control. Half of the samples to which pink cheese extract was added were subjected to bacterial filtration using a microporous porous porcelain filter (Selas VFA-54, 5/8 in. diam). The samples were tested for organisms using plate agar medium and incubating for 48 hr at 37°C as in standard plate method, with no growth observed at 1:10 dilution. The samples were also incubated at room temperature and observed for color change or annatto precipitation. pH was monitored throughout the experiment, before and after pink cheese extract addition and during incubation.

(b) In an effort to simulate conditions leading to pink discoloration, cheeses were exposed to different gases at room temperature. Samples of 6- and 12-month old cheese and yellow portions of defective cheese were incubated separately at room temperature in

air, hydrogen sulfide, ammonia and carbon dioxide for 24 hr.

Desulfhydrase activity of pink cheese

Since sulfide odor was associated with the pink defect, desylfhydrase activity was determined in pink and yellow portions of the defective cheese. A 10% extract in distilled water was adjusted to pH 7.0 and 4 ml aliquot was used in the assay. The method of Chappell and Morris (1968) involving a sulfide ion electrode was used.

RESULTS & DISCUSSION

Preliminary studies

About 100 cheeses having a pink discoloration were examined organoleptically. These observations confirmed previous work since the pink discoloration in these cheeses was always associated with sulfide odor and was predominantly present in cracks, trier sample holes and surfaces of the cheese.

The water content of pink and yellow portions of the defective cheeses did not show any significant difference, although free water was observed in the openings of some of the cheese. Lack of difference may have been due to mode of sampling. It was not entirely possible to separate all yellow portions from pink portions and thus there may have been a redistribution of moisture.

The pink discoloration was found to be stable to air, light, and pH variation. Increase of pH to 10 or decrease to pH 2 did not alter the pink color in defective cheese. Pink portions of cheese when heated to melting in a water bath remained pink.

The pH of the water extract of pink and yellow portions of a defective cheese was found to be 5.7 and 5.9, respectively, after 12 months storage, and was 5.9 and 6.1 in an 18-month old cheese. Thus the pink portions were significantly lower in pH than the yellow portions from the same cheese.

The pink residue isolated from defective cheeses was quite insoluble in nature. Solubility studies indicated (Table 1) that the pink residue was soluble only in 6.6M urea, and thus might be a protein. The pink residue turned blue when a few drops of concentrated sulfuric acid were added. Since this is a characteristic reaction of norbixin (Karrer and Zucker, 1950), the presence of norbixin was therefore suspected. Sodium hydroxide and many organic solvents extracted the color from the pink residue giving rise to a yellow extract and a white residue.

The primary problem faced in the extraction of pink color was that pink color turns yellow when usual extraction procedures are employed. Barnicoat (1950) faced the same problem and was not successful in extracting the pink color. The various steps involved in the extraction of pink color were shown in Figure 1. On the basis of extraction experiments, the following was considered. When annatto color is added during cheese manufacture, color is adsorbed by the casein (Chr. Hansen Laboratory, Inc., Annatto Food Colors Bull.). However, in these studies the pink color was found to be associated with the fat layer. In the extraction procedure, petroleum ether (30–60°C) proved to be a unique solvent. Although it extracted the yellow color from the cheese, it did not change the pink color to yellow.

Identification of yellow extract

The yellow extract obtained by extracting the pink residue with methanol was subjected to thin-layer chromatography along with bixin and norbixin standards. As shown in Figure 2, the yellow extract moved to the same extent as the norbixin standard while the bixin standard had a higher Rf value due to the presence of an ester group. Spots of the yellow extract and norbixin were cut out and eluted with methanol. The visible spectra of extracts were taken which are shown in Figure 3. The spectra of the yellow extract had maxima at 446 mμ, 469

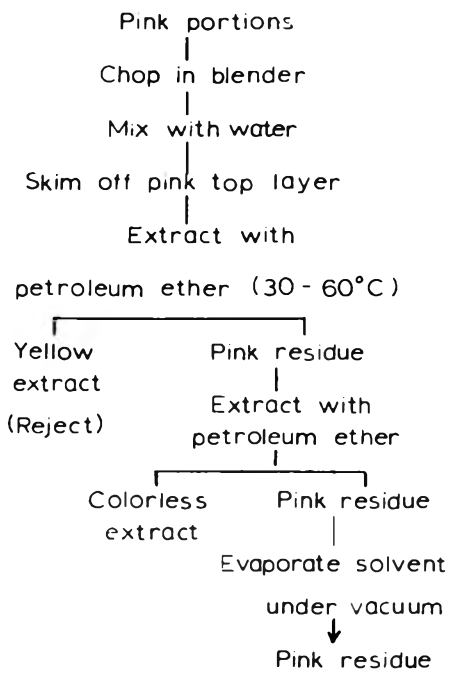

Fig. 1—Scheme for the isolation of pink coloration compounds.

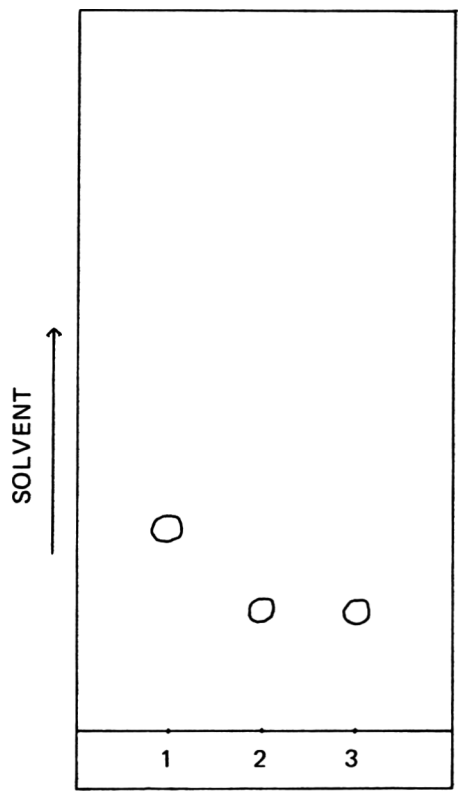

Fig. 2—Thin layer chromatogram of yellow extract, standard bixin and standard norbixin: (1=standard bixin; 2=yellow extract; 3=standard norbixin.)

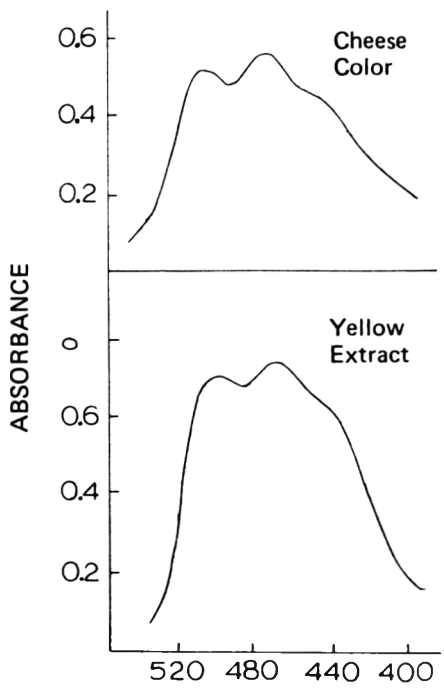

Fig. 3—Absorption spectra of cheese color (norbixin) and yellow extract.

Table 1–Solubility characteristics of pink residue[a]

Solvent	Solubility	Color extracted
Water	–	–
Hydrochloric acid	–	–
6.6M urea	+	+
Petroleum ether	–	–
Methanol	–	+
Chloroform	–	+
Ethanol	–	+

[a] – Negative response; + positive response

mμ and 502 mμ which were similar to the norbixin standard. These data showed conclusively that norbixin is the component of the pink residue responsible for its color.

The yellow chloroform methanol extract was then tested for possible lipid components since extraction of the pink color was achieved from the fat layer. Thin layer chromatography in the neutral lipid solvent system showed no movement indicating the absence of neutral lipids. The presence of norbixin which is polar, sparingly soluble in amylalcohol and insoluble in other organic solvents (Karrer and Zucker, 1950), and the fact that the pink residue was isolated from the fat system suggested an association with phospholipids. The yellow extract therefore was evaporated to dryness to determine nitrogen and phosphorous content. These were 2.6% and 0.17%, respectively. The resolution of the extract by solvent fractionation technique (Jenness and Patton, 1959) indicated the possible presence of lecithin or cephalin.

Table 2–Amino acid content of white residue

Amino acid	Conc μmoles/mg
Lysine	0.387
Histidine	0.128
Arginine	0.123
Aspartic acid	0.440
Threonine	0.227
Serine	0.391
Glutamic acid	1.240
Proline	0.617
Glycine	0.184
Alanine	0.214
Half cystine	0.015
Valine	0.373
Methionine	0.130
Isoleucine	0.288
Leucine	0.468
Tyrosine	0.189
Phenylalanine	0.212

Identification of white residue

The white residue obtained after the removal of color from the pink residue had solubility characteristics corresponding to a protein. The white residue was completely soluble only in 6.6M urea which is a protein solvent. A spot of this solution on a filter paper was dried and sprayed with ninhydrin reagent. Purple color characteristic of a protein was obtained. The UV spectra of the solution, as shown in Figure 4, showed a maxima at 280 mμ, confirming the presence of the protein in the pink residue. The nitrogen content was 10.58%. Since the white residue was isolated from cheese, it was suspected to be a phosphoprotein. The phosphorous content of white residue was 0.98%, which is quite similar to that of casein. This indicated that the protein may be a casein fraction of peptides derived from it. Amino acid analysis of white residue was done in a Beckman Spinco model 120 amino acid analyzer and the amino acid content is summarized in Table 2.

Polyacrylamide gel electrophoresis of the white residue in urea was done to elucidate the nature of the casein fraction. The electrophoretic patterns were similar to those obtained by Ledford et al. (1966) except that the zones following Beta casein were not as clearly defined as Ledford's pattern. Zones corresponding to Beta casein of standard first cycle casein (which was injected along with the sample) were identified in the pattern showing the presence of Beta casein. The two zones above Beta casein were also found in the patterns of Ledford et al. (1966). These authors suggested that these might be Kappa casein. The zones after Beta casein were not clear although as many as five zones were suspected. Increasing the pore size of the gel did not change the pattern. These appear to be unidentified peptide fractions arising out of casein degradation during ripening of the cheese. The white residue is thus mainly Beta casein and several unidentified peptide components.

Model system studies

Having tentatively identified the pink compound and associated material, model system studies were conducted to investigate the development of pink discoloration. Two kinds of model systems were used which were as follows:

Stability of cheese color. In these studies, stability of an alkaline solution of cheese color towards precipitation was tested. Figure 5 shows the pH profile of the system during storage. During the first 2 days there was a sharp drop in pH of samples not subjected to bacterial filtration, following which pH values increased. As soon as the pink cheese extract was added, a reddish tinge was imparted to the sample and the pH showed an immediate drop to 8.0. After 24 hr, the first pink particles became apparent. The pH was 7.5. During the second day, the pH reached a minimum and then slowly started rising. However, pink particles did not disappear on pH increase. Samples subjected to bacterial filtration exhibited similar behavior to that of the control, and although pH values were slightly less, there was no precipitation. The pink particles obtained in the sample not subjected to bacterial filtration were filtered and dried. The color was extracted by methanol, and the extract was subjected to thin-layer chromatography along with norbixin and bixin

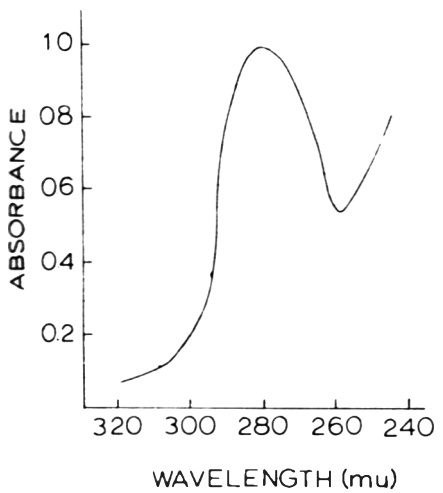

Fig. 4–Ultraviolet spectra of white residue.

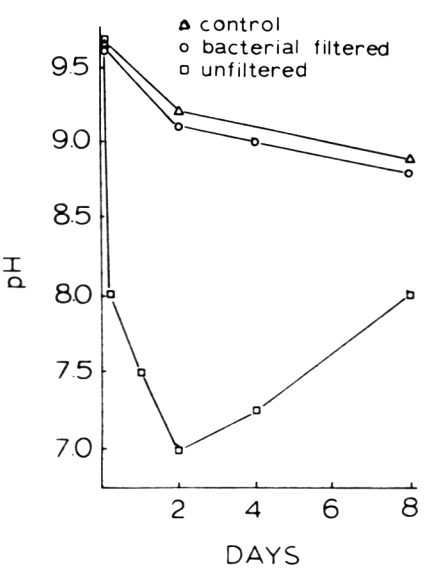

Fig. 5–Effect of addition of pink cheese extract on pH of cheese color solution during storage.

Table 3—Effect of different gases on the color of Cheddar cheese during storage

Gas	Age of normal cheese		Yellow portions from pink defective cheese
	6 months	12 months	
Air	Yellow	Yellow	Localized pink coloration
Ammonia	Deep yellow	Deep yellow	Deep yellow
Hydrogen sulfide	Slight fading of yellow	Slight fading of yellow	More fading; Localized pink coloration
Carbon dioxide	Yellow	Yellow	Localized pink coloration
Nitrogen	Yellow	Yellow	Localized pink coloration

standards. The extract moved at the same rate as standard norbixin, indicating it to be norbixin. These results suggest that bacterial activity may be involved in inducing the precipitation of cheese color, as no precipitate was observed in samples subjected to bacterial filtration. However, there is also the possibility that some enzymes were retained in the Millipore® filter, which could cause such changes. Bacterial growth probably took place in samples not subjected to bacterial filtration, as these samples had a putrid odor. In cheese, proteolytic organisms are presumed to be unevenly distributed, and so the production of hydrogen sulfide from sulfur-containing amino acids during ripening undoubtedly occurs where bacteria are concentrated. Since hydrogen sulfide is acidic, there may be a localized drop in pH at these places. This is probably the reason for an immediate drop in the pH of cheese color on the addition of pink cheese extract and also why pink areas of cheese are lower in pH than yellow areas from the same cheese.

Inducement of pink discoloration. During ripening of Cheddar cheese, hydrogen sulfide, ammonia and carbon dioxide are formed. The basic idea of this experiment was to expose normal and defective (yellow portions) cheeses to different atmospheres of hydrogen sulfide, ammonia and carbon dioxide. Perhaps a preponderance of one of these gases might produce discoloration. Table 3 summarizes the color of cheese under different atmospheres.

It is evident from the table that pink discoloration was not induced under these conditions. However, yellow portions of pink cheese exhibited pink discoloration under all conditions except in ammonia. Ammonia may have inhibited desulfhydrase activity or counteracted the acid condition due to hydrogen sulfide production; otherwise, these results are unexplained. Perhaps there was localized production of H_2S during the gas treatments.

Desulfhydrase activity

Since the literature and our work implicate hydrogen sulfide in pink discoloration, desulfhydrase activities of pink and yellow portions of a defective cheese were determined and found to be 0.07 and 0.05 μmoles H_2S min/ml, respectively. There seems, therefore, to be slightly more activity in the pink portion.

Barnicoat (1950) in his investigation of pink discoloration in New Zealand Cheddar cheese that had no coatings concluded that pink discoloration was due to oxidation of annatto cheese color to norbixin by sulfhydryl-type compounds. Oxidation or reduction of norbixin would change the number of conjugated double bonds. Karrer and Zucker (1950) pointed out that addition to conjugated double bond without other changes in structure results in displacements of visible absorption bands towards larger wavelength by 20–22 mμ and removal has the opposite effect. He also pointed out that if a terminal conjugated double bond is replaced by an epoxide group, the maxima are displaced by 6–9 mμ towards the blue end of the spectrum. The postulation of Barnicoat involving oxidation of norbixin was not found to be correct as absorption spectra of the yellow extract were found to be similar to that of the norbixin standard. There was no shift in the maxima which would have been the case if there had been any oxidation of norbixin. This evidence also ruled out any possibility of reduction of norbixin which would again alter the number of double bonds.

Moir (1933) found that annatto color impregnated filter paper turned pink by exposure to H_2S was stable to pH changes up to pH 5.9. In our work we found that norbixin in association with phospholipid and casein is very stable to pH changes and doesn't solubilize even under alkaline conditions. In contrast, annatto in model systems and on filter paper and in solution readily changes from yellow at pH 7.0 to pink at pH 4.0 and back to yellow at pH 7.0. Perhaps the pink norbixin formed in the presence of H_2S is held tightly by β-casein, phospholipids and filter paper. On the other hand, it is recognized that β-casein and phospholipids may be artifacts recovered as a consequence of the extraction procedure. Even then, the close association could have a protective influence.

Sulfur-containing amino acids like cysteine are gradually made available during curing and thus are available for degradation to H_2S by desulfhydrase activity. This may be the reason why pink discoloration is produced during curing.

In conclusion, (1) identification studies show that the pink color, resistant to pH changes during curing of colored Cheddar cheese, consists of norbixin possibly protected by phospholipid(s) and β-casein; and (2) preliminary observations and model system studies suggest that in the cheese system there may be a localized production of H_2S which in turn leads to a pink microfine precipitate of norbixin.

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IDENTIFICATION AND CHARACTERIZATION OF THE MICROFLORA AND SPOILAGE BACTERIA IN FRESHWATER CRAYFISH
Procambarus clarkii (Girard)

INTRODUCTION

IN RECENT YEARS freshwater crayfish of the genus *Procambarus* have become an increasingly important food product in South Louisiana and other Gulf Coast areas. Lovell and Barkate (1969) reported on the incidence of health-related bacteria in crayfish products; however, no studies have been described in the literature on spoilage bacteria in commercially processed crayfish. Most of the commercially processed crayfish is marketed as hand-peeled tails which are stored at low temperatures, above freezing, until consumed.

The purpose of this study was to isolate and identify bacteria from commercially peeled crayfish tails after various periods of low temperature storage, and to determine which bacteria were actually responsible for spoilage of the crayfish tail flesh.

EXPERIMENTAL

Procurement and preparation of samples

The crayfish samples used in this study were collected from two commercial processing plants in Louisiana. Unwashed peeled tail meat was collected directly from the processing lines, packed in ice and transported to the laboratory for analysis and subsequent storage. Approximately one-half of each sample from each plant was stored in 32-oz screw-cap jars at 0°C and the other half at 5°C. Immediately after the samples were collected and after various storage intervals, 50g of the tail meat were measured into a Waring Blendor and blended for 2 min. Serial dilutions were made using phosphate buffer (Butterfield, 1932) as the diluent. Two sets of duplicate pour plates were made using Milk-Protein Hydrolysate Agar (BBL) and incubated at 23°C for 72 hr and 3°C for 14 days. In preliminary studies of various media, Nutrient Agar (Difco), Brain Heart Infusion Agar (Difco), Tryptone Glucose Extract Agar (Difco), Nutrient Agar with yeast extract (Difco) and Milk-Protein Hydrolysate Agar were evaluated to determine which medium produced the highest number of colonies from crayfish tail meat. Milk-Protein Hydrolysate was selected because it was found to yield a higher total aerobic plate count (APC) than any of the other four media tested.

In addition to bacterial analysis, the same samples of crayfish were evaluated for spoilage by smelling by three experienced judges.

From the samples of tails stored at 0°C from each plant, 20 bacteria were isolated for identification at 0, 8 and 12 days of storage and again after the sample was judged to be spoiled. From samples at 5°C, which were expected to spoil faster than the 0°C samples, 20 bacteria were isolated at 0, 4 and 8 days of storage and again after spoilage.

Isolation and identification

A total of 280 isolated colonies were randomly picked from countable plates and generically identified by the use of an identification scheme set up by Shewan et al. (1960) and modified with the use of *Bergey's Manual of Determinative Bacteriology* (Breed et al., 1957). The identification scheme of Shewan was chosen because of its widely accepted convenience for the generic identification of bacteria commonly found in spoiled seafoods.

Determination of spoilage characteristics

Lerke et al. (1965) isolated bacteria from spoiled fish and distinguished the "spoilers" from the "nonspoilers" by inoculating each organism into fish tissue extract sterilized by microfiltration. The 280 bacterial cultures that were isolated from crayfish tails were inoculated into crayfish substrate that was heat sterilized. Heat sterilization was used because commercially, crayfish are heat processed prior to peeling. The organisms, when grown independently in the sterile crayfish substrate, were evaluated on their ability to produce "spoiled" odors by a trained panel.

To prepare the substrate, live crayfish were scalded in the laboratory for approximately 5 min and peeled. The peeled tail meat was weighed, placed into a Waring Blendor jar, diluted 1:4 with physiological saline solution (0.85%) and blended for 2 min. 10-ml aliquots were placed into 50-ml screw-cap tubes and autoclaved for 15 min at a temperature of 121°C. 18- to 24-hr old cells of each organism were aseptically inoculated into separate tubes of the sterile crayfish medium. The inoculated tubes, along with uninoculated tubes serving as controls, were incubated at 0° and 5°C for 7 and 14 days.

After 7 and 14 days a tube containing each organism in the crayfish substrate was evaluated by three experienced judges by smelling. The odors detected from each tube were scored according to the following scoring system:

3 = Odor not distinguishable from sterile sample
2 = Odor slightly distinguishable from sterile sample
1 = Odor very distinguishable from sterile sample
0 = Odor highly distinguishable from sterile sample

Each organism was classified as a "rapid spoiler," "slow spoiler," or "nonspoiler." A "rapid spoiler" was an organism which received a mean score less than 2.0 at 7 days at 5°C; a "slow spoiler" was an organism which had a mean score less than 2.0 at 14 days at 5°C, but which had a mean score of 2.0 or higher at 7 days; and a "nonspoiler" was an organism which had a mean score of 2.0 or more at 14 days at 5°C.

RESULTS & DISCUSSION

Isolation and identification

The total aerobic plate counts of peeled crayfish stored at 0° and 5°C are shown in Table 1. Spoilage of the tail meat occurred when these counts reached 10^9 per g. The 280 isolates were found to belong to 11 different genera. Tables 2 and 3 show the distribution of the isolated bacteria among the five predominating genera from samples stored at 0° and 5°C, respectively. The three genera, *Achromobacter, Alcaligenes* and *Pseudomonas* were found to comprise approximately 82% of the 280 isolates, while the other 18% was comprised of the other eight genera. In the fresh tail meat, *Micro-*

Table 1—Total aerobic plate count of peeled crayfish stored at 0° and 5°C

Days of storage	0°C	5°C
0	1.6×10^5 [a]	1.6×10^5
4	–	1.8×10^6
8	1.4×10^6	5.6×10^8
12	2.0×10^7	2.8×10^9
24	3.2×10^9	–

[a] Results are the average of two runs

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coccus, Staphylococcus and *Alcaligenes* made up the major portion of the bacterial flora, while *Pseudomonas* and *Achromobacter* predominated in the spoiled product.

Pseudomonas and *Achromobacter* which traditionally comprise the psychrophiles, made up only a total of 12.5% of the isolates in the fresh tail meat; however, after 8 days of storage *Achromobacter* and *Pseudomonas* began to increase. In the spoiled product at 0°C, *Pseudomonas* predominated, whereas at 5°C *Achromobacter* made up the larger portion of the bacteria types. This may indicate that the *Pseudomonas* spoilers are more psychrophilic than the *Achromobacter.*

Determination of spoilage characteristics

Table 4 shows the percentage of "rapid spoilers" and "slow spoilers" found in the peeled tails at various phases of the storage life at the two low temperatures. In this study the percentage of spoilers greatly increased with the length of time that the tail meat was stored. Adams et al. (1964) reported that the percentage of spoilers in different lots of fresh fillets of sole was uniformly well below 10. Although at the end of storage the *Pseudomonas* and *Achromobacter* groups accounted for almost 100% of the population, the percentage of spoilers did not rise along with these groups, but remained low.

In the fresh samples the percentage of spoilers, either "slow" or "rapid", was low; however, in those stored for 8 or more days the percentage of spoilers was high.

Of the 280 isolates, 22.1% were found to be "rapid spoilers," 16.4% were classified as "slow spoilers," and 61.5% were "nonspoilers."

Table 5 shows the number of "rapid spoilers" and "slow spoilers" belonging to each of the genera. The greatest number of "rapid spoilers" belonged to the genus *Pseudomonas* with *Achromobacter* a distant second. This is in agreement with Shaw and Shewan (1968), who reported that *Pseudomonas* spp. were by far the most active spoilers of cod.

The genera containing no "rapid spoilers" were *Aerobacter, Bacillus, Flavobacterium, Lactobacillus, Micrococcus, Sarcina* and *Staphylococcus.* The greatest number of "slow spoilers" also belonged to the genus *Pseudomonas,* with *Achromobacter* a close second. Other genera found to contain "slow spoilers" were *Alcaligenes, Flavobacterium, Aerobacter, Lactobacillus, Micrococcus* and *Staphylococcus.* The only genera containing no "slow spoilers" were *Bacillus, Proteus* and *Sarcina.*

A number of researchers (Lockhead and Landerkin, 1935; Alford et al., 1942;

Table 2—Bacteria found in crayfish tail meat: fresh and stored at 0°C

	Days of storage			
Genus	Fresh	8	12	24 (spoilage)
Achromobacter	12.5[a]	82.5	17.5	22.5
Alcaligenes	15.0	2.5	< 1.0	7.5
Flavobacterium	10.0	5.0	5.0	< 1.0
Micrococcus	30.0	< 1.0	< 1.0	< 1.0
Pseudomonas	< 1.0	7.5	45.0	65.0
Staphylococcus	17.5	2.5	< 1.0	< 1.0
Others[b]	15.0	< 1.0	32.5	5.0

[a] These figures are expressed as percentage of total isolates at the indicated time of spoilage.
[b] Aerobacter, Bacillus, Lactobacillus, Proteus, and Sarcina.

Table 3—Bacteria isolated from crayfish tail meat: fresh and stored at 5°C

	Days of storage			
Genus	Fresh	4	8	12 (spoilage)
Achromobacter	12.5[a]	47.5	65.0	62.5
Alcaligenes	15.0	15.0	15.0	< 1.0
Flavobacterium	10.0	7.5	< 1.0	2.5
Micrococcus	30.0	2.5	< 1.0	< 1.0
Pseudomonas	< 1.0	10.0	17.5	32.5
Staphylococcus	17.5	12.5	< 1.0	< 1.0
Others[b]	15.0	5.0	2.5	2.5

[a] These values are expressed as percentage of total isolates at the indicated time of storage.
[b] Aerobacter, Bacillus, Lactobacillus, Proteus and Sarcina.

Table 4—Percentages of bacterial isolates classified as "rapid spoilers" and "slow spoilers" in crayfish tail meat stored at 5° and 0°C.

		Days of storage				
Type of spoiler	Temp (°C)	0	4	8	12	24
"Rapid spoiler"	5[a]	2.5	5.0	27.5	32.5	–
	0[b]	2.5	–	10.0	35.0	42.5
"Slow spoiler"	5[a]	5.0	17.5	15.0	20.0	–
	0[b]	5.0	–	10.0	30.0	17.5

[a] For samples stored at 5°C, isolations were made on days 0, 4, 8 and 12. Spoilage occurred on day 12.
[b] For samples stored at 0°, isolations were made on days 0, 8, 12 and 24. Spoilage occurred on day 24.

Table 5—Percentage of total bacterial isolates from low-temperature-stored crayfish tail meat, which were classified as spoilage and nonspoilage bacteria, and according to genera

Genus	Rapid spoiler	Slow spoiler	Non-spoiler	Total
Achromobacter	6.1	5.7	32.5	44.3
Alcaligenes	< 1.0	2.5	8.9	12.1
Flavobacterium	< 1.0	< 1.0	3.6	4.3
Pseudomonas	14.6	6.1	4.6	25.4
Others[a]	< 1.0	1.4	11.8	13.9
	23.7	16.7	61.5	

[a] Aerobacter, Bacillus, Lactobacillus, Micrococcus, Proteus, Sarcina and Staphylococcus.

Aschehoug and Vesterhus, 1947; Ayres et al., 1950; Kirsch et al., 1952; Adams et al., 1964; Lerke et al., 1965) have indicated that *Pseudomonas* and *Achromobacter* are the most important genera responsible for spoilage of meats, poultry, fish and shellfish. The data reported in this study indicate that these genera are also the predominant psychrophilic spoilage organisms in commercially peeled crayfish tail meat. Organisms from other genera contribute to spoilage to a lesser extent.

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THE AROMA OF CANNED BEEF: MODELS FOR CORRELATION OF INSTRUMENTAL AND SENSORY DATA

INTRODUCTION

IN ORDER TO be able to interpret sensory data and their changes it is essential to analyse the material instrumentally for chemical properties and to develop models for correlating the sensory and instrumental data in a meaningful way. The practical purpose of determining such relations may be to supplement or complement the panel service by an instrumental technique in quality control work. Other applications can be found in product and process development work.

One may distinguish between mainly two types of approaches:

(a) Categorization of sensory qualities applying cluster analysis, discriminant analysis and related techniques to identify relevant instrumental predictors (e.g., Biggers et al., 1969; Milutinović et al., 1970; Powers, 1968; Powers and Keith, 1968; Powers et al. 1971; Young et al., 1970).

(b) Quantification of sensory qualities enabling the application of parametric statistical techniques like analysis of variance and regression analysis to identify predictors (e.g., Bednarczyk and Kramer, 1971; Biswas and Biswas, 1971; Biswas et al., 1971; Dravnieks and Laffort, 1970; Gacula et al., 1971; Guadagni, 1968; Herrmann, 1971a, b; Kramer, 1969; Persson and von Sydow, 1972; Tanaka and Saito, 1969; Tanaka et al., 1970; Vuataz, 1969, 1971; Vuataz and Reymond, 1971). In particular, for canned beef it has been reported that there exist significant correlations between the "aroma quality" and hydrogen sulfide (Przeździecka and Żółtowska, 1967). A detailed review of the literature on correlation studies concerning odor and taste in food materials is given in ASTM (1968, 1969).

To give a rough outline, psychophysical relations between sensory and instrumental data may be classified into the following main categories:

Ad hoc relations (i)

There are two types of ad hoc relations. One type is pure ad hoc relations intended merely to display and summarize a given set of data by means of numerical fitting. Another type is relations determined by some statistical approach but yielding nongeneralizable results. E.g., many psychophysical regression models (compare type (b) above) are based on the assumption that the instrumental regression variables are fixed, but in reality they may have a certain variance which can often result in significant relations that are not generalizable, but only valid for a certain set of data at a certain occasion. One way to eliminate such ad hoc relations is to test them by repeating the experiments a number of times (Persson and von Sydow, 1972).

Because the relations belonging to this category are not generalizable, they have no predictive power and of course no explanatory power either.

Predictive relations (ii)

This kind of relations is generalizable and can be demonstrated to hold for a certain type of data at all occasions by means of statistical test procedures. The main objective is to obtain mathematical-statistical descriptions of regularities and structures of data. Explanations and interpretations of such relations are subordinate or completely ignored. However, in spite of the limited explanatory power, the predictive power may be sufficiently high for many practical applications, e.g., in quality control work (Biggers et al., 1969; Persson and von Sydow, 1972). It must be pointed out that predictive relations cannot be safely extrapolated outside the tested area. Most psychophysical investigations on food products described in the literature, aim at this type of relations.

Causative relations (iii)

Causative relations describe true stimuli-response connections and not merely statistical correlations. This implies not only high predictive power but also high explanatory power. Since these relations describe a stimulus-response mechanism, extrapolations to areas outside the original investigated one may be possible. Obviously, these kinds of relations have the highest informative value, and therefore have the highest potential degree of usefulness; but at present very few examples of this type have been reported in applied food science (Guadagni and Miers, 1969; von Sydow et al., 1970 and Chang et al., 1966).

The present investigation is of type (b) and aims at finding predictive and if possible causative relations. The regression models applied represent further developments of hypotheses put forward by von Sydow (1971) and Persson and von Sydow (1972).

In the present investigation a psychophysical approach rather than a pure statistical one has been introduced. Stevens' and Fechner's relations have been applied and simple models to handle interactions between chemical compounds have been suggested. The models used have been formulated in analogy with models used in other psychophysical contexts. The models have been tested on data from canned beef using a specially designed computer program for regression analysis. The samples included different heating times and different formulations (Table 1). On the chemical side the compounds were identified using mass spectrometry and quantified by gas chromatography (Persson and von Sydow, 1973). 22 compounds were used (Table 2). The aroma properties were determined by means of a quantitative odor quality technique: a panel was trained to recognize different odor notes and assess the intensities on numerical scales (Persson et al., 1973).

EXPERIMENTAL

Materials and processing

Minced beef from top side round steaks (Biceps femoris and Vastrus labiralis) was added

Table 1—Investigated samples of canned beef[a]

Heating time (min)	F_c-value[b]	B	BF	BC	BFC
15	0.4	+			+
30	10.5	+	+	+	+
45	24.2	+			+
60	38.6	+			+
75	51.9	+			+

[a]Temperature: 121°C. B = 79.3% beef + 20% H_2O + 0.7% NaCl; BF = 66.3% beef + 13% fat + 20% H_2O + 0.7% NaCl; BC = 74.3% beef + carbohydrate + 20% H_2O + 0.7% NaCl; and BFC = 61.3% beef + 13% fat + 5% carbohydrate + 20% H_2O + 0.7% NaCl

[b]Samples containing fat had about 15% lower values

Table 2—22 chemical compounds applied to the models

Peak no.[a]	Compound	Peak no.[a]	Compound
4	Ethanal	S2	Hydrogen sulfide
12	Butanal	S4	Methyl mercaptan
22	Pentanal	S5	Ethyl mercaptan
32	Hexanal	S6	Dimethyl sulfide
47	Heptanal	S7	Methyl ethyl sulfide
9	2-Methyl propanal	S8	Ethylene sulfide
18	2-Methyl butanal	S10	Thiophene
17	3-Methyl butanal	S11	Dimethyl disulfide
		S12	2-Methylthiophene
6a	Furan	S13	3,5-Dimethyl-1,2,4-trithiolane
63	2-Pentyl furan		
13a	2,3-Butandione		
23	2,3-Pentandione		

[a] Peak numbers: 4, 12, 22, etc., and S2, S4, S5, etc., refer to retention orders on a SG 96 column and a Chromosorb G AW column, respectively.

Table 3—Psychophysical models for relations between sensory data (R) and data (S) derived from instrumental measurements (chemical data)

(A) $R = a \cdot \log S + b$
(B) $R = c \cdot S^n + d$
(C) $R = c \cdot S + d$

with fat and carbohydrate (starch) in four formulations and packed into small tinplate cans containing 95g. The cans were processed in small retorts at 121°C for different times. This invesigation included, in all, 12 samples (Table 1). For further details see Persson and von Sydow (1973).

GC-analysis of the volatiles

Data and details about the analytical procedures are given in Persson and von Sydow (1973). The quantitative determination of the volatiles in the headspace gas of canned beef was performed using a sampling technique making it possible to analyse large volumes (up to 500 ml), and an open tubular column gas chromatographic technique. The compounds were detected by flame ionization detector and a sulfur specific flame photometric detector. To analyze the sulfur compounds a sampling device entirely made from glass and Teflon and a packed glass column were used. The amounts were determined in absolute concentration (ppb). The compounds were identified by analyzing the headspace gas of a low temperature distillate on a combined gas chromatograph–mass spectrometer. 95 compounds were identified. The odor properties of the compounds in a low temperature distillate of canned beef were assessed by sniffing the gas chromatographic eluate.

Sensory evaluation

For details in procedure and results, see Persson et al. (1973). The samples were evaluated using the odor quality technique: a panel was trained to recognize different odor notes in a food item and to estimate the intensities of these. 28 odor notes were used and the intensity was estimated using a 10-point scale. The validity of the odor quality assessment technique was tested using a specially designed statistical procedure, showing that most of the odor notes could be measured by uni-dimensional scales. A homogenous group of panelists showing both high consistency and discriminability was selected. In addition, preference tests were performed.

Models applied

To determine relations between the intensity values (R) for an odor quality and the corresponding gas chromatographic data (S) the three basic models listed in Table 3 were applied; considering S as a composed argument a number of hypotheses were tested. The suggested hypotheses, including up to four different compounds at a time, are given in Table 4.

Statistical testing of the models

The validity of the models was tested by means of linear regression analysis. To carry out these analyses a computer program was constructed. Input data are concentrations of compounds and the mean intensity values with the corresponding standard deviations from the odor quality assessments. The sensory data can be obtained directly on punched cards from the computer program performing the statistical analysis of the sensory data (Persson et al., 1973). Corrections for the individual references can be made by taking the difference between the individual intensity values of the reference and of the samples to be estimated, or the ratio between these.

It is possible to select and test any of the three basic models in Table 3 and any of the combinations of compounds (S = f(x)) in Table 4. S = f(x) are calculated for all possible combinations of compounds. The program runs through all odor qualities for every model and for every combination of compounds. (Compare the enumeration techniques suggested by LaMotte and Hocking, 1970; Schatzoff et al., 1968). The regression parameters and the corresponding correlation coefficients are determined for all possible combinations of peaks.

A chi-square statistic is used as a measure of goodness of fit for the regression models. The computation procedure for determining the regression lines has been generalized to include the possibilities that the observational points may have different variances. The conventional assumption of equal variances seems highly improbable when inspecting the data and the above generalization was therefore considered necessary. The simultaneous confidence bands for the two regression parameters are also calculated. The models are ranked according to the chi-square measure. To save computer time it is possible to predetermine suitable numerical limits for the correlation coefficients and the chi-square statistics. Then, all outcomes not fulfilling the present conditions are deleted.

The chi-square statistic is calculated from the residuals according to:

$$\chi^2 = \sum_{i=1}^{N} \left[(n_i/\hat{\sigma}^2 V_i)\left(V_i - (\hat{\alpha} + \hat{\beta} \times U_i)\right)^2 \right] \quad (1)$$

where

N = number of observational points;
n_i = number of (sensory) observations on the i_{th} observational point;
U_i = the independent regression variable (S or log S according to Table 3);
V_i = the dependent regression variable (R or log R according to Table 3);
$\hat{\sigma}^2 V_i$ = the maximum likelihood (ML) estimator of the variance of V at the i_{th} observational point;
$\hat{\alpha}, \hat{\beta}$ = the ML-estimators of the regression parameters.

If the model applied is true, the χ^2-statistic according to equation (1) has a chi-square distribution with N-2 degrees of freedom. Hence (1) can be used as a convenient test statistic. The test is sensitive for detecting deviations from linearity which is a prerequisite for the models applied.

For computational reasons the individual R-values are transformed according to: $R_{ind} = 10 + (R_{obs} - R_{ref})$ to avoid negative values.

In the program it is also possible to introduce multiplicative weighing factors for the different peaks. Particularly, in a sub-experiment of this investigation inverted odor threshold values were used as weighing factors. Since accurate odor threshold data for many compounds are missing, the threshold values were classified into six groups with values: 1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}, approximately corresponding to reported threshold values in ppm-units. For those compounds for which no threshold data could be found in the literature an approximate evaluation based on sniffing data was made to classify them into one of the six groups.

RESULTS

THE 22 COMPOUNDS used in the models are given in Table 2. They were selected so that all were present in amounts above the corresponding odor threshold concentrations taken from the literature or roughly estimated from sniffing data (Persson and von Sydow, 1973). Several of the compounds were present in amounts ranging from 100 to more than a thousand times higher than the corresponding odor threshold value, e.g., hydrogen sulfide, methyl mercaptan,

Table 4—S = f(X), where X is gas chromatographic peak heights (concentrations) and S is calculated stimulus values used in the psychophysical models

(1)	$S = X_i$
(2)	$S = X_i + X_j$
(3)	$S = X_i + X_j + X_k$
(4)	$S = X_i + X_j + X_k + X_l$
(5)	$S = X_i - X_j$
(6)	$S = \sqrt{X_i^2 + X_j^2}$
(7)	$S = \sqrt{X_i^2 + X_j^2 + X_k^2}$
(8)	$S = \sqrt{X_i^2 + X_j^2 + X_k^2 + X_l^2}$
(9)	$S = \sqrt{X_i \cdot X_j}$
(10)	$S = X_i/X_j$
(11)	$S = (X_i + X_j)/X_k$
(12)	$S = (X_i + X_j)/(X_k + X_l)$
(13)	$S = (X_i - X_j)/(X_k - X_l)$
(14)	$S = (X_i + X_j)/(X_k - X_l)$

Table 5—Examples of five outcomes of models for the odor quality "cooked vegetables" using (A) 44 chemical compounds and (B) compounds assumed to contribute directly to this odor quality[a]

		A				B			
Model	S = f(X)	i	j	r[c]	χ^2[b]	i	j	r[c]	χ^2[b]
Stevens' (B)	X_i/X_j	13a	45	0.87	3.6	9	S8	0.83	8.9
	$\sqrt{X_i \cdot X_j}$	–	–	–	–	13a	S6	0.71	9.0
Linear (C)	X_i	21	–	0.84	7.0	–	–	–	–
	X_i/X_j	S10	18a	0.84	3.9	17	22	0.83	4.4
	X_i/X_j	S10	22a	0.87	4.2	–	–	–	–
	$\sqrt{X_i \cdot X_j}$	–	–	–	–	S6	S10	0.81	7.6
	$X_i + X_j$	–	–	–	–	18	S6	0.65	13.3
	$\sqrt{X_i^2 + X_j^2}$	13a	45	0.88	2.1	–	–	–	–

[a]12 samples and 6 persons in the panel. The X-indices corresponding to the peak numbers are given in Table 2 except for 18a = Benzene, 21 = 2-Pentanone, 22a = 3-Pentanone and 45 = 2-Heptanone.
[b]χ^2 = measure of goodness of fit (see text); Critical value for the χ^2-statistic at 5% level: 18.3 and at 1% level 23.2.
[c]r = correlation coefficient

ethyl mercaptan, dimethyl sulfide, 2-methyl propanal, 2-methyl butanal and 3-methyl butanal, and therefore it was assumed that these contribute directly to the aroma. On the other side, some of the 22 compounds have a concentration of the same magnitude as their corresponding odor threshold values, e.g., butanal, methyl ethyl sulfide, dimethyl disulfide, thiophene, 2-methyl thiophene and 3,5-dimethyl-1,2,4,-trithiolane, and therefore were considered to be of secondary importance compared to the first group. Another important aspect when evaluating the relative importance of the compounds, is the degree of change in concentrations between different samples. Compounds, which do not change with heating time and between different formulations can be of importance only as "background" components, but are of minor interest when analyzing how sensory properties are affected by process and formulation parameters. Examples of compounds that have an especially large increase with heat treatment are: the methyl aldehydes, hydrogen sulfide and methyl mercaptan. Examples of compounds which contribute to the aroma, but do not change very much in concentration are: dimethyl sulfide and 2,3-pentandione.

When performing the regression analysis the results showed that the best fitting of data to the models was obtained when the individual R-data had been corrected by taking the difference between the reference and the comparison samples. This was of course expected, and this normalization was used in all calculations.

Table 5 demonstrates eight models and peak combinations for the odor quality "cooked vegetables" using all 44 chemical compounds measurable in all chromatograms, and also using compounds assumed to directly influence this odor quality. The compounds under B were chosen by using empirical sniffing data and information from the literature about aroma compounds in cooked vegetables (Johnson et al., 1971a, b). The outcomes under A and B respectively are of about equal statistical significance. Since the outcomes under A contain purely non-stimuli compounds or such compounds combined with possible stimuli components, such as 2,3-butandione, the results cannot be considered to represent causative relations in contrast to the results under B. The relations under A are of type (i) or (ii), and those under B probably of type (ii) or (iii). The results in Table 5 obviously demonstrate the necessity of distinguishing between stimuli and nonstimuli components.

For each peak combination (Table 4) the statistical optimal relationships between the odor quality "retort flavor, canned beef off-flavor" and the compounds considered to directly contribute to this quality are given in Table 6. Only peak combinations with $r > 0.6$ have been included in the table. The indices S2, S4 and so on, correspond to the peak numbers in Table 2. Significant high correlations between this odor quality and the concentrations of hydrogen sulfide and methyl mercaptan were obtained (Table 6). This can partly be compared with the results obtained by Przeździecka and Żółtowska (1967). They found significant negative correlations between "odor quality" and methyl mercaptan and hydrogen sulfide, respectively. In the other peak combinations are dimethyl sulfide (S6), ethanol (4) and 2-methyl butanal (18) often included in addition to hydrogen sulfide (S2) and methyl mercaptan (S4), suggesting that these compounds directly contribute to the odor quality "retort flavor, canned beef off-flavor" and thus indicating possible causative relationships.

It should be pointed out that the results in Tables 5–8 represent only examples of several, equally interpretable outcomes. For instance, the methyl aldehydes (9, 18, 17) are highly intercorrelated and correlated with methyl

Table 6—Optimal correlation coefficients (r) for each peak combination (cf. Table 4) using a generalized form of Stevens' model (B)[a]

S = f(X)	r[c]	χ^2[b]
X_{S2}	0.71	17.5
X_{S4}	0.67	20.4
$X_{S2} + X_{S6}$	0.73	15.9
$X_4 + X_{S2} + X_{S6}$	0.77	12.6
$X_4 + X_{18} + X_{S2} + X_{S6}$	0.77	12.6
$\sqrt{X_{S2} \cdot X_{S12}}$	0.77	14.2
$\sqrt{(X_{18})^2 + (X_{S2})^2 + (X_{S6})^2}$	0.72	17.0
$\sqrt{(X_4)^2 + (X_{18})^2 + (X_{S2})^2 + (X_{S6})^2}$	0.73	16.8
X_{18}/X_{S8}	0.79	11.0
$(X_{S2} + X_{S4})/X_{12}$	0.79	11.8
$(X_4 + X_{S4})/)X_{12} + X_{S7})$	0.81	9.11

[a]Peaks included are evaluated to contribute directly to the odor quality "retort flavor, canned beef off-flavor." 12 samples and 6 persons in the panel. The X-indices correspond to the peak numbers in Table 2.
[b]χ^2 = measure of goodness of fit (see text); Critical value for the χ^2-statistics at 5% level: 18.3 and at 1% level: 23.2
[c]r = correlation coefficient

mercaptan and hydrogen sulfide and thus all these compounds are giving about the same correlation coefficients.

In Tables 7 and 8, Stevens' model and the linear model have been applied to 15 odor qualities using four of the peak combinations and, in the same way as in Table 6, the compounds included in the models are considered to influence directly the respective odor quality. In these tables only outcomes with $r > 0.5$ or $\chi^2 < 35.0$ have been listed. The indices i and j correspond to the peak numbers. Computations using Fechner's model gave roughly the same results as Stevens' model. These two models in many cases describe very similar relations. Because of the limited power of the test statistic the two models can in many cases not be separated for small sample sizes. Between the linear model and Stevens' model, however, differences could be found, e.g. when the models were applied to the odor qualities "sulfurous" and "burnt, smoky" no significant relations were obtained using Stevens' model, but the linear model gave positive outcomes. "Burnt, smoky" is highly correlated with the ratio between 2-methyl butanal and ethylene sulfide, and "sulfurous" is correlated with the ratio between methyl mercaptan and heptanal (Table 8), which is mainly in accordance with the sniffing data (Persson and von Sydow, 1973) indicating causative relations.

Many other such reasonable relationships can be found in Tables 7 and 8, but for some odor qualities, "like blood, raw meat," no significant relations were obtained. This is probably due to the fact that this odor quality could not be represented by a uni-dimensional intensity scale (Persson et al., 1973).

In Figure 1 the regression line for the odor quality "burnt, smoky," using as S-data the ratio between 2-methyl butanal and ethylene sulfide is illustrated. The linear model (C) was used.

The effect of weighing with odor thresholds is demonstrated in Table 9, where results for the odor quality "cooked cabbage" are given.

DISCUSSION

Models

The above presented functional relations (Tables 3 and 4) are not assumed to be generally valid but to hold only if certain "background" conditions exist. Generally, it is assumed that the relation between sensory response data (R) and the chemical stimuli data (S_i) can be expressed by the following formula:

$$R = F_0(S_1, S_2 \ldots\ldots, S_n) \qquad (2)$$

We have made the assumption that within a specific chemical environment certain chemical compounds can be considered as "background" stimuli components. This means that the above formula can be approximated by:

$$R \approx F_0(S_1 \mid S_2 = C_2, \ldots, S_n = C_n) = F_1(S_1) \qquad (3)$$

where $C_2, \ldots, C_n$ are constants representing the "background" stimuli. The formulas used for (3) are given in Table 4.

This assumption seems intuitively reasonable and has been shown to be valid for other food items. For instance, in investigations of bilberries, von Sydow et al. (1970) found that three components were mainly responsible for the bilberry aroma. When adding these compounds in certain concentrations to a "deodorized" bilberry juice (a juice without bilberry aroma but with a fruity aroma) it was impossible to distinguish this from a natural bilberry juice. But when these compounds were added to water no bilberry aroma was obtained and thus the deodorized bilberry juice acted as "background" stimuli.

If the chemical environment is radically changed, new functional structures will emerge. Thus, results obtained for one particular food item cannot be generalized to very different ones without further investigations.

The basic model (A) (Table 3) exemplifies the logarithmic relation originally proposed by Fechner (1860); (B) is the well-known power law developed by Stevens (Stevens, 1957, 1960, 1961a, b, 1971); and (C) is a special case of (B).

Table 7—Optimal correlation coefficients (r) for four peak combinations using a generalized form of Stevens' model applied to 15 odor qualities.[a]

	S = f(X)															
	X_i			$X_i + X_j$				$\sqrt{X_i \cdot X_j}$				X_i/X_j				
Odor quality	i	r[c]	χ^2[b]	i	j	r[c]	χ^2[b]	i	j	r[c]	χ^2[b]	i	j	r[c]	χ^2[b]	
Meaty (cooked)	22	−0.75	26.6	–	–	–	–	32	S5	−0.78	24.3	22	S8	−0.80	15.3	
Sickly	–	–	–	47	13a	0.78	34.6	32	S2	0.84	20.4	9	63	0.84	16.7	
Musty, mouldy	–	–	–	32	13a	0.72	34.1	32	S5	0.74	25.0	S2	S12	0.76	27.9	
Cooked cabbage	4	0.77	33.2	4	S6	0.74	28.7	–	–	–	–	–	–	–	–	
Garlic, onion	S2	0.60	10.9	4	S2	0.69	8.5	4	S2	0.72	7.7	S4	S7	0.61	10.6	
Animal, goat	S2	0.52	16.5	32	S2	0.53	16.3	13a	S2	0.51	18.3	S2	S5	0.67	11.9	
Nasty-smelling	–	–	–	6a	S4	0.70	20.1	12	S2	0.73	17.0	63	S4	−0.80	15.3	
Burnt, smoky	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
Sweaty	–	–	–	23	63	0.67	6.6	47	13a	0.51	34.9	9	S13	0.52	24.5	
Sour, acid etc.	S5	−0.61	10.3	–	–	–	–	S5	S7	−0.55	12.7	12	S5	0.54	11.1	
Like blood, raw meat	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
Cooked vegetables	S6	0.53	16.8	–	–	–	–	13a	S6	0.71	9.0	9	S8	0.83	8.9	
Sweet	32	0.81	17.3	12	32	0.81	16.7	12	32	0.81	15.7	32	23	0.77	17.8	
"Retort flavor, canned beef off-flavor"	S2	0.71	17.5	S2	S6	0.73	15.9	S2	S12	0.77	14.2	18	S8	0.79	11.0	
Sulfurous	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

[a]The peaks included are considered to contribute directly to the respective odor quality. 12 samples and 6 persons in the panel. The X-indices correspond to the peak numbers in Table 2.

[b]χ^2 = measure of goodness of fit (see text); Critical value for the χ^2-statistic at 5% level: 18.3 and at 1% level: 23.2.

[c]r = correlation coefficient

These models may be derived on the basis of different theoretical assumptions. For instance, they may be considered as solutions to functional equations (Luce and Galanter, 1963; Lesche, 1971; Falmagne, 1971; Krantz, 1971; Åkesson, 1972). The aim of the present study is not, however, to inquire into the theoretical implications of the basic psychophysical models. They are used here only as descriptive tools to relate instrumental measurements to sensory measurements obtained by magnitude estimation. From a statistical point of view (A)–(C) are readily applicable to data because of the simplicity of the parametric structure.

Since there are many chemical compounds involved that may act jointly in a complex fashion as olfactory stimuli, it is difficult to specify the arguments of the models. The suggested hypotheses must all be conceived not as theoretically well-defined relations, but as tentative approaches to describe simple stimuli interactions.

The hypotheses (2)–(5) in Table 4 are obvious examples of the principle of linear summation, representing the simplest possible type of interaction. Models based on linear summation assumptions have been applied in a number of psychophysical investigations; especially in multidimensional analysis (cf. the principles of interdimensional additivity and intradimensional subtractivity as proposed by Beals et al., 1968). In olfaction, this principle has been proved to be valid in different contexts, (e.g., Berglund et al., 1971). A closely related alternative, represented by hypotheses (6)–(8), is the principle of vectorial summation which has been successfully applied in studies of tactual perception (Franzén, 1969).

The basic rationale behind hypotheses (9)–(14) is compatible with that of Helson's (1947, 1948) general adaption theory and of Restle's (1959) set theoretical similarity model. In particular, models based on geometric mean assumption [in analogy with (9)] have been applied for calculating adaptation levels in visual perception studies (Helson, 1959). The vector "content" model (Ekman, 1963, 1965) is based on a similar rationale. The Ekman vector model has recently been demonstrated to handle certain types of intensity summation in odor mixtures (Berglund et al., 1972).

The applied method of determining regression functions does not yield the sort of contradictory and confusing results that have been demonstrated when comparing forward and backward versions of conventional stepwise regression procedures (Effroymson, 1960), particularly in cases where independent variables are linearly associated (Hamaker, 1962; Draper and Smith, 1966). Moreover, the usual stepwise approach is based on the implicit assumption that there exists a best regression equation, whereas there are often several equally, or almost equally good equations involving different sets of regression variables, a fact demonstrated by the approach used here.

As opposed to conventional polynomial regression procedures, the present approach enables improvement of consistency without simultaneous loss of efficiency. This is because the different hypotheses for the composition of the arguments in the regression equations do not involve any change in the number of parameters. In general, when regression models are to be used for rational decision making, as is the case here, one is primarily interested in determining the least mean square estimates rather than the best unbiased ones. In many cases considerable gains in efficiency can be obtained, when reducing the number of parameters by excluding regression variables. Unfortunately, the problem of optimizing decision making in regression studies is a neglected one. Some theoretical guide lines, however, have recently been presented by Rao (1971).

Results

One of the reasons that many potential causative relations were found is to a large extent depending on the analytical approach on the sensory side, since the partition of the aroma into separate quality components makes it easier to find

Table 8—Optimal correlation coefficients (r) for four peak combinations using the linear model applied to 15 odor qualities[a]

	S = f(X)														
	X_i			$X_i + X_j$				$\sqrt{X_i \cdot X_j}$				X_i/X_j			
Odor quality	i	r^c	χ^{2b}	i	j	r^c	χ^{2b}	i	j	r^c	χ^{2b}	i	j	r^c	χ^{2b}
Meaty (cooked)	22	−0.73	26.5	–	–	–	–	32	S5	−0.78	22.2	22	S8	0.84	22.8
Sickly	22	0.76	34.3	47	13a	0.79	27.5	32	S4	0.73	26.6	63	S4	−0.84	17.6
Musty, mouldy	–	–	–	–	–	–	–	22	S5	0.72	28.9	S12	S2	−0.78	25.4
Cooked cabbage	–	–	–	4	S6	0.81	24.9	–	–	–	–	–	–	–	–
Garlic, onion	S2	0.63	10.7	4	S2	0.72	7.6	4	S2	0.76	6.1	21	18	0.69	8.8
Animal, goat	–	–	–	–	–	–	–	13a	S2	0.51	14.1	S2	S5	−0.63	10.4
Nasty-smelling	–	–	–	6a	S4	0.70	22.1	22	13a	0.75	19.8	17	S11	0.68	27.7
Burnt, smoky	–	–	–	–	–	–	–	4	S10	0.89	29.3	18	S8	0.89	19.8
Sweaty	–	–	–	–	–	–	–	–	–	–	–	63	17	−0.63	28.4
Sour, acid, etc	12	0.56	15.8	–	–	–	–	32	13a	0.58	14.9	63	13a	−0.64	12.1
Like blood, raw meat	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cooked vegetables	17	0.57	24.8	18	S6	0.65	13.3	S6	S10	0.81	7.6	17	22	0.83	4.4
Sweet	12	0.74	22.9	12	32	0.71	26.7	12	32	0.76	23.8	20	32	−0.81	18.3
Retort flavor, canned beef off-flavor	S4	0.70	18.0	4	S2	0.74	15.3	S2	S12	0.80	10.7	18	S8	0.82	10.0
Sulfurous	–	–	–	–	–	–	–	4	S12	0.82	32.1	S4	47	0.84	29.2

[a]The peaks included are considered to contribute directly to the respective odor quality. 12 samples and 6 persons in the panel. The X-indices correspond to the peak numbers in Table 2.

[b]χ^2 = measure of goodness of fit (see text); Critical value for the χ^2-statistic at 5% level: 18.3 and at 1% level: 23.2

[c]r = correlation coefficient.

such relations. Another reason is that a headspace technique was used. This technique is gentle to the material and the sample represents in a more natural way the real situation of the food material than if an extraction or a distillation procedure had been used. In addition, the use of an open tubular column gives a high peak resolution, thus implying that a peak represents a single component rather than a group of compounds.

Also, before carrying out regression studies, the metrical test (Persson et al., 1973) was used to exclude those qualities that could not be quantified by unidimensional scales, because inclusion of such qualities will only lead to artificial and misleading results.

It must be pointed out, that the presented relations are not claimed to be definitively causative ones. However, with the technique used a rough selection of possible compounds acting as stimuli for each odor note has been obtained. By using knowledge about the perceptual properties of the applied compounds, obtained from sniffing analysis and from literature sources, probable causative relations have been suggested (Table 5–9).

A more stringent test of these relations can be obtained by including a larger number of different samples of a similar nature in the investigation. Another more direct way of establishing causative relations is to add the pure compounds in known amounts into the food sample followed by sensory estimation of the odor notes concerned.

In Tables 6, 7 and 8 it can be seen that relations having peaks combined in geometric means (combination 9 in Table 4) or in ratios (10–14) give higher correlation coefficients than e.g. arithmetic sums and differences (2–5). This may depend on the fact that the suggested peak combinations have different capacity of handling existing stimuli interactions. For instance, combination (9) contains implicit weighing factors that seem to be of importance (the stimulus component with the lowest concentration value gets the highest weight). One of the reasons that the ratio combinations (10–14) give satisfactory results may be, except for having implicit weighing factors, that the stimuli components in the denominator are, as a matter of fact, inversely related (negatively correlated) and the components in the numerator are directly related (positively correlated) to the sensory intensity. According to this view, the ratio combinations would directly reflect a sensory mechanism. Generally, however, the reasons that certain combinations are superior to others can probably not be explained by such simple and straightforward arguments. Rather, one must assume that the peak combinations constitute "phenomenological units" that cannot be further decomposed, implying that the optimal combinations function as effective stimuli gradients in analogy with suggested mechanisms of visual perception (Gibson, 1959).

Since the positive outcomes for the combinations 9–14 indicate that weighing factors are of importance, it seems reasonable to explicitly insert such factors in those models that contain addition and subtraction operations. In the present version, the components used have equal weights in these types of combinations. A particular form of multiplicative weighing procedure, previously suggested, is to use the inverse of the detection threshold values (Guadagni, 1968). In our investigation, however, this approach did not yield any significant improvement of goodness of fit. Table 9 demonstrates the influence on the correlation coefficient values (r) by dividing the concentration values with odor detection threshold data. One of the reasons for this shortcoming is that threshold values can hardly be considered as units of intensity measurement in particular for compounds present in amounts much higher than the corresponding threshold value (cf. Luce and Galanter, 1960).

On the basis of the models presented here, a more complex one may be constructed using e.g. exponential, logarithmic and trigonometric transformations of the arguments. A principally different approach is to formulate the models in terms of functional sums, e.g., using power functions according to: $R = C_1 S_1^{n_1} + \ldots + C_k S_k^{n_k}$. When using a stepwise polynomial regression approach to fit this kind of models to the data, misleading ad hoc results may be obtained, which have to be excluded by repeating the analysis on independent data sets (Persson and von Sydow, 1972).

From a practical point of view, it is of course desirable that the number of parameters in the models is as low as possible. This is because the models should be testable on experimental materials of limited size and at the same time be used to make accurate predictions. Models formulated in terms of functional sums are then unsuitable since, unavoidably, they will contain a large number of parameters, causing a loss in precision (Rao, 1971).

Technological applications

As mentioned above, the present approach may be used in quality control work to supplement the service of the panel by determining predictive relations (type ii). For that purpose it is not necessary to determine causative relations

Table 9—Influence on the correlation coefficient values (r) by dividing concentrations with odor threshold data[a]

	Without weighing		With weighing	
S = f(x)	r	χ^2 [b]	r	χ^2 [b]
$X_4 + X_{63}$	0.79	28.8	0.79	29.7
$X_4 + X_{S11}$	0.79	29.5	0.80	29.7
$X_4 + X_{47}$	0.78	30.3	<0.70	–
$X_4 + X_{S6}$	0.81	24.9	0.81	4.9
$X_4 + X_{S8}$	0.76	30.3	<0.70	–
$X_4 - X_9$	0.78	30.1	<0.70	–

[a] Model: R = c · S + d (C): Odor quality: Cooked cabbage: 12 samples and 6 persons in the panel. The X-indices correspond to the peak numbers in Table 2.
[b] χ^2 = measure of goodness of fit (see text); Critical value for the χ^2-statistic at 5% level: 18.3 and at 1% level: 23.2
[c] r = correlation coefficient

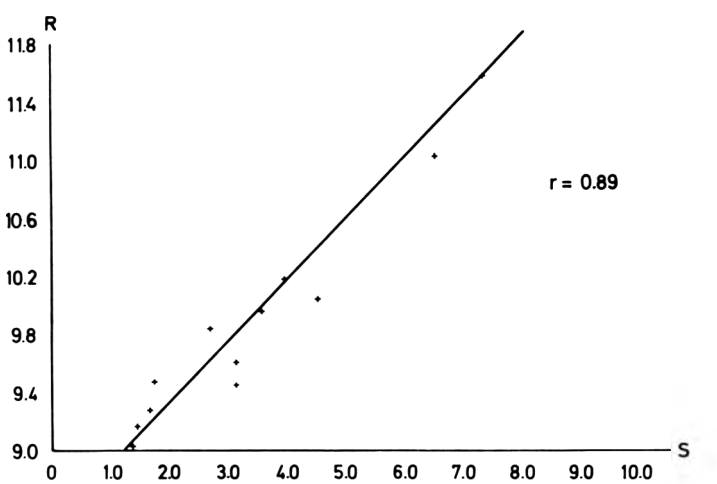

Fig. 1—Regression line for the odor quality "burnt, smoky," using the ratio between 2-methyl butanal and ethylene sulfide as S in the linear model (C).

because nonstimuli compounds may covary with genuine stimuli compounds depending on the fact that they are formed in the same chemical system. This implies, however, that in general it is only possible to predict "inside" the area from which the predictive relations have been derived, because if the chemical system is changed beyond that originally investigated, this co-variation may not exist any longer. The more the chemical system is changed, the higher is the probability that the nonstimuli compounds will loose their predictive power.

In rational product development work, causative relations (type iii) rather than only statistical predictive ones are desirable. When developing a new product the problem is to predict in areas which have not been investigated before. Instead of a trial and error approach, rational guesses can be made by help of causative relations and the operation analysis procedure may therefore be speeded up markedly.

Causative relations can preferably be used in formulation and process optimization. A prerequisite is that it somehow can be determined how the aroma componenets depend on the formulation and the process variables: either by direct gas chromatographic analysis or by using a more theoretical approach (Herrmann, 1970). If causative relations have been established, rational guesses can be made in how to change the formulation and process variables to obtain the desired product. For instance this technique could be especially useful when adding unconventional proteins to conventional foods.

With the present approach we can only obtain approximate relations and one cannot expect accurate predictions in an extremely large area, because the models presuppose a certain chemical environment. Behind the models, however, there are some theoretical considerations: the models are formulated in accordance with often found relations in psychophysical research and it can be supposed that the models, in a simplified way, may reflect certain interactions between the aroma compounds. The main obstacle for a rapid development in this field is the lack of knowledge of the perceptual mechanism in the olfactory sense. At present it seems therefore extremely difficult to establish causative relations in a more rational way on the basis of some well-established psychophysical theory of olfaction.

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EFFECT OF POSTMORTEM CONDITIONS ON CERTAIN CHEMICAL, MORPHOLOGICAL AND ORGANOLEPTIC PROPERTIES OF BOVINE MUSCLE

INTRODUCTION

OUR EARLY WORK (Busch et al., 1967) on excised bovine muscles demonstrated that postmortem aging at 16°C caused a markedly greater improvement in tenderness than aging at 2°C. This improved tenderness was related to the small amount of isometric tension developed by muscle strips (Busch et al., 1967) and to the relaxed appearance of sarcomeres of myofibrils (Stromer and Goll, 1967a,b; Stromer et al., 1967) from muscle stored at 16°C. Others also have shown that sarcomere length is an important factor in tenderness (Gothard et al., 1966; Herring et al., 1965a,b; 1967; Howard and Judge, 1968; Locker, 1960; Takahashi et al., 1967). Other changes possibly related to tenderness at the subcellular level are the loss and disruption of Z-line structure of the myofibril (Fukazawa et al., 1969; Henderson et al., 1970; Stromer et al., 1967) and the shearing or breaking of myofibrils at the Z-line, termed fragmentation (Davey and Gilbert, 1967, 1969; Fukazawa et al., 1969; Sayre, 1970; and Takahashi et al., 1967). Henderson et al. (1970) have further shown that Z-line degradation occurred more rapidly in myofibrils from muscle stored at higher temperature than from muscle stored at lower temperature.

Some of the recent fundamental information about the effect of postmortem conditions on subcellular changes in the myofibril could be applicable to commercial aging of beef carcasses. We (Busch et al., 1967) and others (Sleeth et al., 1957, 1958) had previously shown that aging at higher temperatures improved tenderness. These observations indicate that higher storage temperature accelerates certain reactions in the muscle cell, which affect tenderization of meat. But in another study (Parrish et al., 1969), we found essentially no difference in tenderness between treatments when we aged carcasses at 2°C continuously, and 15°C for short initial periods before moving them to age at 2°C. Consequently, our objectives in this study were to further investigate and relate changes in certain chemical, morphological and organoleptic properties of beef muscle aged in the carcass immediately after slaughter at two different temperatures and sampled at different postmortem times.

MATERIALS & METHODS

BEEF WEIGHING about 454 kg, 14–20 months old and meeting USDA Choice grade standards were used in this study. Immediately after slaughter, samples were removed from longissimus and semitendinosus of a side and then both sides were moved to aging coolers, one side into a 2°C and the other into a 16°C cooler. At 1 day postmortem, samples (longissimus and semitendinosus) were removed from both sides and at analogous anatomical locations; also at this time rib sections and semitendinosus muscles were removed and placed in plastic bags and further aged at 2° and 16°C. Subsequent samples were removed at 3 and 7 days postmortem. All samples were analyzed after collection for ATPase activity, fragmentation and sarcomere length, Warner-Bratzler (WB) shear force and sensory evaluation score. Number of sides sampled is given in the tables of the Results section. It was not possible to sample all sides for all determinations because of the large number of analyses and evaluations carried out at each particular time.

Organoleptic evaluation of rib and semitendinosus steaks were carried out by a 10-member panel. The panel consisted of five male and five female panelists and had a mean age of 26 years. Each panelist was served a 1.27 cm × 2.54 cm section obtained from the same location of a steak at each sitting, in an individual booth illuminated with red light. Panelists drank water before evaluating the next sample to remove sensory characteristics of the previous sample. Tenderness, juiciness and flavor of 2.54 cm thick steaks broiled to an internal temperature of 65°C were evaluated on an 8 point hedonic scale, with 8 being the most desirable. The values of the 10 panelists were averaged, and this average represented the quality attributes of that particular steak. Percentage increase in tenderness was calculated by using at-death organoleptic values as a base and finding the difference in tenderness for subsequent postmortem times.

Steaks for Warner-Bratzler (WB) shear also were broiled to an internal temperature of 65°C, cooled to 25°C and then three cores, each 1.27 cm diam, were removed from the medial, central and lateral positions of rib steaks and from three areas of equidistance of semitendinosus steaks. Each core was sheared across grain twice and the average of the six shear values represented the WB shear value for a particular steak. WB shear values are reported as kg shear force/cm^2.

Percentage cooking loss was computed for each steak by obtaining weights before and after cooking.

Isolation of myofibrils from longissimus and semitendinosus muscle was carried out by a modified procedure of Goll and Robson (1967). Muscle was minced and suspended in 10 vol of 0.25M sucrose, 1 mM EDTA, 0.05M Tris, pH 7.6 and centrifuged at 1000 × G for 10 min. After centrifuging, the supernatant was decanted, resuspended in 5 vol of 0.25M sucrose, 1 mM EDTA, 0.05M Tris, pH 7.6 and centrifuged at 1000 × G for 10 min. The supernatant was decanted again, and the sediment was resuspended in 0.05M Tris, pH 7.6, 1 mM EDTA and passed through a wire mesh strainer to remove connective tissue. The suspension was centrifuged at 1000 × G for 10 min and the supernatant again decanted. Sedimented myofibrils were resuspended in 0.15M KCl twice more and centrifuged each time at 1000 × G for 10 min. After the last centrifugation, myofibrils were resuspended in 0.15M KCl, and protein concentration was determined by the biuret procedure (Gornall et al., 1949) modified by Robson et al. (1968).

Sarcomere lengths of 25–50 myofibrils per sample were measured with a Vicker's image splitter attached to a Zeiss Photomicroscope.

A drop of myofibrillar suspension in 0.15M KCl was placed on a glass slide and used for microscopy examination. Phase microscopy observations were made with a Zeiss Photomicroscope by using a green interference filter with a 100X planachromatic objective in the optical path and recorded on Adox KB14 film.

ATPase assays were carried out according to Goll and Robson (1967). Conditions of the ATPase assay are detailed in Table 5 of the Results section.

RESULTS

POSTMORTEM AGING of carcasses immediately post slaughter at 16°C for 1 day was effective in improving palatability, especially tenderness of steaks from longissimus. Rib steaks from longissimus aged for 1 day postmortem at 16°C were about as tender as rib steaks from longissimus aged for 7 days at 2°C (37 vs. 41% increase in tenderness) (Table 1). Although the panel recognized improved tenderness of steaks from sides aged for 1 day at 16°C, they did not detect an appreciable change in tenderness of steaks from longissimus until 3 days of post-

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Table 1—Effect of postmortem aging temperature and time on sensory properties of longissimus[a]

Postmortem time (days)	Flavor		Juiciness		Tenderness		Percent increase (tenderness)	
	2°C	16°C	2°C	16°C	2°C	16°C	2°C	16°C
0	4.9 ± 0.2(5)	4.9 ± 0.2(5)	6.3 ± 0.1(5)	6.3 ± 0.1(5)	4.6 ± 0.3(5)	4.6 ± 0.3(5)	–	–
1	5.9 ± 0.2(14)	6.4 ± 0.2(6)	6.9 ± 0 .1(14)	6.5 ± 0 .3(6)	4.9 ± 0.3(14)	6.3 ± 0.1(6)	6	37
3	6.1 ± 0.2(9)	6.2 ± 0.1(6)	6.6 ± 0.3(9)	6.4 ± 0.2(6)	5.7 ± 0.4(9)	7.0 ± 0.1(6)	24	52
7	6.2 ± 0.1(12)	–	6.7 ± 0.1(12)	–	6.5 ± 0.2(12)	–	41	–

[a]Figures are mean values, ± standard errors of mean, based on an 8-point hedonic scale with 8 being the most desirable. Number in parentheses is the number of sides represented.

Table 2—Effect of postmortem aging temperature and time on sensory properties of semitendinosus[a]

Postmortem time (days)	Flavor		Juiciness		Tenderness		Percent increase (tenderness)	
	2°C	16°C	2°C	16°C	2°C	16°C	2°C	16°C
0	4.9 ± 0.1(5)	4.9 ± 0.1(5)	5.8 ± 0.1(5)	5.8 ± 0.1(5)	2.8 ± 0.3(5)	2.8 ± 0.3(5)	–	–
1	5.5 ± 0.1(9)	5.2 ± 0.1(6)	5.5 ± 0.3(9)	5.2 ± 0.4(6)	3.7 ± 0.2(9)	4.2 ± 0.3(6)	32	50
3	5.8 ± 0.1(9)	5.5 ± 0.2(6)	5.8 ± 0.3(9)	5.1 ± 0.4(6)	5.1 ± 0.1(9)	5.5 ± 0.3(6)	82	96
7	5.9 ± 0.1(9)	–	6.2 ± 0.3(9)	–	6.0 ± 0.3(9)	–	115	–

[a]Figures are mean values, ± standard errors of mean, based on an 8-point hedonic scale with 8 being the most desirable. Number in parentheses is the number of sides represented.

mortem aging at 2°C. Flavor of rib steaks from sides postmortem aged at 16°C was also more desirable.

Tenderness of semitendinosus was improved by aging at 16°C (Table 2), but the effect of temperature treatment on tenderness differences was not as pronounced for semitendinosus as it was for longissimus. Furthermore, aging of semitendinosus improved flavor at 1 day, but aging time or temperature of storage had little effect on flavor thereafter, and slightly decreased juiciness at both 16° and 2°C storage.

WB shear values (Table 3) decreased significantly for both postmortem aging treatments at 1 day, but thereafter little difference was noted between the two treatments with the steaks from the 16°C aging treatment having the lower values.

No difference was observed in percentage cooking loss for longissimus steaks, but about an 8% loss occurred in semitendinosus between 0 and 1 day postmortem (Table 4). Time and temperature of postmortem aging, therefore, had little affect on cooking loss.

ATPase activity of myofibrils isolated from at-death, 2° and 16°C aged longissimus and semitendinosus showed that Ca^{++}-, Mg^{++}- and EGTA-modified ATPase activity increased with postmortem storage (Table 5) and that myofibrils from muscle aged at 16°C had greater, but in general not significantly greater, ATPase activity values than did myofibrils from muscle aged at 2°C. A twofold increase occurred in both Ca^{++}- and Mg^{++}-modified myofibrillar ATPase during postmortem aging of longissimus at 2° and 16°C. EGTA-modified ATPase activity remained essentially unchanged in myofibrils from muscle aged at 2°C and increased at 1 day, decreased somewhat at 3 days and again increased at 7 days postmortem in myofibrils from longissimus aged at 16°C.

The effects of postmortem aging conditions and cookery on the dimensions (Table 6) and morphology (Fig. 1–4) of myofibrils from bovine muscle were also investigated. At-death myofibrils from both longissimus and semitendinosus were prepared from excised, unrestrained samples and isolated and homogenized in cold isolating media. Both of these steps in preparing myofibrils for microscopy are likely explanations for the shortness of sarcomeres from at-death muscle of 1.86μ (Table 6). Sarcomeres of myofibrils from 1-, 3- and 7-day aged longissimus were longer than those from at-death muscle, but their individual mean values were about the same. Sarcomeres of myofibrils from aged semitendinosus, however, increased in length during each postmortem aging interval. Broiling of rib steaks, in addition to aging, affected change in sarcomere lengths. Shortening and distortion of the typical banding pattern of myofibrils from cooked at-death longissimus prevented their measurement. Sarcomere lengths of myofibrils from aged longissimus steaks broiled to 65°C internally, however, were measurable and they were shorter than sarcomeres of myofibrils from uncooked longissimus.

Figure 1 is a phase micrograph showing the appearance of myofibrils from at-death and from longissimus stored at 2°C for 1, 3 and 7 days postmortem. Myofibrils from at-death (1a) and 1-day 2°C (1b) stored muscle contained numerous sarcomeres; however, myofibrils isolated from 3 day 2°C (1c) stored longissimus showed

Table 3—Effect of postmortem aging temperature and time on Warner Bratzler Shear (kg/cm^2) of longissimus and semitendinosus muscle[a]

Postmortem time (days)	Longissimus		Semitendinosus	
	2°C	16°C	2°C	16°C
0	4.80 ± 0.26(14)	4.80 ± 0.26(14)	6.82 ± 0.52(14)	6.82 ± 0.26(14)
1	3.77 ± 0.17(27)	3.41 ± 0.19(23)	4.72 ± 0.28(11)	4.36 ± 0.23(8)
3	3.40 ± 0.16(19)	2.97 ± 0.19(16)	3.87 ± 0.22(11)	3.66 ± 0.34(8)
7	2.97 ± 0.15(27)	2.60 ± 0.15(15)	3.34 ± 0.17(11)	3.26 ± 0.22(7)

[a]Figures are mean values (kg/cm^2), plus or minus standard error. Number in parentheses is the number of sides represented.

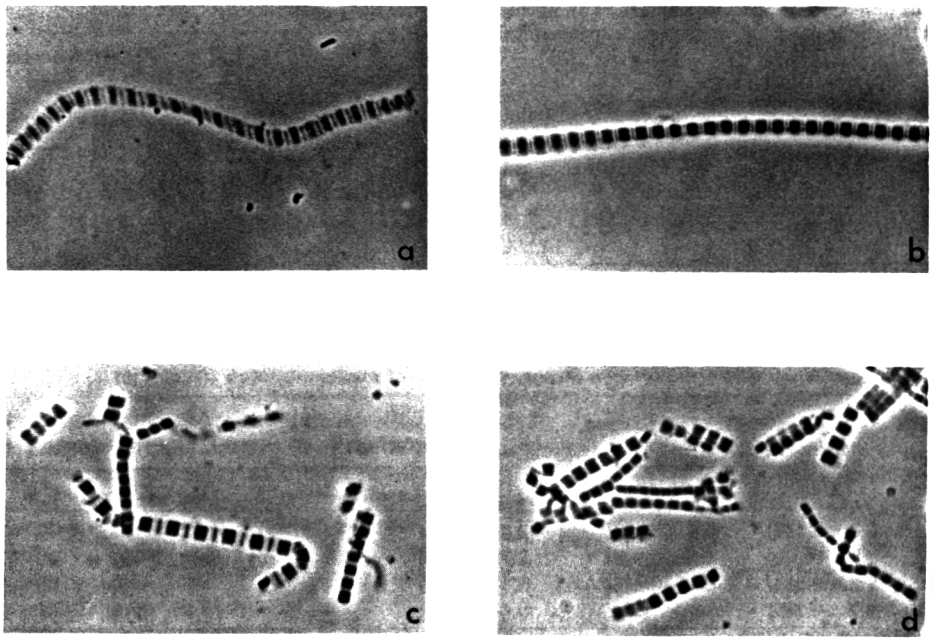

Fig. 1—Effect of postmortem aging at 2° C on the structure of myofibrils from bovine longissimus: (a) at death; (b) 1 day postmortem; (c) 3 days postmortem; (d) 7 days postmortem. (1400 x).

fragmentation (fewer sarcomeres per myofibril) and partial loss of Z-lines. Conversely, myofibrils from longissimus stored for 1 day at 16°C showed some fragmentation (2a) and further storage of longissimus aged for 3 (2b) and 7 days (2c) at 16°C resulted in greater fragmentation (Fig. 2).

Figures 3 and 4 are phase micrographs of myofibrils from rib steaks broiled to 65°C from muscle aged 1, 3 and 7 days postmortem at 2°C and 16°C storage, respectively. Myofibrils from cooked longissimus appeared morphologically similar to uncooked myofibrils, but they had somewhat shorter sarcomeres than myofibrils from postmortem aged muscle.

Figure 5 shows the effect of postmortem aging conditions on the interrelationships between tenderness measurements and sarcomere length of longissimus. Organoleptic tenderness of longissimus at 2°C did not change much between at-death and at 1 day, although sarcomere length increased extensively. Improvement did occur in tenderness between 1 and 3 days postmortem, but very little

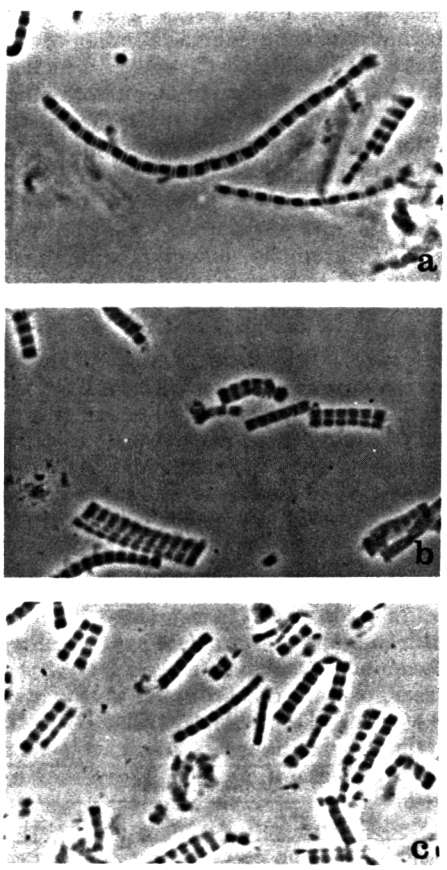

Fig. 2—Effect of postmortem aging at 16° C on the structure of myofibrils from bovine longissimus: (a) 1 day postmortem; (b) 3 days postmortem; (c) 7 days postmortem. (≈ 1350×)

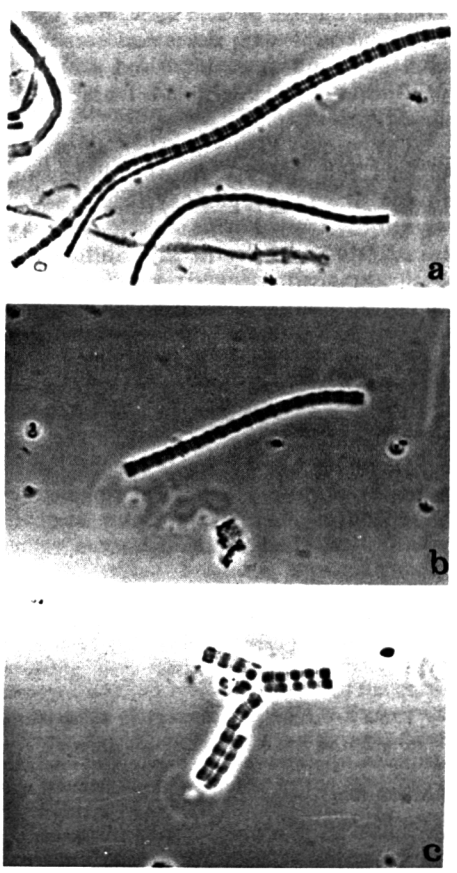

Fig. 3—Effect of broiling to an internal temperature of 65° C on the structure of myofibrils from bovine longissimus aged at 2° C for 1, 3 and 7 days postmortem: (a) 1 day; (b) 3 days; (c) 7 days. (≈ 1350×)

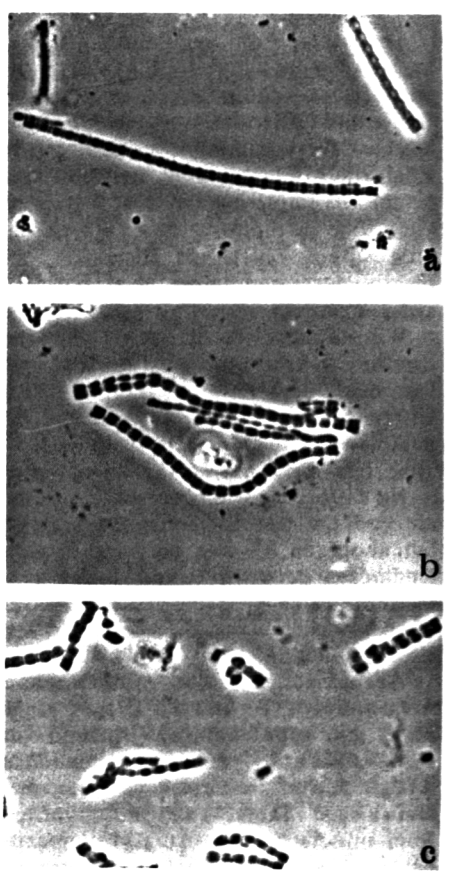

Fig. 4—Effect of broiling to an internal temperature of 65° C on the structure of myofibrils from bovine longissimus aged at 16° C for 1, 3 and 7 days postmortem: (a) 1 day; (b) 3 days; (c) 7 days. (≈ 1350×)

change occurred in sarcomere length. The paradox of improved tenderness and no change in sarcomere length may partially be explained by the increased fragmentation of myofibrils from longissimus aged at 2°C which we observed at 3 days postmortem. Tenderness of longissimus aged at 16°C increased significantly at 1 day, and this increase may be related to both increase in sarcomere length and fragmentation, although sarcomeres of myofibrils from cooked longissimus were no longer than were sarcomeres of myofibrils from uncooked, at-death longissimus. It appears, however, that sarcomere length and WB shear values are inversely related and that the amount of shear force may be a function of sarcomere length. Sensory tenderness response, on the other hand, probably reflects a more complex phenomenon, i.e., it may also reflect and be interrelated to flavor evaluation.

DISCUSSION

RESULTS of our study show that tenderness of bovine longissimus can be markedly improved by aging carcasses at 16°C for 1 day. Aging muscle in the carcass and at a higher aging temperature seems important in accelerating tenderness from four aspects: (1) muscle is attached to bone, which minimizes shortening while it is undergoing rigor mortis; (2) minimal muscle shortening is effected, which should eliminate any adverse effect of shortening on tenderness; (3) greater fragmentation of myofibrils occurs at or near the Z-line, which seems to result in improved tenderness; and (4) greater loss of calcium sensitivity occurs, which indicates some kind of a postmortem change in the actin-myosin interaction.

Our main purpose of this study was to take practical advantage of both postmortem storage temperature and bovine muscle attachment. Muscle removed from a carcass immediately after exsanguination shortens and when it is stored at 2°C it shortens further and toughens extensively; however, when it is stored at 16°C it does not shorten appreciably and gradually increased in tenderness with postmortem aging (Busch et al., 1967). Leaving the semitendinosus intact in the bovine carcass while it is undergoing rigor mortis, and while it also has the potential to shorten minimizes the detrimental effect of shortening on tenderness (Goll et al., 1964), although McCrae Et al. (1971) demonstrated that several lamb muscles were extremely tough because of the cold shortening effect if the carcass was frozen with minimal delay postmortem even though the muscles remain attached to the skeleton during the chilling and freezing operation. Leaving bovine muscle intact allows a slow rate of decline in postmortem carcass temperature even at 2°C; this probably brings about more rapid degradation of energy supplies before muscle reaches a temperature conducive to cold shortening, and this promotes more chemical reactivity on the protein constituents of the myofibril to hasten fragmentation and tenderization of the myofibril. Because of the insulating effect of muscle mass and subcutaneous fat cover, cold shortening of beef muscle aged in the carcass, even in 2°C environments, is an unlikely deterrent to tenderness. This especially seems to apply to the semitendinosus where we found little difference in tenderness due to aging temperature treatment. Cold shortening and associated toughening in lamb muscle, however, is a problem (Marsh et al., 1968). Studies on rate of bovine muscle cooling needs to be systematically carried out to determine its effect on and relationship to physical and chemical changes associated with palatability characteristics.

Fragmentation, the breaking of myofibrils into a series of myofibrils with smaller number of sarcomeres, because of either removal or disintegration of Z lines, seems to have a beneficial effect on tenderness. Fragmentation was observed at 16°C for 1 day and an improvement in sensory tenderness was also observed at that time. Increased fragmentation of myofibrils from longissimus aged at 2°C was not observed until 3 days of postmortem aging. Concomitantly the panel recognized an improvement in tenderness of steaks from longissimus without change in sarcomere length between 1 and 3 days postmortem aging. Myofibrillar structure that has undergone fragmentation probably reduces the force necessary to bite or chew meat by an organoleptic panel; consequently, meat is more tender. Improved tenderness, therefore, seems more a function of fragmentation of myofibrils than a change in sarcomere length of longissimus aged in the carcass because tenderness improved with essentially no change occurring in sarcomere length of myofibrils from longissimus after 1 day of postmortem aging at both 2° and 16°C. Increased sarcomere length and fragmentation may, however, both be important in the tenderization of

Table 4—Effect of postmortem aging temperature and time on percent cooking loss of longissimus and semitendinosus muscle[a]

Time/temp of postmortem storage	Longissimus	Semitendinosus
0 day	17.7 ± 0.9(14)	17.6 ± 1.0(14)
1 day, 2°C	16.0 ± 0.7(26)	25.4 ± 1.0(15)
1 day, 16°C	18.9 ± 1.4(18)	25.3 ± 1.1(12)
3 day, 2°C	16.6 ± 0.7(22)	24.2 ± 1.1(15)
3 day, 16°C	16.0 ± 0.7(19)	25.2 ± 0.7(12)
7 day, 2°C	18.1 ± 1.1(25)	27.0 ± 1.4(15)
7 day, 16°C	20.2 ± 2.1(14)	26.2 ± 1.1(7)

[a]Percent of uncooked weight, plus or minus standard error of mean. Number in parentheses is the number of sides represented.

Table 5—Effect of postmortem aging temperature and time on myofibril ATPase activity of longissimus and semitendinosus muscle[a,b]

Time/temp of postmortem storage	1 mM Ca^{++}	1 mM Mg^{++} and 0.5 mM Ca^{++}	0.1 mM EGTA and 1 mM Mg^{++}	0.1 mM EGTA and 5 mM Mg^{++}
		Longissimus		
0 day	0.074 ± 0.007(5)	0.059 ± 0.005(5)	0.020 ± 0.003(5)	0.029 ± 0.003(4)
1 day, 2°C	0.117 ± 0.008(17)	0.080 ± 0.004(17)	0.023 ± 0.002(16)	0.033 ± 0.001(4)
1 day, 16°C	0.136 ± 0.008(6)	0.093 ± 0.006(6)	0.028 ± 0.002(6)	
3 day, 2°C	0.133 ± 0.010(15)	0.089 ± 0.006(14)	0.017 ± 0.002(13)	0.028 ± 0.003(5)
3 day, 16°C	0.140 ± 0.007(6)	0.104 ± 0.004(6)	0.022 ± 0.003(6)	
7 day, 2°C	0.159 ± 0.004(17)	0.110 ± 0.002(17)	0.019 ± 0.002(16)	0.034 ± 0.002(5)
7 day, 16°C	0.146 ± 0.003(6)	0.111 ± 0.003(6)	0.031 ± 0.003(6)	
		Semitendinosus		
0 day	0.071 ± 0.008(5)	0.048 ± 0.005(5)	0.018 ± 0.002(5)	0.026 ± 0.002(4)
1 day, 2°C	0.119 ± 0.005(5)	0.086 ± 0.004(5)	0.021 ± 0.003(5)	0.034 ± 0.003(4)
3 day, 2°C	0.122 ± 0.011(5)	0.085 ± 0.007(5)	0.021 ± 0.003(5)	0.031 ± 0.002(5)
7 day, 2°C	0.124 ± 0.006(5)	0.088 ± 0.005(5)	0.022 ± 0.002(5)	0.032 ± 0.003(5)

[a]Conditions of assay: 0.50–0.75 mg myofibril protein/ml, 1 mM ATP, 26°, 50 mM KCl, 50 mM Tris-Ac, pH 7.0 with the exception that 5 mM Mg^{++} contains 5 mM ATP.

[b]Figures are μ moles Pi/min/mg protein expressed as mean values plus or minus standard error. Number in parentheses is the number of sides represented.

semitendinosus, although increase in tenderness does not seem to parallel increase in sarcomere length.

Several researchers have observed Z-line degradation of postmortem aged skeletal muscle (Davey and Dickson, 1970; Davey and Gilbert, 1969; Fukazawa and Yasui, 1967; and Henderson et al., 1970) and Z-line degradation may be the underlying basis for fragmentation of postmortem aged muscle (Davey and Gilbert, 1969; Fukazawa et al., 1969; Goll et al., 1970; 1971b; Sayre, 1970; Takahashi et al., 1967). Sayre (1970) found a general pattern of fragmentation corresponding to tenderness; however, he did not find fragmentation to be an accurate index of tenderness of chicken pectoralis major.

Another possible contributing factor to tenderization is the increase that occurs in myofibrillar ATPase activity, indicative of a change in the actin-myosin interaction or degradation of certain protein constituents of the myofibril. Arakawa et al. (1970), however, did not find degradation of troponin-tropomyosin (TP-TM) complex from postmortem aged rabbit muscle. Goll et al. (1971b) have, however, demonstrated that trypsin treatment and postmortem aging affects subcellular components of muscle similarly. Also, trypsin affects the actin-myosin interaction (Goll et al., 1971a) and removes Z-lines (Stromer et al., 1967). The demonstration of subcellular changes by trypsin indicates that proteolysis in the muscle cell may be responsible for changes in the actin-myosin interaction and loss of Z-line structure and fragmentation in the myofibril. Busch et al. (1972) have recently shown the presence of calcium activated proteolytic enzyme isolated from skeletal muscle capable of removing Z-lines from myofibrils.

Another aspect contributing to tenderness that requires further investigation is cookery. In this study heat induced shortening of at-death longissimus resulted in reduced tenderness and distorted myofibril banding patterns. Myofibrils isolated from cooked-aged longissimus, however, were morphologically similar to those from uncooked longissimus, although those from cooked longissimus had shorter sarcomere lengths. These results confirm earlier observations reported by Schmidt and Parrish (1971). Shortening of sarcomeres from aged longissimus by broiling at 65°C probably occurs because of heat dehydration of myofibrillar proteins. Consequently the beneficial tenderness changes brought about by cookery may occur predominantly in the change of connective tissue protein, collagen.

Aging carcasses at 16°C offers a very practical method of accomplishing rapid improvement in tenderization without modification of carcass handling and use of costly refrigeration. Our earlier work (Rey et al., 1970) has shown that there was little microbiological difference between carcasses aged at 16°–22°C and those at 2°C. We are continuing our studies of carcass aging in an effort to determine the most practical means of handling carcasses for optimum quality.

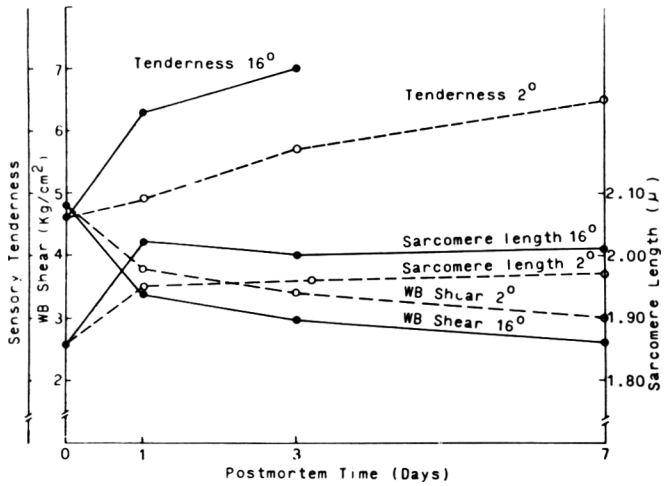

Fig. 5—Interrelationships of sensory tenderness, W-B shear and sarcomere length of bovine longissimus postmortem aged at 2° and 16°C.

Table 6—Effect of postmortem aging time and temperature and heating on sarcomere lengths (microns) of myofibrils from bovine longissimus and semitendinosus muscle[a]

Time/temp of postmortem storage (°C)	Longissimus	Semitendinosus
0 day	1.81 ± 0.08[a](3)[b]	1.86 ± 0.06(5)
1 day, 2°C	1.95 ± 0.03(5)	2.17 ± 0.02(6)
1 day, 16°C	2.02 ± 0.04(3)	2.24 ± 0.03(3)
1 day, 2°–65°C	1.86 ± 0.02(6)	
1 day, 16°–65°C	1.83 ± 0.02(6)	
3 day, 2°C	1.96 ± 0.03(7)	2.34 ± 0.02(8)
3 day, 16°C	2.00 ± 0.03(6)	2.30 ± 0.02(5)
3 day, 2°–65°C	1.86 ± 0.02(6)	
3 day, 16°–65°C	1.97 ± 0.02(6)	
7 day, 2°C	1.97 ± 0.02(9)	2.47 ± 0.02(9)
7 day, 16°C	2.01 ± 0.03(5)	2.46 ± 0.02(5)
7 day, 2°–65°C	1.92 ± 0.03(6)	
7 day, 16°–65°C	1.85 ± 0.01(6)	

[a]Figures are mean values, plus or minus standard error. Number in parentheses is the number of sides represented.

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EFFECT OF POSTMORTEM AGING ON CHICKEN MUSCLE LIPIDS

INTRODUCTION

THE SUBTLETY and complexity of the changes which occur in muscle during postmortem aging accentuate the need for the complete analysis of muscle lipids and a thorough description of the changes in these compounds concomitant with aging. Others (Fishwick, 1968; Davidkova and Khan, 1967) have suggested that the significance of postmortem changes in avian muscle lipids might reside in alterations in lipoprotein membrane structures and/or possible interaction of free fatty acids with the protein components in muscle. While loss of integrity in membrane structures as a result of lipolysis in muscle is still largely hypothetical, both Fishwick (1968) and Davidkova and Khan (1967) produce strong evidence that the majority of free fatty acids found in frozen avian muscle, stored for several months, originate from membrane phospholipids. Regarding the interaction between free fatty acids and muscle proteins, Anderson and Ravesi (1968) have demonstrated a relationship between decreases in protein extractability and production of free fatty acids in aged cod muscle, while studies by Ikeda and Taguchi (1968) and Taguchi and Ikeda (1968a, b) indicate that lecithin markedly affects the properties of fish muscle actomyosin. No comparable investigations have been conducted with avian muscle proteins.

Our previous studies (Hay et al., 1972; 1973a, b) have been directed toward the effects of postmortem aging on the biochemical and morphological properties of chicken muscle, with emphasis placed on the protein components. The fact that lipids may be integral to the myofibrillar structure and that cell organelles known to markedly influence postmortem changes in muscle contain a large proportion of lipid constituents heightened our interest in the significance of lipid alterations during the initial period of aging, when most of the critical changes in the myofibrils are known to occur.

This paper presents the results of detailed analysis of both neutral and phospholipid fractions from chicken breast muscle. The primary objective was to determine if changes in the phospholipids had occurred simultaneously with the events of rigor mortis and resolution of rigor, during the first 48 hr of postmortem cold storage.

The following abbreviations are used:

DG – diglyceride
DPG – diphosphatidyl glycerol
FAME – fatty acid methyl ester
FFA – free fatty acid
LPC – lysophosphatidyl choline
LPE – lysophosphatidyl ethanolamine
MG – monoglyceride
PC – phosphatidyl choline
PE – phosphatidyl ethanolamine
PI – phosphatidyl inositol
PS – phosphatidyl serine
Sph – sphingomyelin
St – sterol
StE – sterol ester
TG – triglyceride

MATERIALS & METHODS

Materials

Egg PC, egg LPC, soybean PE, beef LPE, beef PS, soybean PI, monopalmitin and dipalmitin were obtained from the Hormel Institute, Austin, Minn. A mixture of StE, St, TG, FFA, FAME and Sph were obtained from Sigma Chemical Co., New York. The compounds were free of impurities as judged by thin-layer chromatography (TLC). All solvents were redistilled prior to use. All other chemicals were of the highest available purity.

Samples

Since the relative amounts of individual lipids have been shown to be influenced by age (Marion and Miller, 1968; Wangen et al., 1971) it was decided to use 10-wk old broilers throughout this study. The broilers, strain Hubbard, reared at the University of Alberta Animal Center were fed a commercial broiler starter feed for 6 wk and a commercial broiler finisher for the remainder of the time.

Lipid Extraction

Approximately 25g breast muscle (pectoralis superficialis) was removed from chickens at 0 and 48 hr postmortem. The stored carcasses were plucked, eviscerated and washed thoroughly in cold water and microbial growth was prevented by wrapping the carcasses in paper towels which had been soaked in 10 mM sodium azide. The muscle was trimmed free of all visible fat and homogenized in a Waring Blendor for 3 min with 500 ml chloroform:methanol (2:1, by vol). The homogenate was allowed to stand for 30 min and then filtered to remove precipitated protein. The extract was equilibrated with 0.2 vol of 0.74% KCl in deionized water and allowed to stand overnight to permit phase separation. The upper phase was aspirated and washed three times with chloroform:methanol:0.37% KCl aqueous (3:48:47, by vol) (Folch et al., 1957). The total lipid was taken to dryness by rotary evaporation and redissolved in 10 ml chloroform:methanol, (2:1, by vol). To determine the total weight of lipid, three 50 μl aliquots were removed, dried and then weighed using a Cahn-Gram Electrobalance.

Chromatographic lipid fractionation

To separate the neutral lipids from the phospholipids the total lipids were taken to dryness and applied to a silicic acid column (1.5g silicic acid, 0.5g celite). The neutral lipids were eluted with 60 ml diethyl ether and the phospholipids with 50 ml chloroform:methanol (1:1, by vol) and then 40 ml methanol.

The neutral lipids were concentrated by distillation, weighed and separated into their individual components using the double development solvent system devised by Freeman and West (1966). The lipids (150 μg) were applied as a double spot to a chromatoplate with a 0.3 mm layer of MN-silica gel G (Macherey, Nagel & Co.). The plates were developed in the first direction with diethyl ether:benzene:ethanol: acetic acid (40:50:2:0.2, by vol), dried and developed in the second direction with diethyl ether:hexane (6:94, by vol). The plates were vacuum dried for 30 min and charred with 50% H_2SO_4. The neutral lipid components were identified by comparison with authentic standards. The individual lipids were separated by preparative TLC and located by spraying with 2',7'-dichlorfluorescein and viewing under ultra-violet light. The separated bands were scraped off and eluted with chloroform:methanol (4:1, by vol). Cholesterol and cholesterol esters were quantitated by the colorimetric method of Searcy and Bergquist (1960), and TG and FFA by gas-liquid chromatography using methyl heptadecanoate as an internal standard (Morrison and Smith, 1964).

The total phospholipids were concentrated by rotary evaporation, weighed and separated into their individual components by two-dimensional TLC according to the procedure described by Rouser et al., (1970). The phospholipids (600 μg) were applied as a single spot to chromatoplates made with a 0.4 mm layer of MN-silica gel N prepared as a slurry of 20g in 60 ml water containing 1.5g magnesium acetate. The plates were developed in the first dimension with chloroform:methanol:28% aqueous ammonia (65:25:5, by vol), vacuum dried for 60 min and then developed in the second dimension with chloroform:acetone:methanol: acetic acid:water (3:4:1:1:0.5, by vol). The plates were dried in a vacuum oven for 30 min. The phospholipids were detected on TLC plates by charring with 50% sulphuric acid. Lipids containing NH_2 were detected with ninhydrin

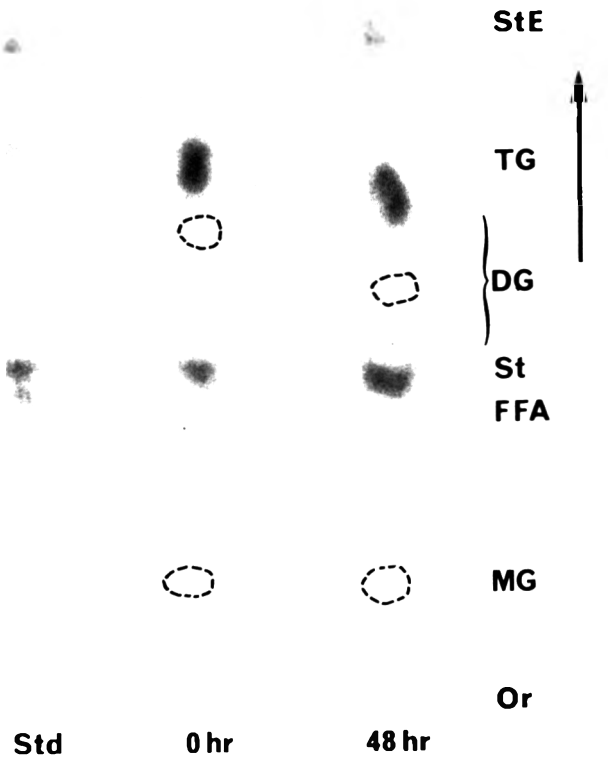

Fig. 1—Double development TLC of total neutral lipids isolated from chicken breast muscle aged 0 and 48 hr. The chromatoplates were developed in solvent system 1, diethyl ether:benzene:ethanol:acetic acid (40:50:2:0.2, by vol) followed by second development in solvent system 2, diethyl ether:hexane (6:94, by vol). The standard consists of a mixture of cholesterol ester, FAME, TG, cholesterol and FFA. (Load: approx. 150 μg; Detection: by charring after spraying with 50% H_2SO_4.)

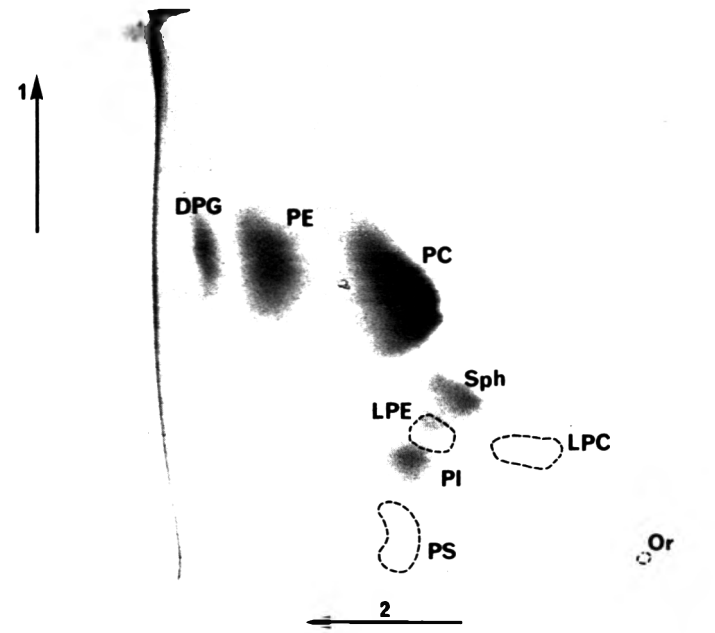

Fig. 2—Two dimensional TLC of phospholipids isolated from whole chicken breast muscle aged 0 hr. The chromatoplates were developed in the first direction (arrow 1) with chloroform:methanol:28% aqueous ammonia (65:25:5, by vol) and in the second direction (arrow 2) with chloroform:acetone:methanol:acetic acid:water (3:4:1:1:0.5, by vol). (Load: approx. 600 μg; Detection: by charring after spraying with 50% H_2SO_4.)

(Skidmore and Entenman, 1962) and phosphates with a spray reagent developed by Vaskovsky and Kostetsky (1968). Identification of the phospholipids was confirmed by comparison with authentic standards. Preparative TLC was also performed and the individual phospholipids were detected by spraying with rhodamine 6G and viewing under ultraviolet light. The individual lipids were scraped from the plates and eluted with 5 ml chloroform:methanol (1:1, by vol) and 5 ml methanol. Phosphorus was determined by the method of Morrison (1964).

FAME were prepared from TG, PC, PE, Sph, PI, DPG and PS using the BF_3-methanol procedure described by Morrison and Smith (1964). Gas-liquid chromatography of the methyl esters was conducted on a Varian 600C gas chromatograph equipped with flame ionization detector and 6 ft × 1/8 in od stainless steel columns packed with 15% EGSS – X on 100–120 mesh Gas Chrom P (Applied Science Labs.). The column temperature was 180°C and the carrier gas helium was used at a flow rate of 25 ml/min. Relative peak areas were measured as retention distance × peak height. GLC correction factors were calculated using different standard methyl ester mixtures approximately the same as those of lipids being analysed (Hormel Institute). Correction factors for minor components were obtained by interpolation. Reference retention data for methyl esters were obtained from known mixtures of methyl esters and from published data (Ackman, 1969).

RESULTS & DISCUSSION

THE 0 HR BREAST MUSCLE contained approximately 1% lipid of which the phospholipid content was estimated to be 54%. Separation of the individual neutral lipids is shown on Figure 1. The neutral lipid fraction consisted mainly of TG with small amounts of St and StE and trace amounts of FFA, mono- and diglyceride (Table 1). The individual phospholipids were satisfactorily separated using the solvent system described by Rouser et al. (1970) and the phospholipids, PC, PE, PI, PS, DPG, Sph, LPE and LPC were identified (Fig. 2). Except for the differences in the relative proportions of PS and PI, the quantitative analysis of the individual phospholipids (Table 2) is in good agreement with previously reported values (Davidkova and Khan, 1967; Peng and Dugan, 1965; Marion and Miller, 1968).

The FA composition of the individual lipids is shown in Table 3. When the FA composition of the individual lipids are compared to previously reported values, minor variations are evident (Marion and Miller, 1968; Marion and Woodroof, 1963; Peng and Dugan, 1965). These differences probably reflect variations in the dietary intake, since Marion and Woodroof (1963) and Marion (1965) for example, have observed that the FA pattern of chicken breast muscle tends to assume the FA pattern of dietary fat. Each lipid contains a unique and characteristic FA pattern. The predominant FA in PC are 16:0, 18:1 and 18:2, in PI and PE are 18:0 and 20:4, in PS are 16:0, 18:0 and 18:1, in DPG are 18:1 and 18:2, in Sph are 16:0, 18:0 and long chain saturated fatty acids. In TG the predominant FA are 16:0, 18:0, 18:1 and 18:2. The selective incorporation of specific fatty acids into phospholipids has been observed in

other tissues (Gray, 1960; Nelson, 1962).

The chicken breast muscle aged 48 hr (see Table 1 and 2) shows an increase in the relative amounts of LPC, LPE and FFA with a corresponding reduction in PC and to a lesser extent PE and TG. The increase in LPC and LPE coupled with the reduction of PC and PE would appear to have resulted from phospholipase A activity. This is supported by the presence of long chain polyunsaturated FA in the FFA fraction extracted from muscle aged 48 hr (Table 3). Long chain polyunsaturated FA were not detected in TG from 0 hr muscle. Nevertheless the reduction in TG observed after 48 hr indicates that some lipase action may also have occurred. Thus the increase in FFA is probably the result of a combination of phospholipase and lipase activity. Since the reduction in PC exceeds the increase in LPC it is conceivable that the LPC has been further hydrolysed at both the 1 and 2 positions leaving glycerophosphatidyl choline, a water soluble substance which would not be detected in this analysis, probably at a slower rate than PC is converted to LPC. Lipolytic activity of this type has already been reported on long term cold storage studies of fish muscle (Olley and Lovern, 1960; Lovern and Olley, 1962) and bovine muscle (Awad et al., 1968). No evidence of lipolysis of PI, PS, Sph or DPG were found in this study.

The lipolysis observed in this study, though not as pronounced as in the long term cold storage studies, decidedly demonstrates a rapid occurrence coinciding with the time that tenderness is developing in muscle. The role that lipolysis may play in the course of events leading to tenderness in meat is worthy of speculation. In the first instance, the lipolytic enzymes could exert a direct effect on the myofibrils. There is evidence to suggest that phospholipids may form an integral part of the contractile machinery (Briggs, 1963; Fujino et al., 1961; Taguchi and Ikeda, 1968a, b; Harsanyi and Garamvolgi, 1969). We have observed several compositional differences in the phospholipids extracted from chicken breast myofibrils compared to the results shown in the present study; however, these results are complicated by the possible activation or release of lipases and phospholipases from other muscle sites during preparation of the myofibrils, and are not included for that reason. But, if lipids do in fact constitute an integral part of the myofilament assembly, then lipolysis could have an important part to play in the events leading to the development of tenderness in meat. Currently, studies are being directed toward resolution of this problem.

Alternatively, the effects produced by lipolysis may be indirect. The FFA produced may for instance cause denaturation of the proteins. Dyer and Frazer (1959), Olley et al. (1962); Olley and Duncan (1965), demonstrated a relationship between FFA production and protein denaturation in frozen fish muscle and King et al. (1962) showed that small amounts of 18:2 and 18:3 reduced the solubility of cod actomyosin. Another possible indirect effect might be that since the phospholipids are situated predominantly in lipoprotein membrane systems, lipolysis could markedly affect the permeability properties of these structures leading to the release of substances which in turn could alter or affect the

Table 1—Composition[a] of neutral lipids from whole chicken breast muscle at 0 hr and 48 hr postmortem

	Time of postmortem storage	
Neutral lipid	0 hr	48 hr
Cholesterol	12	12
Triglyceride	78	75
Cholesterol ester	10	9
Monoglyceride	Trace	Trace
Diglyceride	Trace	Trace
Free fatty acid	Trace	4

[a]Expressed as percentage of total neutral lipids; results represent the average of two separate determinations which varied no more than 2%.

Table 2—Composition[a] of phospholipids from whole chicken breast muscle at 0 hr and 48 hr postmortem

	Time of postmortem storage	
Component	0 hr	48 hr
Phosphatidyl choline	53	44
Phosphatidyl ethanolamine	20	17
Sphingomyelin	6	6
Lysophosphatidyl ethanolamine	3	7
Lysophosphatidyl choline	3	6
Phosphatidyl serine	4	5
Phosphatidyl inositol	7	8
Diphosphatidyl glycerol	4	5

[a]The concentrations of component phospholipid fractions are expressed as percentages of the total lipid phosphorus; results represent the average of three separate determinations which varied by no more than 1%.

Table 3—Fatty acid composition[a] of individual lipids from chicken breast muscle 0 hr postmortem lipid fraction

Fatty Acid	PC	PE	Sph	PI	PS	DPG	TG	FFA[b]
12:0			0.3			0.5		1.4
14:0			1.4	0.4	0.6	1.0	0.8	3.0
15:0			0.6	0.4		0.6	0.2	1.3
16:ald	3.7	5.4			0.9			
16:0	29.0	4.6	17.8	3.8	13.7	9.5	24.3	23.4
16:1	0.8	0.3	1.6	0.6	2.3	2.3	3.9	1.7
17:0			0.8	1.3	1.1	0.6	0.7	0.2
17:1			0.6	0.3	0.4	1.4	0.4	
18:ald		5.3						
18:0	5.8	17.1	36.2	35.7	29.7	7.8	10.6	21.8
18:1	24.4	8.5	4.8	4.9	25.2	29.3	43.4	18.9
18:2	15.7	6.3	2.1	5.6	9.9	40.6	10.7	11.7
18:3		3.3		4.3			0.3	0.7
19:0			0.5					0.4
20:0			3.6		0.6		0.3	
20:1			0.5	0.3	1.6	2.1	1.8	
20:2	0.3	3.6	0.9	4.3	1.4			0.9
20:3	2.2	1.3		5.7	1.9			2.2
20:4	6.8	16.4		25.5	6.0			4.9
20:5	0.3	0.5				2.5		
22:0			5.7					
22:1			1.2		0.5			
22:3	1.0	3.9		2.0	1.4			
22:5	0.8	2.5		2.3	1.6			
22:6–24:4	7.6	19.9		2.4				5.7
23:0			1.4					
24:0			4.3					
24:1			9.6					
25:0			2.1					
26:0			3.4					
Unknown	1.6	1.1	0.6	0.2	1.2	1.8	2.6	2.8

[a]Expressed as percentage of the total area under the peaks on the chromatogram tracing; results represent the average of two separate determinations.
[b]Analysis of FFA from chicken breast muscle aged 48 hr.

myofibrillar constituents. An argument of this nature would be extremely pertinent if applied to lysozomal fractions which are thought to contain a variety of catheptic enzymes. Destruction of their membrane system would bring about the release of these catabolic enzymes which could in turn bring about a degeneration of the macromolecular assemblies.

The absence of even insignificant amounts of DG in 48 hr aged muscle precludes the possibility of phospholipase C activity up to 48 hr.

In conclusion, while many workers have demonstrated long term storage effects on muscle lipids, little attention has been focussed on more immediate postmortem effects. That phospholipase activity is present early in aging has been satisfactorily demonstrated in this study, and further investigation into the role of this lipolysis in postmortem phenomena are currently under way.

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EFFECT OF POSTMORTEM AGING ON CHICKEN BREAST MUSCLE SARCOPLASMIC RETICULUM

INTRODUCTION

THE SARCOPLASMIC RETICULUM (SR) is an extensive membrane system surrounding muscle fibrils, which controls the degree of contraction and relaxation in this tissue by regulating the concentration of free Ca^{++} ions in the sarcoplasm. This regulation is achieved through an ATP dependent Ca^{++} transport system in the SR membranes, and storage of Ca^{++} within vesicles of the SR (Carvalho, 1972). Contraction is thought to occur when Ca^{++} is either released from the vesicles through changes in permeability or when bound Ca^{++} is released from the membrane. The released Ca^{++} stimulates the contractile elements in the myofibrils. In the relaxed state, the SR system maintains sarcoplasmic Ca^{++} concentration below 10^{-7}M (Feinstein, 1966), insufficient to initiate contraction.

Postmortem aging of pork (Greaser et al., 1967; 1969a) and bovine (Goll et al., 1971) muscle has been shown to decrease the ability of the SR to accumulate Ca^{++}. This decrease has been proposed by Goll et al. (1971) as the principal reason for the onset of rigor mortis. While the reasons for this loss in functional integrity of the SR are not fully understood, uncoupling of the Ca^{++} pump by proteolysis, postmortem pH decline, postmortem loss of ATP, and temperature changes have been suggested as possible contributing factors.

Chicken muscle is unique in that it enters rigor mortis very rapidly postmortem, and that resolution of rigor and ensuing tenderness development are normally complete within 24 hr (deFremery and Pool, 1960). Our previous studies on this system (Hay et al., 1973a, b, c) have been directed towards postmortem changes in lipids, myofibrils and myofibrillar protein, respectively. In light of the significant role played by Ca^{++} ion release in the onset of rigor mortis, it was deemed appropriate to investigate the effects of postmortem aging on the functional properties of the SR in order to elucidate further the factors contributing to the postmortem changes in chicken muscle.

MATERIALS & METHODS

Preparation and purification of sarcoplasmic reticulum

Approximately 400g muscle samples were obtained from the pectoralis major of 18–20 wk old broilers, 5 min, 48 hr and 168 hr postmortem. To minimize bacterial contamination the carcasses were wrapped in paper towels, soaked in 10 mM sodium azide and stored at 2°C. Muscle samples were homogenized with 4 vol of ice cold 0.3M sucrose (Schwarz/Mann, Orangeburg, N.Y.) in a Waring Blendor, using 15 sec bursts with a 15 sec interval between each burst. A schematic representation of the procedure used in the fractionation of the muscle homogenate is shown in Figure 1. The myofibrils were sedimented at 3,800 × G for 10 min. The supernatant was strained through four layers of cheese cloth and mitochondria sedimented at 15,000 × G for 20 min. The resulting supernatant was strained through four layers of cheese cloth and a crude SR fraction recovered by sedimentation at 27,000 × G for 3 hr. The sedimented material was transferred to a glass homogenate tube where it was gently hand mixed with 0.6M KCl, 5 mM histidine (pH 7.3) to remove contaminating actomyosin (Martonosi et al., 1968). The homogenate was then centrifuged for 1 hr at 90,000 × G. The sedimented SR was resuspended in 0.3M sucrose and layered on top of a sucrose linear density gradient (35%–65%, w/v). The tubes were spun at 26,000 rpm (120,000 × G) for 2

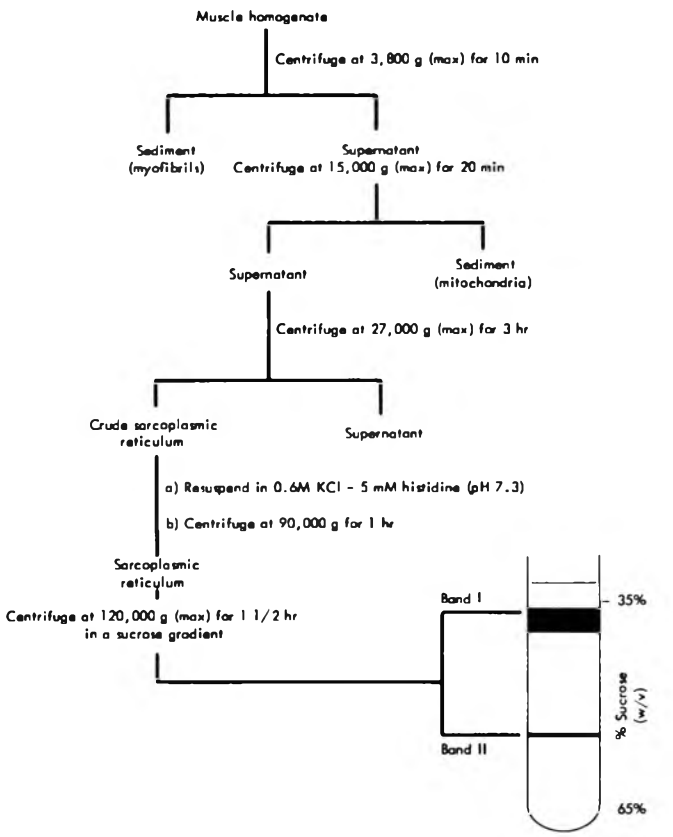

Fig. 1—Flowsheet for the procedure used in the fractionation of the muscle homogenate.

hr in the SW 27 swinging bucket rotor (Beckman Instruments, Palo Alto). The centrifugation resulted in the formation of two bands (Fig. 1). The upper band (Band 1) was withdrawn with a Pasteur pipette and stored at −20°C.

ATPase activities

The ATPase activities of SR were determined after the method of Greaser et al. (1969a) at 25°C. The incubation medium was 0.1M KCl, 5 mM histidine (pH 7.2), 5 mM ATP, 5 mM $MgCl_2$ and 0.1 mM $CaCl_2$. SR protein concentration (Oyama and Eagle, 1956) was between 0.1 and 0.2 mg/ml. Aliquots were removed at fixed time intervals, added to cold 15% TCA, and inorganic phosphate determined by the method of Fiske and Subba Row (1925).

Calcium ion uptake

The Ca^{++} uptake of the SR was measured according to Meissner and Fleischer (1971), in 3 ml of a solution consisting of 0.1M KCl, 5 mM $MgCl_2$, 5 mM potassium oxalate, 10 mM histidine (pH 7.3), 5 mM ATP and 0.1 mM $CaCl_2$ (containing 0.1 μc $^{45}Ca^{++}$). An SR protein content of 0.01–0.03 mg/ml was used. The reaction was carried out at 23°C for 15 min and terminated by Millipore® filtration. A sample without added SR served as the control. Ca^{++} uptake was measured as a decrease in $^{45}Ca^{++}$ in the filtrate.

Electron microscopy

The sarcoplasmic reticulum fraction was obtained as a pellet and fixed in 3.5% glutaraldehyde in 0.1M phosphate buffer pH 7.4 for 4 hr at 4°C. Following a buffer wash overnight, the samples were post-fixed in 2% phosphate buffered osmium tetroxide for 2 hr at room temperature. The samples were dehydrated with graded ethanol solutions and proplyene oxide and embedded in araldite. Sections were stained using uranyl acetate and lead citrate solutions. The sections were viewed using a Phillips 300 electron microscope.

Polyacrylamide disc gel electrophoresis in SDS

The procedure used was identical to that described by Hay et al. (1973c). 8½% gels were used, and the SDS protein complexes were prepared by incubating the SR in the presence of 1% SDS and 0.5 mM DTT at 40°C for 30 min. 50 μg of the SR was applied to the gel and electrophoresis was performed at 20°C at a constant current of 5 mA/tube for 5 hr. The gels were stained in a solution of Coomassie Brilliant Blue for 2 hr and destained in a quick gel destainer (Canalco Co.) for 25 min. The molecular weights of the protein components were determined by comparison of their mobilities with proteins of known molecular weight (Weber and Osborn, 1969).

Lipid analyses

Lipid was extracted from an amount of freeze-dried sarcoplasmic reticulum which contained approximately 10 mg protein, by shaking overnight at 4°C with 19 vol of chloroform: methanol (2:1 v/v). The mixture was filtered through a sintered glass filter funnel to remove proteinaceous material and the lipids purified by the Folch procedure (Folch et al., 1957). The total lipid extract was taken to dryness by rotary evaporation and the total weight determined gravimetrically using a Cahn-Gram electrobalance. The neutral lipids were separated from the phospholipids on a silicic acid column (1.5g silicic acid, 0.5g celite). The neutral lipids were eluted with 60 ml diethyl ether and the phospholipids with 50 ml chloroform:methanol (1:1, by vol) followed by 40 ml methanol. Each fraction was concentrated and weighed.

Table 1—pH, calcium accumulation and ATPase determination by the fragmented sarcoplasmic reticulum from chicken pectoral muscle

Time post-mortem (hr)	pH	Ca^{++} uptake (μmoles Ca^{++}/mg protein[a])	ATPase activity (μmoles Pi/min/mg protein[a])
0	6.0	1.6	.07
0[b]	6.6	1.6	.06
48	5.8	2.2	.07
168	5.6	4.8	.08

[a]Values represent the average of two determinations from three different muscle preparations.

[b]Chicken injected with sodium pentobarbital prior to exsanguination.

Table 2a—Ca^{++} uptake assays of the sarcoplasmic reticulum from chicken breast muscle

	pH 7.2 25°C	pH 5.6 25°C	pH 5.6 2°C
Ca^{++} uptake (μmoles of Ca^{++}/mg protein[a])	1.5	0.7	0.4

[a]Values represent the average of three determinations from a single muscle preparation.

Table 2b—Ca^{++} uptake assays at pH 7.2 and 25°C of the sarcoplasmic reticulum from 0 hr chicken breast muscle after pre-incubation of the SR for 1 hour at 2°C, 25°C and 37°C at pH 5.6

	Temperature		
	2°C	25°C	37°C
Ca^{++} uptake (μmoles of Ca^{++}/mg protein[a])	1.7	0.7	0.4

[a]Values represent the average of three determinations from a single muscle preparation.

RESULTS

LINEAR DENSITY gradient profiles of the principal SR fractions are shown in Figure 1. Occasionally the upper band (Band I) was resolved into two bands of close proximity, but this did not occur regularly enough to permit separate characterization of this subfraction. Band I contained the highest Ca^{++} uptake and ATPase activity, a feature observed in similar studies of the SR from rabbit (Seraydarian and Mommaerts, 1965) and porcine (Greaser et al., 1969b) skeletal muscle. The SR from 48-hr and 168-hr preparations yielded similar profiles though the intensity of the lower band (Band II) appeared to diminish with aging.

The ATPase activities of the sarcoplasmic reticulum are shown in Table 1. The activity is less than that reported for SR isolated from porcine skeletal muscle (Greaser et al., 1969a) under similar assay conditions. Reduction in portmortem pH did not contribute to this reduced activity, since intravenous injection of sodium pentobarbitol prior to slaughter to prevent pH fall had no effect on either the ATPase or Ca^{++} uptake activities (Table 1). No loss in ATPase activity was observed with aging.

The Ca^{++}-accumulating ability of the SR is also shown on Table 1. The value for the 0-hr preparation is lower than the value obtained from rabbit skeletal muscle under similar assay conditions (Meissner and Fleischer, 1971). However, as is seen in the 48- and 168-hr preparations, aging of the muscle resulted in an increase in the ability of the sarcoplasmic reticulum to sequester Ca^{++}.

The effect of pH and temperature on the uptake of Ca^{++} by the SR are shown

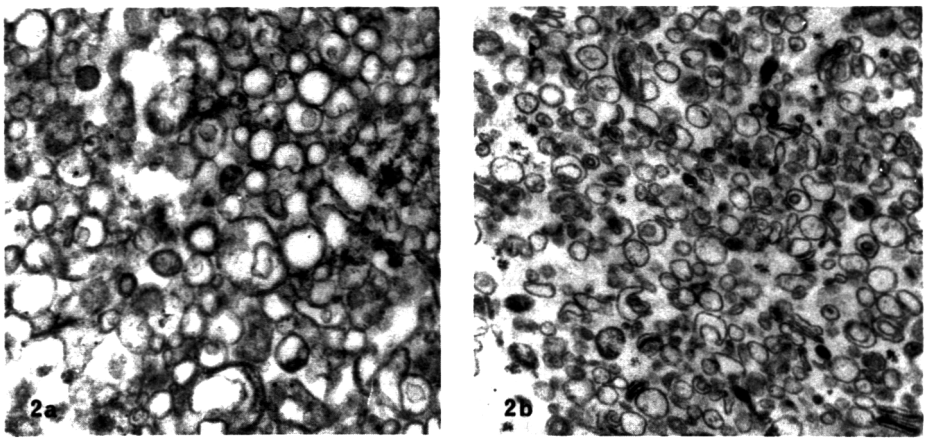

Fig. 2–Electron micrographs of sarcoplasmic reticulum Band 1 from chicken breast muscle at (a) 0 hr and (b) 7 day postmortem. Magnification ≈ 25,000 X.

in Table 2a and b. In 2a the incubations with $^{45}Ca^{++}$ were modified according to temperature and pH as indicated, demonstrating the sensitivity of the SR Ca^{++} uptake system to these variables. In 2b the SR was preincubated at 0°C, 25°C and 37°C (all at pH 5.6) for 1 hr prior to the assay at 25°C and pH 7.2 (Greaser et al., 1969b). These results clearly demonstrate the fragility of the SR Ca^{++}-accumulating system, and the dramatic effect of mild heat treatment on this system.

The electron micrographs of the sarcoplasmic reticulum from muscle aged 0 hr and 168 hr are shown on Figures 2a and b. The 0-hr preparation consists of vesicles ranging from 80 mμ to 900 mμ in diameter. The general morphological features are similar to the fragmented sarcoplasmic vesicles observed by Greaser et al. (1969c) from pig and Yu et al. (1968) and Meissner and Fleischer (1971) from rabbit skeletal muscle systems. The 168-hr preparation appears to consist mainly of vesicles approx 110 mμ in diameter, some of which contain distinct electron dense cores (see Fig. 2b).

The SDS-gel electrophoresis patterns of SR protein are shown on Figure 3. The major band (M.W. 100,000) is presumably the Ca^{++}-activated ATPase (McLennan et al., 1971; Meissner and Fleischer, 1971) or M-band protein (Purcell and Martonosi, 1972) reported in other studies. The minor bands in the 35,000–75,000 dalton region cannot be identified though they are likely to include several proteins which have recently been shown to have an affinity for Ca^{++}. Only one minor change can be detected in the gel profiles of the SR from aged muscle, that is the appearance of a band at 30,000 daltons. The origin of the protein is unknown, though it may prove to be more than coincidental that a protein with a similar molecular weight was also detected in the myofibrillar proteins from aged chicken muscle (Hay et al., 1973c).

The relative amounts of total lipid and phospholipid found in this study (Table 3) are similar to the values obtained in lipid studies of the sarcoplasmic reticulum from human (Takagi, 1971), rat (Fiehn et al., 1971), and rabbit (Meissner and Fleischer, 1971) skeletal muscle. The sarcoplasmic reticulum from aged muscle contains a slightly higher proportion of phospholipid.

DISCUSSION

FROM OUR STUDIES it is clear that there is no significant impairment of the ATPase activity from chicken muscle sarcoplasmic reticulum following postmortem storage. These results agree with the findings of Goll et al. (1971) in studies with bovine muscle, but are difficult to reconcile with the data presented by Greaser et al. (1969a, b) who reported an increase in Ca^{++}-activiated ATPase in one study and a decrease in another study.

The increase in Ca^{++}-accumulating ability of SR following postmortem aging is surprising and unexpected. Greaser et al. (1967; 1969a, b) and Goll et al. (1971) in studies of porcine and bovine muscle, respectively, showed that postmortem aging causes the SR to lose its ability to sequester Ca^{++}. Greaser et al. (1969b) attributed this loss to the drop in pH coupled with the high muscle temperature after death. They did in fact demonstrate that SR very rapidly loses its ability to accumulate Ca^{++} when subjected to the postmortem condition of pH and temperature normally encountered in pork muscle. However, this explanation was questioned by Goll et al. (1971) who suggest that proteolysis is the principal factor responsible for the loss of Ca^{++}-accumulating ability of SR from bovine muscle. Thus the retention of the Ca^{++}-accumulating ability of the SR from chicken muscle throughout postmortem aging is probably due to either the rapid lowering of the temperature of the chicken muscle postmortem or the absence of significant proteolysis as judged by the similarity of gel patterns of SR proteins throughout the postmortem aging periods.

The results of the present study raise an interesting argument in relation to the theory that loss of Ca^{++} sequestering properties by the sarcoplasmic reticulum is one of the chief factors responsible for the development of rigor mortis in certain muscle systems as claimed by Greaser et al. (1969b) and Goll et al. (1971). Schmidt et al. (1970) have questioned this assumption by showing that rigor mortis can develop under conditions where there is little loss of calcium-binding ability of the sarcoplasmic reticulum per se. It is clear from our studies that rigor mortis development in chicken muscle is not due to the loss of Ca^{++}-accumulating ability of the SR.

It is safe to assume that Ca^{++} is released from sarcoplasmic reticulum during postmortem aging, since binding of Ca^{++} to troponin is an essential prerequisite for the chain of events leading to the interaction of actin and myosin manifested during rigor development (Ebashi et al., 1968). To account for this efflux of Ca^{++} from the SR several explanations may be advanced. Firstly, it is reasonable to suggest that the depletion of ATP may be responsible for the postmortem changes in chicken muscle. ATP depletion has already been shown to be clearly allied to the development of rigor mortis in this muscle (de Fremery and Pool, 1960). Hasselbach and Makinose (1964) have demonstrated that the Ca^{++}-activated ATPase pump, essential for the removal of Ca^{++} from the myofibrillar system, is inhibited by ADP. Moreover, Panet and Selinger (1972) have shown that the Ca^{++} released from the sarcoplasmic reticulum is markedly enhanced by the addition of low concentrations of ADP and Pi. The release of Ca^{++} has been shown to bring about a chemico-osmotic mechanism for ATP synthesis. This could conceivably be a response by the muscle system postmortem to conserve its depleted stocks of ATP.

Temperature is also an important consideration when speculating upon the causes of the release of Ca^{++} from the SR. The rapid cooling of the carcass following slaughter may have a direct effect upon Ca^{++} release. Lowering of the temperature in a muscle system pretreated with caffeine to 1–3°C has been shown to cause strong contractures (Conway and Sakai, 1960). It is noticeable from our

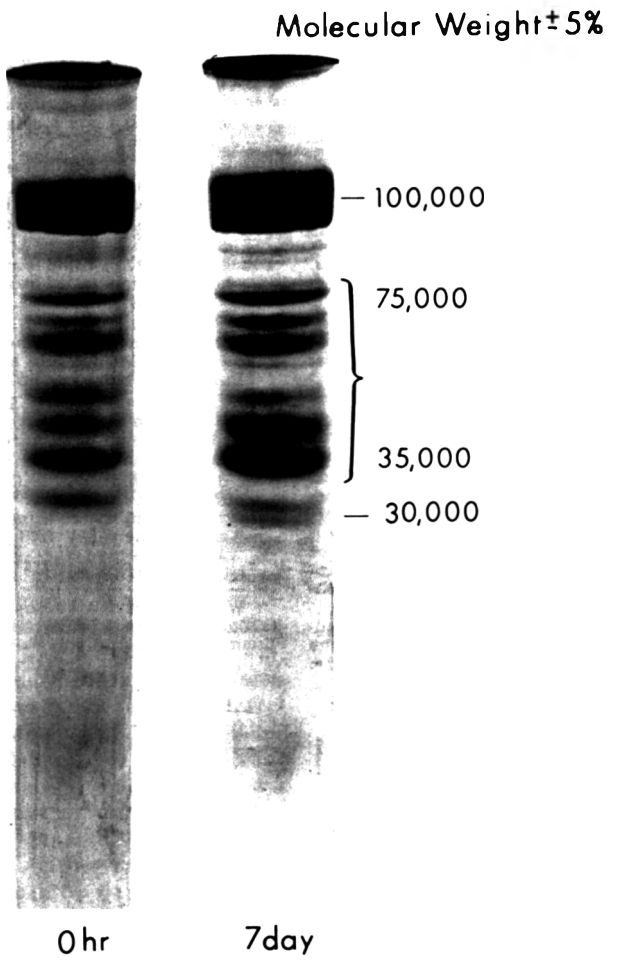

Fig. 3–SDS-gel electrophoresis of sarcoplasmic reticulum proteins from chicken breast muscle at 0 hr and 7-day postmortem. The conditions for the electrophoresis are described in Materials & Methods.

studies (Table 2a) that lowering of Ca^{++} uptake assay medium from 25°C to 2°C causes a reduction in Ca^{++} uptake by the SR. Thus either the Ca^{++}-binding sites are blocked off by the lowering of the temperature or, alternatively, the ATPase activated pump is less efficient at the lower temperature causing a reduction in uptake of Ca^{++}.

pH is another factor which could influence the release of Ca^{++} from the sarcoplasmic reticulum. Carvalho and Leo (1967) have shown that below pH 6.2 in the presence of ATP, H^+ replaces Ca^{++} at the FSR binding sites. Bertrand et al. (1971) have also shown that low pH causes a marked reduction in the affinity of the sarcotubular membranes to bind Ca^{++}. The results of the experiments reported in Table 2a and b show that sarcoplasmic reticulum incubated or assayed at pH 5.6 has a reduced capacity to bind Ca^{++}.

The increase in Ca^{++}-accumulating ability with postmortem aging is difficult to explain. There appeared to be no marked morphological changes nor was there any marked alteration with aging in the polyacrylamide gel patterns of SR protein. The increased lipid content of sarcoplasmic reticulum from aged muscle could partly account for the enhanced Ca^{++}-accumulating ability. However, this is debatable in view of the fact that The and Hasselbach (1972) have shown that neither the number of Ca^{++}-binding sites nor their affinity for Ca^{++} is affected when membranes are deprived of phospholipids. The increase in Ca^{++}-binding capacity may be due to a greater concentration of actively Ca^{++} sequestering sarcoplasmic reticulum fragments in the 7-day preparation compared to the 0-hr case. Greaser et al. (1969d) have reported that the actual Ca^{++} transporting membranes constitute only 20% of the total membranous fragments as judged electron microscopically by the number of vesicles which contained calcium oxalate deposits. Differences in Ca^{++}-accumulating ability due to contamination of the SR preparations by other sub-cellular fragments seems unlikely in view of the similarity in SDS-gel electrophoresis profiles and electron micrographs of SR vesicles from the chicken muscle at the various postmortem aging periods.

Table 3–Lipid content of fragmented sarcoplasmic reticulum from chicken breast muscle aged 0, 48 and 168 hr[a]

	0 hr	48 hr	168 hr
Total lipid[a]	52	59	62
Phospholipid[b]	70	77	79

[a]Values represent % (w/w) of total sarcoplasmic reticulum.
[b]Values represent % (w/w) of total lipid.

In conclusion, the results presented here clearly indicate that degeneration of the SR in postmortem chicken muscle is not occurring to an extent that will destroy the Ca^{++} sequestering ability of this organelle. Since temperature and pH have such a marked effect on the Ca^{++} uptake in 0-hr myofibrils, it is reasonable to suggest that the differences between porcine and bovine muscle SR and chicken muscle SR are a direct result of the rapid cooling experienced by the much smaller chicken carcass. Consideration of the temperature dependence clearly suggests that while SR degradation might be an acceptable explanation for large animal carcasses, other mechanisms such as ATP depletion, pH or low temperature induced permeability changes are responsible for the Ca^{++} release leading to rigor onset in chicken muscle.

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ON THE INTERACTION OF MYOGLOBIN AND HEMOGLOBIN WITH MOLECULAR OXYGEN AND ITS LOWER OXIDATION STATES AND WITH CYTOCHROME c

INTRODUCTION

THE POSSIBLE INTERACTION of myoglobin and hemoglobin with the superoxide anion became of interest to us during a study in which it was observed that gamma irradiation of deoxygenated solutions of ferrimyoglobin and ferrihemoglobin causes reductive oxygenation of the pigments to the oxyferrous form, while in the presence of oxygen the ferriheme pigments are oxidized to the ferryl (+4) oxidation state (Giddings and Markakis, 1972). Rabani and Stein (1962) reported that radiation-generated $\bar{e}_{aq}$ and O_2^- reduce ferricytochrome c, and that irradiation of oxygenated solutions favors reduction by O_2^-. However, H_2O_2 is the principal stable reactive oxygen derivative in irradiated oxygenated aqueous solutions, and we determined that H_2O_2 was the causative agent in said oxidation of the two ferrihemoproteins. A simple reduction in irradiated deoxygenated solutions (as in the case of cytochrome c) could be explained solely by the hydrated electron (Pecht and Faraggi, 1972); however, the observed reductive oxygenation cannot, and led to speculation about the possibility of O_2^- involvement. Although the reductive oxygenation could be due to a sequential interaction of ferrihemoprotein with radiation-generated $\bar{e}_{aq}$ and O_2 in that order, one could not rule out the possibility of an "oxygenation" of ferrimyoglobin or ferrihemoglobin by O_2^-.

It was further speculated that the reverse of oxygenation by O_2^-, namely the dissociation of O_2^- (or the conjugate acid, $HO_2^{\cdot}$) from the oxyhemoproteins, might occur during autoxidation. Such a reversible dissociation of O_2^- from myoglobin and hemoglobin was alluded to years ago (George and Stratmann, 1954; Kikuchi et al., 1955), but has since received little attention. However, the recently advanced $(Fe^{3+}O_2^-)^{VI}$ structure for the oxyhemoproteins (Wittenberg et al., 1970) suggests that a dissociation of O_2^- (or $HO_2^{\cdot}$) from, and a liganding of O_2^- to the hemoprotein heme-iron may occur under favorable conditions. The mechanism(s) of oxymyoglobin and oxyhemoglobin autoxidation and factors influencing same relate directly to fresh red meat color stability. Thus this study was undertaken to attempt to determine experimentally if the superoxide anion does in fact dissociate from the oxyhemoproteins during autoxidation. Also, in connection with the reductive oxygenation of the ferrihemoproteins during gamma irradiation of deoxygenated solutions of same, the possibility of an interaction of the ferrihemoproteins with superoxide anion was investigated.

MATERIALS & METHODS

THE AEROBIC xanthine-xanthine oxidase system was selected as a generator of superoxide anions since it is known with certainty to univalently reduce the molecular oxygen biradical, it can be put into solution along with the hemoproteins under study, and, it is known to cause interaction between O_2^- and another soluble hemoprotein, ferricytochrome c, under the same conditions employed herein (Bray et al., 1970; Fridovich, 1970). To attempt to ascertain whether or not ferrimyoglobin indeed interacts with O_2^- in this system, competitive inhibition by ferrimyoglobin of ferricytochrome c reduction by the anion was tested. In an attempt to monitor for O_2^- dissociation during oxymyoglobin autoxidation, ferricytochrome c reduction by the anion, and competitive inhibition of same by "tiron" (Muraoka et al., 1967; Miller, 1970) was employed.

Highly purified ferrimyoglobin was prepared from bovine semitendinosus muscle essentially according to the procedure of Hardman et al. (1966). Ferrous myoglobin and oxyferrous myoglobin were prepared from this by reduction with either dithionite or borohydride, followed by dialysis and deionization in the oxyferrous case. Type III horse heart and Type V beef heart ferricytochromes c (Sigma) were solubilized and exhaustively dialyzed against deionized water, and the pH adjusted, after assuring complete oxidation with a minute amount of ferricyanide. Human ferrihemoglobin (Miles Laboratories) was further purified by Sephadex G-100 column chromatography (Aebi et al., 1964). Ferrous and oxyferrous hemoglobin were prepared as per myoglobin. Milk xanthine oxidase (E.C.1.2.3.2,N.B.Co.) was dialyzed against 0.01M $NaPO_4$ (pH 7.8) prior to use. Heme-free globin was prepared by the 2-butanone method of Teale (1959) as modified by Yonetani (1967). Tiron (4,5-dihydroxy-m-benzene disulfonic acid, disodium salt, Eastman Co.) was added to solutions as the dry powder. Xanthine (2,6-dihydroxypurine, Sigma Co.) was dissolved in 0.1N NaOH and adjusted to 1.5×10^{-3}M with deionized water. Other chemicals and gases were of the highest purity available. Irradiation and spectrophotometry were carried out as described in the previous paper (Giddings and Markakis, 1972). Anaerobic techniques for electron-transfer experiments were done essentially as described by Dixon (1971).

RESULTS & DISCUSSION

FIGURE 1 shows the absorption spectra of (a) untreated ferricytochrome c; (b) ferricytochrome c in the presence of 2.5×10^{-7}M ferrimyoglobin after completion of the xanthine-xanthine oxidase reaction; (c) deoxygenated ferricytochrome c solution after completion of the xanthine-xanthine oxidase reaction; (d) aerobic ferricytochrome c solution after 100 Krad of gamma irradiation; (e) aerobic ferricytochrome c solution, no myoglobin present, after completion of the xanthine-xanthine oxidase reaction; and (f) aerobic ferricytochrome c solution after addition of H_2O_2.

The xanthine oxidase reaction caused very rapid, essentially complete reduction of ferricytochrome c in the presence of ample oxygen (curve E), whereas the presence of ferrimyoglobin markedly diminished (curve B) and the absence of oxygen practically eliminated (curve C) the ferricytochrome c reduction. (The apparent slight reduction in the latter case was likely due either to residual oxygen, or to a nonoxygen requiring secondary reduction mechanism). Thus the oxygen-requiring process was operating, and myoglobin clearly interfered with ferricytochrome c reduction by the system. Further evidence of this is contained in Table 1. Curves D and F of Figure 1 illustrate the facts that neither H_2O_2 addition nor irradiation to 100 Krad in the presence of oxygen changes the oxidation state of ferricytochrome c even though aerobic irradiation can produce O_2^- and $HO_2^{\cdot}$ as well as H_2O_2 (Klug et al., 1972). As pointed out in the introduc-

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tion, under the same conditions of irradiation ferrimyoglobin and ferrihemoglobin are oxidized to the ferryl state by radiation-generated H_2O_2.

Table 1 further illustrates the inhibitory capacity of ferrimyoglobin and the virtual lack of inhibition in the case of heme-free globin. Earlier reports (Fridovich, 1962; McCord and Fridovich, 1968) singled out the globin moiety of ferrimyoglobin as the inhibitory entity, but this was later attributed to "superoxide dismutase" contamination of their myoglobin preparations (McCord and Fridovich, 1969). The rigorous myoglobin preparation procedure employed in the present work, plus supporting evidence from preliminary checks rules out "superoxide dismutase" contamination of the ferrimyoglobin preparation. Moreover if the heme moiety of one heme-protein (ferricytochrome c) is known to engage in a redox reaction with a nonspecific reactant like the superoxide anion, then one might expect the anion to also interact with the heme of other hemoproteins.

Figure 2 compares the visible and Soret spectra of: (a) ferrimyoglobin and (b) ferrylmyoglobin (displaced downward), and (d) ferrihemoglobin and (c) ferrylhemoglobin. The aerobic xanthine-xanthine oxidase system oxidized the ferric (+3) heme iron of both ferrimyoglobin and ferrihemoglobin to the ferryl (+4) oxidation state. In addition to the typical ferryl spectra, the +4 oxidation state of the hemoprotein iron was established by the fact that addition of ferrocyanide ions instantaneously reduced the pigments back to the ferric state. Of the two principal products of reduced xanthine oxidase oxidation by oxygen, O_2^- and H_2O_2, peroxide is known to oxidize ferrimyoglobin and ferrihemoglobin to the ferryl state. Since O_2^- is known to reduce ferricytochrome c we expected it to also reduce, and possibly oxygenate ferrimyoglobin and ferrihemoglobin in a manner similar to that proposed for other ferric hemoproteins (Odajima and Yamazaki, 1972; Hirata and Hayaishi, 1971). However, since H_2O_2 does not reduce ferricytochrome c the results depicted in Figure 1 and in Table 1 point to O_2^- as the causative agent in the oxidation of ferrimyoglobin and ferrihemoglobin by the xanthine oxidase system since the oxidation presumably is directly linked with the inhibition of ferricytochrome c reduction. As demonstrated further on, however, there is at least one other possible inhibitory mechanism involving myoglobin.

Table 2 compares the relative reaction rates of the three hemoproteins as ΔA/min at the appropriate wavelengths, per total absorbance change. Cytochrome c reduction and uric acid production (xanthine oxidation) reached completion during the same initial 1 min interval.

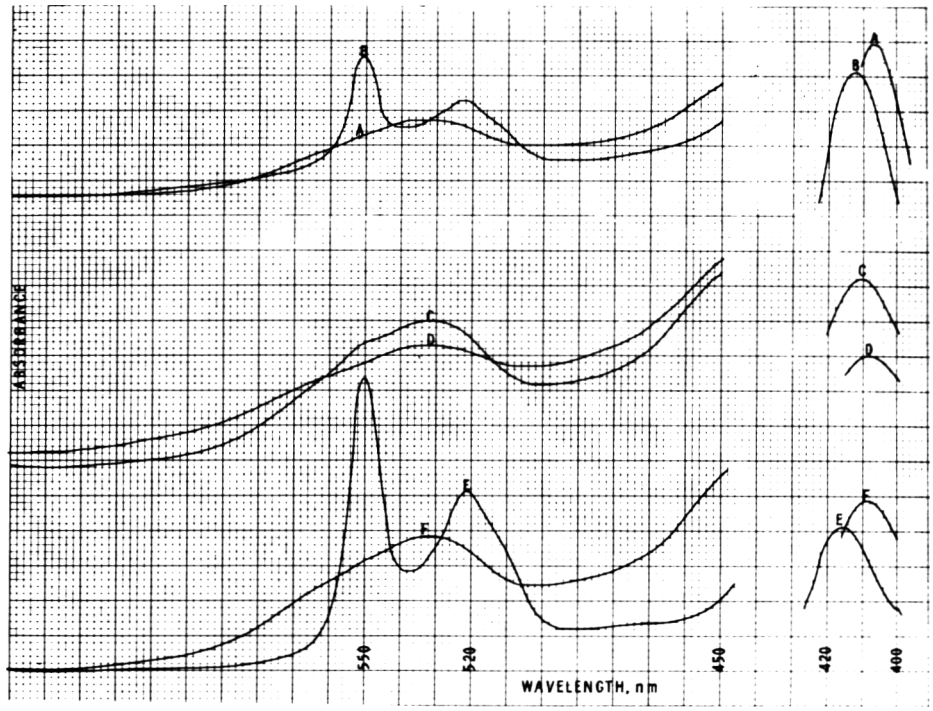

Fig. 1—Effect of various treatments on cytochrome c oxidation state. Conditions: 10^{-5} M ferricytochrome c, pH 7.8 and 25°C for the six treatments shown. Spectra: (A) untreated ferricytochrome c; (B) ferricytochrome c in the presence of 2.5 × 10^{-7} M ferrimyoglobin at the completion of xanthine oxidase reaction; (C) deoxygenated ferricytochrome c solution at the completion of xanthine oxidase reaxtion; (D) ferricytochrome c after exposure to 100 Krad of gamma irradiation; (E) ferricytochrome c, no myoglobin present, after completion of xanthine oxidase reaction; (F) ferricytochrome c after addition of 0.05 ml of 30% H_2O_2 per 3 ml reaction mixture. Reaction solutions for B, C and E contained 5 × 10^{-5} M xanthine, 10^{-5} M EDTA and 10^{-7} M xanthine oxidase in the total 3 ml.

Table 1—Effect of ferrimyoglobin and heme-free globin on the xanthine-cytochrome c reductase activity of milk xanthine oxidase[a]

Amount of globin or ferrimyoglobin	Ferricytochrome c reduction ΔA550/min
None	0.26
2.8 × 10^{-7} M (4.8 μg/ml) globin	0.26
2.8 × 10^{-6} M (48 μg/ml) globin	0.24
2.2 × 10^{-8} M (0.4 μg/ml) ferrimyoglobin	0.20
5.6 × 10^{-8} M (1 μg/ml) ferrimyoglobin	0.16
1.1 × 10^{-7} M (2 μg/ml) ferrimyoglobin	0.13
2.5 × 10^{-7} M (4.5 μg/ml) ferrimyoglobin	0.08

[a] Conditions: 10^{-5} M ferricytochrome c; 5 × 10^{-5} M xanthine; 10^{-5} M EDTA; 10^{-7} M xanthine oxidase; indicated amounts of globin and ferrimyoglobin.

However, ferrimyoglobin and ferrihemoglobin oxidation continued for approximately an additional 2 min indicating that at least the latter 2 min of their oxidation was caused by H_2O_2 and not O_2^-. If the apparent inhibition by ferrimyoglobin of ferricytochrome c reduction is due to a redox reaction between it and O_2^-, it seems odd that O_2^- would reduce ferricytochrome c and oxidize ferrimyoglobin and hemoglobin. However, the anion is believed to be able to act as either a reductant or an oxidant (McCord and Fridovich, 1969). Also, the midpoint reduction potentials of the reacting couples, as shown in Table 3, support such a possibility although O_2^- is thought to usually act as a reductant and $HO_2^{\cdot}$ as an oxidant (Hart, 1972). It seems to be preclusive to experimentally determine unequivocally whether O_2^- as well as H_2O_2 oxidizes ferrimyoglobin and ferri-

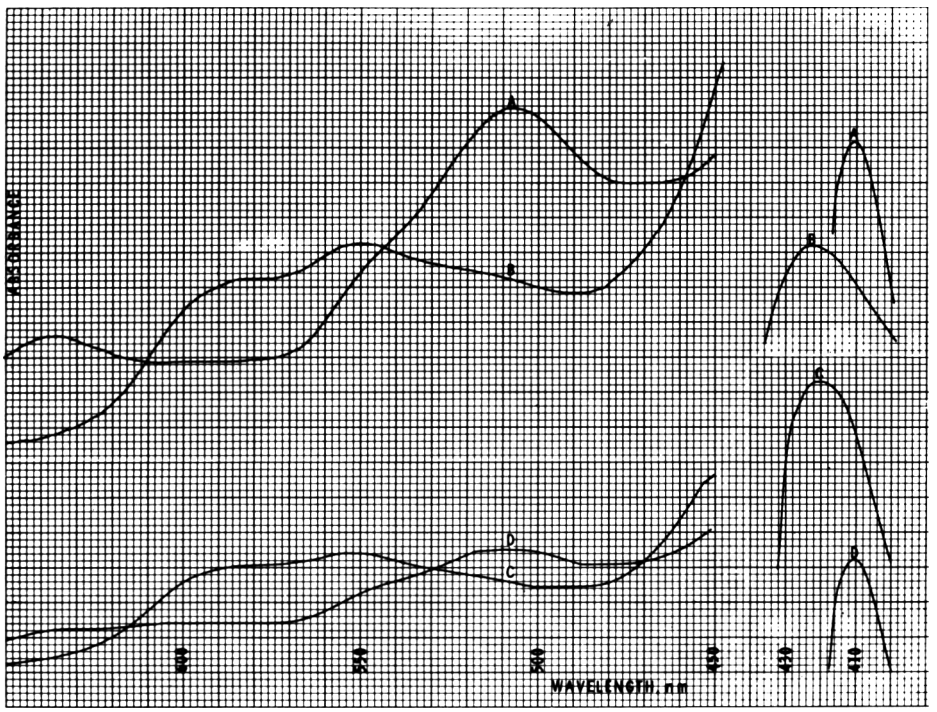

Fig. 2–Oxidation of ferrimyoglobin and ferrihemoglobin to the ferryl state by the aerobic xanthine oxidase system. Conditions: 10^{-5} M ferrimyoglobin or ferrihemoglobin; 5 × 10^{-5} M xanthine; 10^{-5} M EDTA; 10^{-7} M xanthine oxidase. Reaction solutions were in equilibrium with the atmosphere at 25°C and pH 7.8. Reactions were initiated by addition of xanthine. Spectra: (A) ferrimyoglobin solution before xanthine addition; (B) "A" after completion of xanthine oxidase reaction; (D) ferrihemoglobin solution before xanthine addition; (C) "D" after completion of xanthine oxidase reaction.

Table 2–Comparative reaction rates of ferricytochrome c, ferrimyoglobin and ferrihemoglobin with the xanthine oxidase generated superoxide anion and rate of production of uric acid from xanthine[a]

Product monitored	ΔA/min	Total ΔA	ΔA/min / total ΔA	Approx time to complete reaction
Ferrocytochrome c	0.26 @ 550 nm	0.26	1.0	1 min
Ferrylmyoglobin	0.10 @ 582 nm	0.25	0.4	2.5–3 min
Ferrylhemoglobin	0.05 @ 578 nm	0.13	0.38	2.5–3 min
Uric acid[b]	0.36 @ 290 nm	0.32	1.13	1 min

[a] Conditions: 10^{-5} M ferrihemoprotein; 5 × 10^{-5} M xanthine; 10^{-5} M EDTA; 10^{-7} xanthine oxidase.
[b] Based upon 1 unit of activity = 1 μmole uric acid produced per min @ pH 7.8 and 25°C. This is equivalent to 0.2 unit of activity per cuvette for all reactions, and a specific activity of approx 2.2 units per mg of xanthine oxidase. Uric acid production was measured with no hemoprotein present.

hemoglobin under these experimental conditions. In similar situations catalase is usually employed to determine whether or not H_2O_2 is a required reactant. However, in this case catalase inhibition of ferrylmyoglobin (hemoglobin) formation could be attributed to its interacting with either O_2^- or its disproportionation product, H_2O_2, or both, for catalase inhibition of ferricytochrome c reduction by the xanthine oxidase system (Muraoka et al., 1967) indicates that catalase can interact with O_2^-, since catalase removal of only H_2O_2 should enhance ferricytochrome reduction by the system. Moreover "superoxide dismutase" which catalyses the disproportionation of O_2^- to H_2O_2 and O_2 (McCord et al., 1971), and compounds such as tiron which also interacts with O_2^- to form H_2O_2, are of no value in this case (in contrast to the case of ferricytochrome c reduction by O_2^-) since they generate stable H_2O_2 as rapidly as they remove O_2^- (Weser and Voelcker, 1972). The unequivocal answer to this question presents experimental difficulty also due to the rapid (i.e., milliseconds) spontaneous disproportionation of the unstable anion to H_2O_2 and O_2.

Another possible inhibitory mechanism that is supported by favorable redox potentials (Table 3) is that of a direct, intermolecular, univalent redox reaction (electron transfer) between ferrylmyoglobin (produced by ferrimyoglobin oxidation by O_2^- and/or H_2O_2) and ferrocytochrome c (produced by O_2^- reduction of ferricytochrome c). Evidence in support of this possibility was obtained while testing for O_2^- generation during oxymyoglobin and oxyhemoglobin autoxidation in sealed Thunberg cuvettes. Concurrent autoxidation and ferricytochrome c reduction was observed to occur gradually with time; however, tiron exhibited no inhibition whatever, and periodic scans of visible and Soret spectra during each experiment indicated that oxyhemoprotein autoxidation and ferricytochrome c reduction proceeded proportionately at about the same rate. Lack of tiron inhibition tends to rule out O_2^- mediation of the ferricytochrome c reduction.

Employing a painstaking procedure involving deoxygenated solutions of dithionite or borohydride-reduced myoglobin or hemoglobin in sealed Thunberg cuvettes, oxygen-free nitrogen gas, and deoxygenated ferricytochrome c solution in a second cuvette sidearm (a slight positive N_2 pressure being employed during handling and interchange of sidearms) it was established through periodic spectral scans that deoxyferrous myoglobin and hemoglobin also reduce ferricytochrome c, themselves becoming concurrently oxidized. Since the oxyferrous and deoxyferrous hemoproteins did not similarly oxidize in the absence of ferricytochrome c or in the presence of ferrocytochrome c, and since ferricytochrome c did not undergo reduction in the absence of the reduced forms of the other hemoproteins, nor in the presence of their ferric forms, it is concluded that the ferrous forms of bovine myoglobin and human hemoglobin undergo a direct one-equivalent redox reaction (electron transfer) with bovine and equine ferricytochrome c. Subsequently, the same approach was followed in testing the possibility of a similar redox reaction between ferrylmyoglobin and ferrocytochrome c. The two were mixed, each at the 5 × 10^{-5} M level in 0.05M $NaPO_4$ (pH 7.5). Before mixing, horse heart ferricytochrome c was reduced with a small amount of dithionite followed by deionization and then dialysis against the buffer. Ferrimyoglobin (horse muscle, Sigma type I) was oxidized to the ferryl state with a slight excess of H_2O_2 followed by removal of residual H_2O_2 with catalase (beef liver, Worthington) which was mixed into the ferrylmyoglobin solution to a level of 10^{-7} M. The catalase preparation was pretested for

Table 3—Midpoint reduction potentials of selected redox couples[a]

Redox couple	E'_o (volts)	Source of E'_o value
O_2/O_2^-	−0.7	Wang, 1970
Mb^{3+}/Mb^{2+}	0.046	Mahler and Cordes, 1971
$Cyt.b^{3+}/Cyt.\ b^{2+}$	0.035	Mahler and Cordes, 1971
	0.038	Dutton et al., 1970
Hb^{3+}/Hb^{2+}	0.17	Mahler and Cordes, 1971
$Cyt.C_1^{3+}/Cyt.C_1^{2+}$	0.22	Mahler and Cordes, 1971
	0.22	Dutton et al., 1970
$Cyt.C^{3+}/Cyt.C^{2+}$	0.25	Mahler & Cordes, 1971
	0.25–0.3	Dutton et al., 1970
O_2/H_2O_2	0.27	Wang, 1970
	0.3	Mahler and Cordes, 1971
$H_2O_2/\cdot OH+H_2O$	0.4	Wang, 1970
Mb^{4+}/Mb^{3+}	0.84	George and Irvine, 1955
O_2^-/H_2O_2	1.3	Wang, 1970
$H_2O_2/2H_2O$	1.35	Wang, 1970
$\cdot OH/O\bar{H}$	2.3	Wang, 1970

[a] Mb = Myoglobin; Hb = Hemoglobin; Cyt.b reduces Cyt.C_1 in mammalian mitochondrial electron transport chain; Cyt.C_1 reduces Cyt.C in mammalian mitochondrial electron transport chain; The self-dismutation of O_2^- is probably mainly via the $2HO_2^\cdot \rightarrow H_2O_2 + O_2$ mechanism below pH 9 (Behar et al., 1970)

activity, and the ferrocytochrome c and ferrylmyoglobin were verified spectrophotometrically before mixing. Periodic spectral scans, starting immediately upon mixing, indicated concurrent oxidation of ferrocytochrome c and reduction of ferrylmyoglobin. Thus the reaction: $Cyt.c^{2+} + Mb^{4+} \rightarrow Cyt.c^{3+} + Mb^{3+}$ can at least partly explain the apparent inhibition of ferricytochrome c reduction by myoglobin during the xanthine oxidase reaction. The possibility of a competitive inhibition (myoglobin interception of O_2^-) also occurring cannot be ruled out, however, although unequivocally demonstrating same under the conditions employed presents considerable experimental difficulty.

In connection with mechanisms of oxymyoglobin autoxidation the same approach was taken to test whether ferrylmyoglobin can accelerate autoxidation of oxymyoglobin in a manner analogous to the autocatalytic peroxidation of ferrocytochrome c by ferricytochrome c plus H_2O_2 (Mochan and Degn, 1969). Periodic spectral scans indicated that when solutions of stable oxymyoglobin and ferrylmyoglobin are admixed a redox reaction between the two takes place, the sole product of which is ferrimyoglobin. Recently a report of the first comprehensive study of a redox reaction between myoglobin and cytochrome c appeared (Wu et al., 1972). In it the authors report a direct intermolecular electron transfer between sperm whale deoxyferrous myoglobin and horse heart ferricytochrome c. Thus it appears that electron transfer between hemeprotein couples having favorable redox potentials may be a rather general phenomenon.

The question of whether or not ferrimyoglobin (and ferrihemoglobin) are oxidized by O_2^- ($HO_2^\cdot$) as well as by H_2O_2 remains an open one. The results reported herein do indicate however, that O_2^- does not reduce or reductively oxygenate ferrimyoglobin or ferrihemoglobin under the same conditions under which the anion rapidly reduces ferricytochrome c. As to the reductive oxygenation of ferrimyoglobin and hemoglobin during irradiation of anoxic solutions of same, the results suggest that this is due to a sequential interaction of the hemoproteins with radiolytically produced $\bar{e}_{aq}$ and O_2 in that order, although one might expect $\bar{e}_{aq}$ to react preferentially with any O_2 present.

Regarding the possibility of dissociation of O_2^- from oxyhemoproteins during autoxidation the results suggest that this does not occur under the conditions employed. McCord et al. (1971) express the view that such autoxidation by oxygen belongs to a group of processes all of which generate O_2^- via a one-electron reduction of the O_2 biradical. However, Castro (1971) presents a theoretical argument against such an electron transfer from low valent transition metals to high field (low spin) Π bonding axial ligands (e.g., O_2, NO, CO, CN^-). He argues that such strong Π bonding ligands in fact render the hemoprotein heme-iron less susceptible to oxidation, and that oxyhemoproteins undergo autoxidation only when (1) a second electron from a source other than the heme iron is donated to the liganded oxygen (a two equivalent reduction of O_2), or (2) the liganded oxygen (superoxide anion) is protonated, thus destabilizing the complex and allowing for dissociation of the hydroperoxy radical ($HO_2^\cdot$), or (3) the globin moiety undergoes a conformational change such that it becomes less effective in exerting a stabilizing influence on the heme-oxygen complex. Castro's arguments are supported by other theoreticians (Halton, 1972; Wang, 1970; Williams, 1970; Ingraham, 1966; Taube, 1965; Nicholls, 1965). Indications are then that oxygen must be protonated in order to dissociate from the oxyhemoprotein complex via one-electron autoxidation, even though the molecular oxygen biradical largely withdraws an electron from the heme-iron coordination 'shell' during oxygenation of ferrous myoglobin or hemoglobin (Weiss, 1964; Pauling and Weiss, 1964; Maggiora et al., 1965; Wittenberg et al., 1970; Halton, 1972).

The proposed requirement for protonation is consistent with the fact that autoxidation of oxyhemoproteins is enhanced by lowering the pH of oxyhemoprotein-containing systems, including fresh red meat. Under such conditions a pH induced globin conformational change may also contribute to destabilization of the iron-oxygen bond. Such a dissociation of $HO_2^\cdot$ from oxymyoglobin or oxyhemoglobin is consistent with the oxygen utilization-evolution stoichoimetry meticulously determined by Brown and Mebine (1969) if one views oxyhemoprotein autoxidation as proceeding via the following variations of the same basic mechanism (steps 1 and 2 being common to each):

(A)

$$2MbO_2 + 2H^+ \rightarrow 2MbO_2H \rightarrow 2Mb^{3+} + 2HO_2^\cdot \quad (1)$$

$$2HO_2^\cdot \rightarrow H_2O_2 + O_2 \quad (2)$$

$$MbO_2 + H_2O_2 \rightarrow Mb^{3+} + H_2O + HO\cdot + O_2 \quad (3)$$

$$MbO_2 + HO\cdot \rightarrow Mb^{3+} + OH^- + O_2 \quad (4)$$

(B)

$$Mb^{3+} + H_2O_2 \rightarrow Mb^{4+} + H_2O + HO\cdot \quad (3a)$$

$$MbO_2 + Mb^{4+} \rightarrow 2Mb^{3+} + O_2 \quad (4a)$$

$$MbO_2 + HO\cdot \rightarrow Mb^{3+} + HO^- + O_2 \quad (5)$$

Each reaction sequence involves net utilization of 0.25 mole of and net evolution of 0.75 mole of O_2 per mole of ferrihemoprotein formed. Further, inability to detect H_2O_2 formation during autoxidation (Keilin, 1961), in spite of good experimental (Shtamm et al., 1970) and theoretical evidence that H_2O_2 is formed, is accounted for through its removal by reactions (3,3a) as rapidly as it is generated. The mechanistic model is consistent with both relevant theoretical and experimentally demonstrated myoglobin and hemoglobin chemistry, and it represents (H^+) induced one-equivalent autoxidation especially well. However it, like all similar proposed mechanisms, does not account for any number of possible side reactions in a matrix as complex as meat, and

therefore it is no doubt a gross oversimplification of events occurring at the molecular level as oxymyoglobin oxidizes at the surfaces of fresh red meat cuts.

Recently a report appeared in which the authors interpret their experimental results as indicating that autoxidation of shark oxyhemoglobin occurred via three different pathways under their conditions, one of them being O_2^- dissociation. This conclusion was based on concurrent epinephrine oxidation, part of which was inhibitable by superoxide dismutase (Misra and Fridovich, 1972a). Previously the authors cited this as the preferred chemical method for detecting O_2^- generation by various systems (Misra and Fridovich, 1972b). However, many compounds, including ferrihemoproteins, are known to oxidize epinephrine to adrenochrome (Valerino and McCormack, 1971), and $HO_2^\cdot$ very likely can as well. Further, $HO_2^\cdot$ most likely can react with superoxide dismutase copper as do O_2^-, and H_2O_2 (Symonyan and Nalbandyan, 1972), and probably $\cdot OH$ and other reactive species. Therefore, in our view their results are not unequivocal for O_2^-, and, in light of the foregoing discussion, $HO_2^\cdot$ dissociation is at least as satisfactory an interpretation.

Finally, in connection with the theory that autoxidation ensues only upon approach of either a second metal ion or a proton, the phenomenon of maximal autoxidation rate at half saturating $_pO_2$ might be explained by an "outer sphere" electron transfer (Castro, 1971) from the heme of deoxyferrous to the liganded oxyanion of oxyferrous hemoprotein molecules. If such an intermolecular two-equivalent reduction of oxygen predominates at low oxygen pressures one would expect an autoxidation rate maximum at half-saturating oxygen pressure at which the equilibrium amounts of the oxyferrous and deoxyferrous forms are equal. Below this pressure the fraction of oxygenated molecules drops off sharply, and above it the fraction of oxygenated molecules rises sharply to the saturation level. This would also be consistent with the known stability of oxyhemoprotein solutions under conditions of saturating O_2 plus near neutral pH. Moreover, it can explain the finding of George and Stratmann (1952) that while autoxidation at saturating $_pO_2$ followed simple first order (in unoxidized myoglobin) kinetics, at well below saturation the observed rate constants showed a complex second order variation with oxygen pressure according to the product of the concentrations of Mb^{2+} and MbO_2.

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JFS RESEARCH NOTES

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A Research Note
POST-SLAUGHTER pH VARIATION IN BEEF

INTRODUCTION

LACK OF UNIFORMITY in meat quality has long been a major problem in the meat industry. Although extensive work has been done to develop standards on the basis of muscle mass and muscle-to-fat ratio, little attention has been given to the use of biochemical processes causing variation in the properties of muscle for assessing meat quality. Among the biochemical processes that might be useful in this regard are above normal antemortem and faster than normal postmortem glycolysis and onset of rigor mortis. All these processes have been shown to have deleterious effects on the quality of beef (Khan and Lentz, 1973; Marsh, 1964; Webb et al., 1967), pork (Bendall and Wismer-Pedersen, 1962; Briskey, 1964; Sink et al., 1965; Tarrent et al., 1972) and poultry (DeFremery and Pool, 1960; Khan and Nakamura, 1970). More recently, pH changes in beef muscle within 1 hr after slaughter has been associated with the required aging time and tenderness, and appear to have potential as a practical method of segregating carcasses which would probably result in tough meat (Khan and Lentz, 1973). To determine the potential value of such a measurement information on frequency distribution of post-slaughter pH in commercially processed beef carcasses is desirable.

EXPERIMENTAL

THE pH MEASUREMENTS were made on over 1200 carcasses (choice steers and heifers) in two meat packing plants in central Canada. In one of the plants (plant A) measurements were made on five days during 1 wk of operation. At the other plant (plant B) measurements were made on 10 days during two separate weeks, three months apart. Information on the age and sex of the animals, length of time for which they were held before slaughtering and on the kind of feed used during the holding period was also collected.

All measurements were made after the carcasses were split into halves and moved off the killing floor. (1 hr post-slaughter). In all cases a combination electrode was inserted directly into the Semimembranosus muscle. During splitting of the carcass, this muscle is exposed near the aitch (ischium) and pelvic (tuber ischii) bones, and the pH measurement can be made without damaging the carcass. To determine between-muscle variation within a single animal, 10 carcasses were cut into primal cuts within 1 hr post-slaughter and the pH of 12 major muscles was measured.

RESULTS & DISCUSSION

1 HR POST-SLAUGHTER pH values varied widely between comparable animals (Table 1). The frequency distribution of post-slaughter pH also varied between the weeks of measurements. The largest number of carcasses in plant A had post-slaughter pH values of 6.6 or higher while the corresponding range for plant B was between 6.4–6.6. The number of carcasses having post-slaughter pH values of 6.25 or lower, varied by 2–22% between plants and time of measurement Post-slaughter pH values of 6.25 or lower indicate above normal ante- and/or early-mortem glycolysis and resulting faster

Table 1—Frequency distribution of post-slaughter pH of carcasses in two packing plants

Post-slaughter pH	Plant A[a] %	Plant B[b] % 1st wk	Plant B[b] % 2nd wk
5.95 or below	–	1.8	2.5
5.96–6.05	–	4.2	5.0
6.06–6.15	0.5	3.6	5.8
6.16–6.25	1.4	10.3	9.2
6.26–6.35	1.5	8.5	10.0
6.36–6.45	5.2	8.5	15.8
6.46–6.55	12.0	17.6	19.2
6.56–6.65	18.0	19.4	17.5
6.66–6.75	22.8	18.2	8.3
6.76–6.85	22.6	6.7	5.0
6.86–6.95	10.9	1.2	0.8
6.96–7.05	4.9	–	0.8
7.06–7.15	0.4	–	–

[a]Measurements made on 946 carcasses during 1 wk
[b]Measurements made on 165 carcasses during the first week and on 120 carcasses during the second week

than normal onset of rigor mortis which have been associated with less tender meat (Khan and Lentz, 1973). Carcasses having a low post-slaughter pH occurred in all groups of animals, irrespective of sex, holding time at the plant or the kind of feed during the holding time. Although plant B appears to have a higher incidence of low pH than plant A, no clear pattern as to the effect of processing conditions, source of procurement or breed of the animals could be established. These results indicate that a substantial number (up to 22.5%) of carcasses particularly in plant B had a pH value which would probably result in tough meat. This percentage would appear to justify the use of the pH test for segregating these carcasses.

Within a single animal, between muscles variation in 1-hr post-slaughter pH was 0.4 units or less (Table 2). Gluteus medius, Psoas major, Longissimus dorsi, had lower pH values than the average pH for all the muscles; Obliquus abdominis internus and Gastrocnemius had higher pH values than the average; and Gracilis, Semimembranosus and Semitendinosus had pH values closer to the average pH for all the muscles.

The results indicate a large variation in post-slaughter pH in comparable beef carcasses. Factors which probably lead to more than normal antemortem glycolysis and consequently to variation in the post-slaughter pH are, struggling at slaughter, anoxia following loss of the muscle blood supply and release of adrenalin following antemortem stress or the shock of stunning (McLoughlin, 1970). These factors have been related to the nervous stimuli reaching the muscle before or during slaughtering and may vary between animals (Bendall, 1966). Excessive antemortem glycolysis and rapid postmortem glycolysis have been shown to cause rapid onset of rigor mortis (Briskey, 1964; Khan and Lentz, 1973; Marsh, 1964). These conditions bring about changes in the properties of sarcoplasmic and myofibrillar proteins (Bendall and Wismer-Pedersen, 1962). Since the loss of solubility of myofibrillar proteins in beef has been directly correlated to toughness (Hegarty et al., 1963), it is plausible to conclude that similar changes may affect tenderness in low post-slaughter pH beef.

Table 2–Typical 1-hr post-slaughter pH values for 10 major muscles from a high post-slaughter and a low post-slaughter pH carcass

	Post-slaughter pH	
Muscle	High	Low
Biceps femoris	6.9	6.2
Gastrocnemius	6.9	6.3
Gluteus medius	6.7	6.1
Gracilis	6.8	6.2
Longissimus dorsi	6.7	6.1
Obliquus abdominis internus	7.0	6.3
Psoas major	6.6	5.9
Semimembranosus	6.7	6.2
Semitendinosus	6.7	6.2
Triceps brachii	6.9	6.3
(Avg pH for all muscles)	(6.8)	(6.2)

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A Research Note

EXAMINATION OF BONE CONTENT IN MECHANICALLY DEBONED POULTRY MEAT BY EDTA AND ATOMIC ABSORPTION SPECTROPHOTOMETRIC METHODS

INTRODUCTION

THE USE OF mechanical deboning procedures by the poultry industry has increased the yield of meat taken from the carcass, resulting in increased economical returns to the processor. Accompanying this technological advance has been a concern for the efficiency of bone removal from the resulting comminuted product. The Code of Federal Regulations (1971) states that the bone content of mechanically deboned poultry meat must not exceed 1%.

Basically, two approaches have been investigated in monitoring bone levels in this type of meat product. The first approach involves a physical separation of the tissue and fine bone particles and calculation of the percent bone residue present by weight (Kamm and Coffin, 1968; and Hill and Hites, 1968). A second approach involves a chemical determination of calcium present from which a percent bone solids (PBS) is calculated. This latter approach has been found to be more accurate than the physical methods (Kamm and Coffin, 1968).

The purpose of this study was to compare bone solids of five types of mechanically deboned poultry meat by two chemical methods of calcium determination.

EXPERIMENTAL

THE UNCOOKED MATERIAL to be deboned consisted of broiler backs, broiler necks and backs, whole spent layer carcasses and turkey racks. More specific details about the substrates are outlined in Table 1. All deboned meat was prepared for this laboratory by two Pennsylvania-based processing plants. Each used Bioun Deboning Separator-Strainers (Food Masters Inc., Boston, Mass.). 10-lb aliquots of deboned meat were placed in plastic bags and held at −18°C until tests were conducted.

EDTA titration method

Calcium content was determined by a modified method of Steagall (1966) as described in the technical bulletin "Raw Meat Deboner" (Beehive Machinery, Inc., 3360 Southwest Temple, Salt Lake City, Utah).

Atomic absorbance spectrophotometer method

A 10.0-g aliquot of sample was ashed and dissolved in 5 ml concentrated hydrochloric acid. The dissolved ash was diluted to 100 ml with demineralized water. A 25-ml portion of the diluted sample was then pipetted into a 50 ml volumetric flask with 10 ml of 5% lanthanum oxide-25% hydrochloric acid solution and diluted to the mark with demineralized water. A portion of this preparation was submitted to a Perkin-Elmer Atomic Absorption Spectrophotometer, Model 290 (Perkin-Elmer Co., Norwalk, Conn.). The percent absorption read from the spectrophotometer was converted to absorbance via a conversion chart supplied with the equipment. The calcium level for the diluted sample was determined from a standard curve. A dilution factor of 200 was multiplied to the observed calcium concentration to give a percent calcium present in the initial sample.

Conversion of percent calcium to PBS

The procedure for converting percent calcium to PBS was the same for both the EDTA titration and the atomic absorption spectrophotometer methods. The equation used for determining PBS were:

For young birds:

$$PBS = (\text{percent calcium} - 0.015\%)\,6.25$$

For mature birds and turkeys:

$$PBS = (\text{percent calcium} - 0.015\%)\,4.55$$

The factor 0.015% represents an approximate normal level of calcium present in the tissue. Factors 6.25 and 4.55 are recommended by the U.S. Dept. of Agriculture for use in determining bone levels from percent calcium.

Table 1—Description of mechanically deboned poultry meat analyzed in this investigation

Substrate	Identification	Age (wk)	Sex	Avg live weight (lb)	Machine capacity (lb/hr)
Broiler backs	Commercial	7–8	♂	3–4	3500
Broiler necks and backs (1:2)	Commercial	7–8	♂	3–4	3500
Spent layer carcasses	White Leghorn	> 52	♀	3–4	3500
Turkey racks	Broad breasted white	24	♂	25–26	2000
Turkey racks	Broad breasted white	52	♀	17–18	2000

Table 2—Average PBS for mechanically deboned poultry meat as determined by spectrophotometric and EDTA methods[a,b]

Substrate	PBS by method: Spectrophotometer	PBS by method: EDTA
Young male turkey racks	0.32	0.41
Mature female turkey racks	0.55	–
Broiler necks and backs	0.70	0.79
Broiler backs	0.79	0.82
Spent layer carcasses	1.21	1.44

[a]Each value represents a mean of two replications; each replicate consists of three observations.

[b]Means within each method connected by the same vertical line are not significantly different, $P < 0.01$

Data were examined statistically using a student's t-test (Steel and Torrie, 1960).

RESULTS & DISCUSSION

THE AVERAGE BONE solids for the five types of deboned meats are presented in Table 2. Deboned meat from racks of the young tom turkeys was found to contain the least amount of bone. Other samples in order from the least to the greatest amount of bone solids were mature female turkeys, broiler necks and backs, broiler backs and spent layer carcasses had increasing bone solids, respectively. There was a significant difference among PBS values of all the types of mechanically deboned poultry meat, except between the meat of broiler necks and backs and broiler backs ($P < 0.01$). Only the deboned meat from the spent layer carcasses had higher bone solids than is legally allowed. This would indicate that adjustments need be made to the deboning equipment for spent layer carcasses in order to bring the final bone solids values within the required limit of 1%. It is interesting to note that the deboned meat from the more mature types of poultry, mature female turkey racks and spent layer carcasses, had higher bone solids than the younger counterparts, young male turkey racks and broiler parts. This reflects the higher degree of calcification of the bones, causing more fragmentation when passing through the deboner, resulting in an increased level of bone particles. Only a very slight difference was found between the two broiler substances.

There was a high correlation ($r = 0.99$) between PBS values as determined by EDTA and spectrophotometric methods. However, all EDTA values were slightly higher than the spectrophotometric values. These higher values could be attributed to the presence of magnesium. Approximately the same method can be used for determining magnesium levels.

It is evident from the foregoing data that pre-slaughter age, reflecting the level of bone calcification, is an important factor affecting bone content of the mechanically deboned poultry meat. Of particular concern would be the spent layer carcasses which averaged more than the legal 1% limit. The EDTA and spectrophotometric methods gave very similar results.

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A Research Note

EFFECT OF FRANKFURTER CURE INGREDIENTS ON N-NITROSODIMETHYLAMINE FORMATION IN A MODEL SYSTEM

INTRODUCTION

OUR LABORATORY has recently confirmed the presence of dimethylnitrosamine (DMNA) in a small number of samples of commercial frankfurters (Wasserman et al., 1972). In view of the carcinogenic nature of most N-nitrosamines, this finding could be of significance from a public health standpoint. However, the random manner in which positive samples appeared made it impossible to relate DMNA formation to residual concentration of sodium nitrite in the frankfurters. Frankfurters containing measurable concentrations of DMNA can be prepared in the pilot plant when approximately ten times the legally-permissible level of sodium nitrite is used (Fiddler et al., 1972a). Inhibition or reduction of DMNA formation was demonstrated in the presence of sodium ascorbate (Fiddler et al., 1972b) which is commonly used as an accelerator for the development of the characteristic cure color. The effect of the cure components on DMNA formation in frankfurters is being investigated. We feel that valuable information can be obtained through the use of model systems because the isolation, determination and confirmation of nitrosamines in meat products are complex and time-consuming procedures. This paper reports the effect of cure ingredients on DMNA formation under conditions approximating those used in the processing of frankfurters.

EXPERIMENTAL

THE CURE INGREDIENTS were purchased from commercial suppliers and were of food grade purity or better. Samples of each ingredient used in this study were obtained from at least two sources. With the exception of NaCl, which was used at 2.5% concentration, the other cure ingredients were tested at the maximum levels permitted in comminuted meat products, based on the weight of meat (85% of the emulsion weight). In the model system the following concentrations of cure ingredients were added as desired to 25 ml of pH 5.6 buffer solution (0.5M KH_2PO_4 and NaOH) in which 0.444 mM dimethylamine hydrochloride had been dissolved: sodium nitrite ($NaNO_2$) (3.3 mg, 156 ppm); sodium nitrate ($NaNO_3$) (36.1 mg, 1720 ppm); glucono-Δ-lactone (GDL), sodium acid pyrophosphate (SAPP), sodium tripolyphosphate (STPP) (106.3 mg, 5000 ppm); ascorbic acid (AscH) (10.0 mg, 468 ppm) and sodium ascorbate (NaAsc), sodium erythorbate (NaEry) (11.6 mg, 547 ppm). Sodium nitrite was always added after the other cure components. The reaction mixture, in a 100 ml round-bottom flask equipped with a condenser and magnetic stirring bar, was heated and stirred for 2 hr at 71°C (160°F). After cooling, the mixture was extracted three times with 30 ml CH_2Cl_2, then the combined extracts were concentrated to 1 ml using a Kuderna-Danish concentrator. The amount of DMNA formed was determined by GLC and the identity confirmed by mass spectrometry. The details of the analytical procedures have been reported previously (Fiddler et al., 1972a).

RESULTS & DISCUSSION

THE FORMATION of DMNA resulting from the reaction of frankfurter cure ingredients with dimethylamine is shown in Table 1.

Sodium nitrite, alone or in combination with NaCl or $NaNO_3$, yielded approximately the same amount of DMNA [60 μg/liter (ppb)] indicating the latter salts had no effect on DMNA formation. Glucono-Δ-lactone (GDL) and SAPP are recommended for use as cure color accelerators. Sodium acid pyrophosphate was only recently approved for use in comminuted meat products (Federal Register, 1972). When used with $NaNO_2$, SAPP had no effect on the formation of DMNA; GDL, however, led to the production of almost twice as much of the nitrosamine. The action of GDL may be due to the fact that this compound reduced the pH of the buffer solution to pH 5.45–5.50 whereas SAPP had no effect. In equimolar concentrations, GDL is a stronger acid than SAPP, thus tending to favor formation of DMNA which has an optimum pH of formation of 3.4 (Mirvish, 1970). In some preliminary studies with frankfurters prepared with 1500 ppm $NaNO_2$, we have also noted an increase in DMNA present with the use of GDL (Fiddler et al., 1972c). A recent publication by van Logten et al. (1972) which appeared during the preparation of this paper reports that canned meat cured with large amounts of $NaNO_2$ had higher concentrations of nitrosamines when GDL was present compared to $NaNO_2$ alone. These authors, however, attached no significance to this observation.

Phosphates, particularly sodium tripolyphosphate (STPP), are used in many meat preparations to increase water retention, thereby decreasing loss due to shrinkage during processing. Although

Table 1—Effect of sodium ascorbate and sodium erythorbate on the formation of DMNA in model systems containing dimethylamine, $NaNO_2$ and other cure components

Cure component[b]	DMNA formed, μg/liter (ppb)[a]		
	No reductant added	NaAsc added	NaEry added
None	63	39	28
NaCl	59	35	27
$NaNO_3$	59	32	37
GDL	117	32	33
SAPP	58	28	27
STPP	43	27	25
AscH	38	—[c]	—[c]
$NaNO_3$ + GDL	101	28	31
NaCl + STPP	49	31	30
NaCl + SAPP + STPP	55	30	31

[a]Confirmed by mass spectrometry
[b]Concentrations used are described in Experimental
[c]Not studied

STPP is presently not permitted in frankfurters, it could enter this product when trimmings of meat products containing STPP are used in the formulation. STPP alone was found to inhibit DMNA formation to some extent in our model system.

The reductant NaAsc markedly inhibits the formation of DMNA. Similar results have been obtained with frankfurters prepared with a high concentration of $NaNO_2$ and the reductant (Fiddler et al., 1972b). Free ascorbic acid (AscH) has about the same inhibitory activity as the sodium salt. Sodium erythorbate (NaEry) or isoascorbate, is used more commonly by meat processors. In the model system when used alone with $NaNO_2$, NaEry appeared to have a somewhat greater inhibitory effect on DMNA formation than NaAsc. However, in frankfurter preparations the activity of both reductants was the same (Fiddler et al., 1972c). Combinations of the other cure ingredients with either NaAsc or NaEry resulted in similar levels of inhibition of DMNA formation. It is interesting to note that the enhancing effect of DMNA formation by GDL alone is nullified in the presence of the reductants. The mechanisms of these reactions in the model systems and comminuted meats are under investigation.

To date there has been a good correlation between our frankfurter study and the model system which does not contain meat or meat byproducts. However, it is still not known at this time whether the results obtained from this model system study are applicable to comminuted meats.

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The mention of commercial items is for your convenience and does not constitute an endorsement by the USDA.

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A Research Note

SOME OBSERVATIONS ON THE COLOR MEASUREMENT OF CANNED TUNA

INTRODUCTION

AN IMPORTANT FACTOR in the commercial value of canned tuna is the color of the product. It is known that the green luminous reflectance (Y) is the psychophysical correlate of the lightness, which is directly related to the visual score (Barrett et al., 1965). Since the visual color of canned tuna is judged mainly on the basis of the lightness parameter, it was, therefore, recommended by Little (1969a) that the Y values be used as the only means for routine objective color evaluation of canned tuna.

Two of the most important factors which influence the color measurement of a sample are the physical state and the manner in which it is presented to the instrument. The particle size has a great influence on the scattering of the incident light which, in turn, will affect the color of the sample. The results of color measurement of canned tuna using different sample holders and the effect of various sample preparations on the color values of canned tuna are reported herein.

MATERIALS & METHODS

THE FISH used in these experiments were frozen skipjack (*Euthynnus pelamys*), yellowfin (*Thunnus albacares*) and albacore (*Thunnus alalunga*). After being thawed, they were cooked in steam and packed in ½-lb cans in soybean oil.

Methodology

For each measurement four cans were opened, drained by gravity and mixed thoroughly in a bowl. One-half was taken and ground for 2 min in a Hobart grinder. The other half was pressed with a 13-lb weight for 10 min and was then ground for 2 min.

Sample holders

One sample holder (A) was an empty 8 cm diam can made of stainless steel with a well about 1.2 cm deep. The ground sample was placed in the sample holder and pressed gently a few times with a tapper. The color measurements were also carried out using this sample holder and a Corning micro cover glass (22 mm square and 0.1 mm thick) mounted on the orifice of the search unit of a Photovolt colorimeter.

The second sample holder (B) was the same as described by Little (1969a).

A Hobart silent cutter was used for grinding the tuna. An attachment to this silent cutter permitted the use of an ordinary meat grinder (Keystone) with two interchangeable plates with 1/10 in. and 1/20 in. diam holes.

Some color evaluations were also performed by passing the drained, oil-expressed tuna meat through a stainless steel ¼ in. mesh sieve.

Color measurement

Reflectance characteristics of the samples were measured relative to a standard MgO plate using a tristimulus Photovolt colorimeter.

RESULTS & DISCUSSION

Variation of color values from application of different sample holders

As indicated earlier, one of the major factors influencing color measurement is the manner in which the sample is presented to the colorimeter. Sample holder B had a lucite cover placed on top of the tuna meat before the sample was presented to the instrument for color evaluation. This cover was a transparent object which absorbed some of the incident and reflected light according to Lambert's law during the color measurement.

The incident light after passing through the lucite is reflected at randomly oriented internal interfaces, and only a fraction of this reflected radiation is transmitted back through the initial interface (Clydesdale, 1972). There is also a significant possibility of refraction and diffusion at the boundary. Therefore, one expects to get lower reflectance values using sample holder B than A which lacks a cover.

Table 1 shows the tristimulus green values of canned skipjack using two different sample holders A and B. Color measurements were carried out at three different time intervals: 0, 5 and 20 min after the samples were prepared by grinding them with a Hobart grinder.

The results indicated that in all cases higher Y values were obtained when the drained, oil-expressed canned tuna meat was not pressed. Perhaps the increase was due to the increase in reflection of light in the presence of free and excess oil and juices on the surface of the unpressed sample.

Sample holder B, on the other hand, resulted in lower Y values in both unpressed and pressed tuna samples when compared with holder A. However, this

Table 1—Tristimulus green (Y) values of chunk skipjack canned tuna[a]

Sample holders	Tuna sample[b]	Y_1 At zero time	Y_2 After 5 min	Y_3 After 20 min
A	ground and unpressed	33.33 ± 0.83	32.35 ± 1.03	31.08 ± 0.76
A	ground and pressed	31.55 ± 1.45	30.72 ± 1.24	30.15 ± 1.48
A	ground and unpressed with a cover slip	33.85 ± 1.22	32.36 ± 0.54	31.25 ± 0.42
A	ground and pressed with a cover slip	30.90 ± 1.81	30.38 ± 1.70	29.50 ± 1.65
B	ground and unpressed	30.74 ± 1.74	30.53 ± 1.54	30.25 ± 1.63
B	ground and pressed	29.32 ± 1.51	29.07 ± 1.74	28.85 ± 1.82

[a] Tristimulus green (Y) values are percent reflectance at CIE-XYZ system using a green filter having a maximum absorption band at 555 nm.

[b] Composite of four cans were used for each measurement. Five measurements were made and five readings were obtained in each measurement. The standard deviations were calculated and tabulated.

Table 2–Effect of different sample preparations on the green reflectance (Y) values of chunk packed skipjack tuna[a]

Types of grinder used for sample preparation[b]	Y_1 At zero time	Y_2 After 5 min
Hobart[c]	30.35 ± 2.09	29.63 ± 1.61
Keystone with 1/20 in. holes plate[d]	30.27 ± 1.13	30.18 ± 1.00
Keystone with 1/10 in. holes plate	28.86 ± 1.20	28.74 ± 1.20
Sieve, with 1/4 in. holes	28.42 ± 1.89	27.40 ± 1.80

[a] See footnote a, Table 1
[b] See footnote b, Table 1
[c] Hobart grinder is an electric grinder with a rotary blade and bowl.
[d] Keystone meat grinder is an ordinary screw-type hand grinder which could be attached to a Hobart grinder and operated electrically.

sample holder showed an insignificant change in Y values even after 20 min delay in color measurement. This was in contrast to holder A which showed a two-point drop in Y values after 20 min of exposure to air. The darkening and discoloration was obviously a direct result of oxidation of heme-pigments present on the surface of the sample.

The results obtained with sample holder A and a Corning micro-cover glass mounted on the orifice of the search unit of the Photovolt colorimeter showed no change in Y values as compared to holder A with no cover slip. It was thought that the use of a cover slip would reduce the variation of Y values by forming a relatively smooth surface of tuna meat and eliminating unnecessary reflections due to the roughness of the surface. However, the results showed that the standard deviations were in the same order as in other cases, and the fact that no reduction in Y values were observed, as in case of B holder, indicated that there was none or very slight light absorption because of small thickness of the cover slip (0.1 mm as compared to 1.5 mm in holder B).

Measurement of green reflectance of canned yellowfin and albacore tuna resulted in the same kind of observations. Since skipjack tuna meat has a higher content of heme-pigments than yellowfin and albacore, one would expect to see a sharp decrease in Y values as the oxidation progresses, but the results showed a similar (about two points) change in Y values after 20 min of surface oxidation. This indicated that either the available pigments which undergo oxidation are a minor constituent of the whole pigment or the rate of oxidation and, thus, discoloration is slow (Little, 1969b). It should be noted that the absorption of light by meat is only partially due to the heme-pigments (Clydesdale and Francis, 1971).

Based on the above facts and findings, it is recommended that sample holder A be used in quality control laboratories for color evaluation of canned tuna. One would save a considerable amount of time by using this sample holder.

Effect of sample preparation on the luminous reflectance, Y

The luminous green reflectance values of canned skipjack tuna prepared by different methods are presented in Table 2. The results indicated that the particle size of the ground tuna meat had a pronounced effect on the Y reflectance values. The highest Y values were obtained when a Keystone meat grinder plate with 1/20 in. holes was used. It also gave the least standard deviation. The Hobart grinder yielded results with a slightly higher standard deviation.

The conventional ¼ in. sieve, which is used in most quality control laboratories, resulted in smaller Y reflectance values and relatively high standard deviations. This is, perhaps, due to the fact that tuna meat which is passed through the sieve by force, not only lacks a definite shape and size, it is also composed of relatively large-sized particles, which could decrease the internal diffusion and result in lower reflectance values. On the other hand, an ordinary meat grinder will result in more or less uniform-sized particles and its use is much easier, less cumbersome and less time consuming.

When the plate with 1/20 in. holes was replaced by a plate with 1/10 in. holes, the green reflectance values decreased significantly.

Experiments performed with a grinder having plates with holes smaller than 1/20 in. resulted in difficulties with the passage of ground tuna through the plate's holes.

It is interesting to note that the green reflectance values decreased very slightly after 5 min of air oxidation when a Keystone grinder was used. There is not a good explanation for the anomaly. Perhaps when tuna meat is ground up in this manner, the free oil and juices coat the particles to an extent that reduces penetration of oxygen and the rate of discoloration. This is in contrast to the sieve and Hobart grinder which have no such mixing and sizing effects. The results reported here indicate that a regular meat grinder, such as a Keystone with a plate with 1/20 in. holes, has a great advantage over a sieve and other types of more expensive devices in that it produces much more uniform sized particles which, in turn, yield higher reflectance values and lower deviations. It is also less time consuming and more economical. The higher reflectance values are, perhaps, a result of physical scale shift. Additional work is in progress to establish the relationship between the above scale and visual evaluation of the color.

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A Research Note

QUANTITATIVE CHANGES IN WHOLE MYOFIBRILS AND MYOFIBRILLAR PROTEINS DURING FROZEN STORAGE OF TRUE COD

INTRODUCTION

AN INCREASE in toughness of fish portions during frozen storage has been suggested as the primary cause of decreased organoleptic acceptability (Tarr, 1965). At the ultrastructural level, this is manifested by a hardening of the sarcolemma, and the myofilaments becoming more fibrous (Tanaka, 1965). Previous studies have suggested that this is the result of insolubilization of the myofibrillar proteins (Dyer, 1951; Luijpen, 1957; Ravesi and Anderson, 1969; and Babbitt et al., 1972). At present no studies, to the author's knowledge, have centered on the effects of frozen storage on the whole myofibril unit. With this in mind, experiments were undertaken to determine (a) if there is a decrease in the quantity of whole myofibrils which can be extracted from frozen, stored fish portions; and (b) if the decrease correlates with decreases in total myofibrillar protein extractability.

EXPERIMENTAL

PACIFIC TRUE COD *(Gadus macrocephalus)* was obtained 1 day post-catch and filleted. Portions were blocked in 1 × 3.75 × 21 in. stainless steel trays and frozen in a −40°C blast freezer. 24 hr later, the blocks were sawed (in a 0–2°C room) into 1 × 3.75 × 1.6 in. portions, packed in evacuated moisture-vapor proof film, and held at −40°C until analysis at 0, 3 and 6 months.

Whole myofibrils were extracted from fresh and stored samples as previously described (Childs, 1973). 8g of fish muscle were used for each extraction. For quantification, the final myofibril pellet was washed out of the centrifuge tube with copious amounts of deionized water, dried at 105°C for 12 hr, and the dry weight of the extracted myofibrils determined.

Total extractable myofibrillar protein was measured by an adaptation of methods described by Ravesi and Anderson (1969). 5g of minced fish muscle was homogenized for 5 min in 145 ml of 5% NaCl and 0.02M $NaHCO_3$. A Virtis 45 homogenizer with a baffle plate to prevent foaming was used at medium speed. The volume of the homogenate was determined and an aliquot centrifuged at 2000 × G for 20 min. The protein content of the supernatant was measured by biuret analysis (Snow, 1950). Data were calculated as g protein/g muscle on a wet weight basis.

RESULTS

THERE WAS a marked decrease in the amount of whole myofibrils which could be extracted from frozen, stored portions (Fig. 1). Regression analysis of time in storage (x) vs. total extracted myofibrils (y) provided a correlation coefficient of −0.998 and a regression equation given by:

$$y = -0.047(x) + 0.301$$

Total extractable myofibrillar protein also decreased during frozen storage (Fig. 2). Previous workers have suggested that this decrease should be logarithmic (Love and Olley, 1965; and Connell, 1968). Regression analysis of time in storage (x) vs. log total extractable protein (y) provided a correlation coefficient of −0.982 and a regression equation given by

$$y = -0.019(x) - 0.779$$

Regression analysis was also performed for an arithmetic relationship of time in storage (x) and total extractable protein (y). In this case the correlation coefficient was −0.988 and the regression equation was

$$y = -0.006(x) + 0.166$$

From these data, it is not possible to define the kinetics of the decrease in extractable myofibrillar protein, because the spread of means is not adequate to allow possible differences in logarithmic and arithmetic plots to be expressed. Because of the small range in means, t-function analyses were performed and the means were found to be significantly different at $\alpha = 0.10$.

Regression analysis was also performed to detect possible correlations between decreases in total extractable myofibrillar protein (x) and extractable whole myofibrils (y). A correlation coefficient of 0.986 was calculated and the regression equation was found to be

$$y = 7.27(x) - 0.906$$

When log total extractable myofibrillar proteins was used as x, the correlation

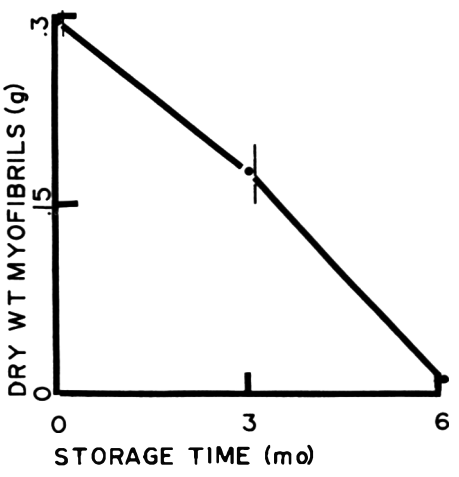

Fig. 1—Decrease in the amount of extractable myofibrils in frozen stored true cod. Points are the mean of at least four replicates ± standard error.

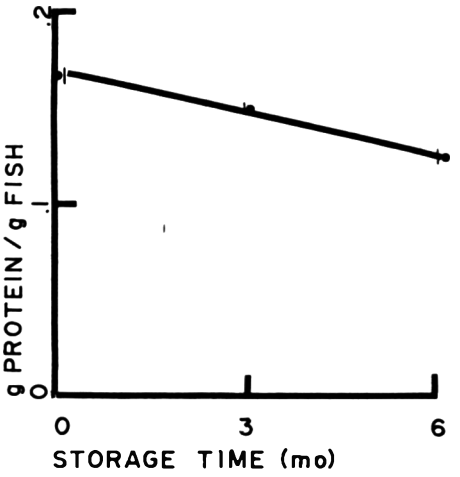

Fig. 2—Decrease in the amount of extractable myofibrillar protein in frozen, stored true cod. Points are the mean of at least four replicates ± standard error.

coefficient was 0.992 and the regression equation was defined as

$$y = 2.39(x) - 2.62$$

Thus a correlation does exist, but from these data no cause-effect relationship should be inferred.

DISCUSSION

RESULTS obtained in this study suggest that during frozen storage of true cod the extractability of whole myofibrils decreased more rapidly than the combined extractability of component myofibrillar protein. It is not possible at this time to rule out the possibility that a portion of the difference may be artifactual, and result from limitations in available methodology. For example, Ravesi and Anderson (1969) have shown that the amount of myofibril protein which can be extracted from frozen fish muscle is a function of blending time, with longer periods allowing more extraction. Because a shorter homogenization time is used during whole myofibril isolation than total myofibrillar protein extraction, the greater decrease in extractability of whole myofibrils during frozen storage may be a partial reflection of extraction methodology. Therefore, it is necessary to undertake studies with known denaturants to determine if the whole myofibril is insolubilized before all of its component proteins. Such a study utilizing formaldehyde is in progress in this laboratory.

It should be emphasized that these studies have been limited to Pacific true cod, and at this time the utility of quantity of extractable myofibrils as an index of storage time cannot be generalized to other species. Work with other species is in progress. In addition, work must be performed to ascertain the effects of pH, biological condition and bacterial spoilage on the quantity of extractable whole myofibrils.

There is a continuing desire to correlate changes observed in frozen fish muscle with organoleptic acceptability. While some have observed meaningful correlation, others have not (Dyer, 1951; Luijpen, 1957). In this laboratory, judges have been able to differentiate between fresh and frozen-stored samples when both were available for simultaneous scrutiny. A 20% decrease in textural preference of frozen-stored samples relative to fresh samples was noted. However, when judged at separate times no differences could be ascertained. Further, Crawford and Law (1972) have found flavor panels to prefer breaded, deep-fried frozen, stored hake over fresh portions. These observations would suggest that the consumer may not be able to differentiate frozen from fresh portions, or may actually prefer frozen, stored portions. From a practical, experimental point of view it would thus seem more desirable to determine the effects of changes noted in this study on utility of fish muscle in further processed items, rather than search for the elusive key to organoleptic correlation.

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A Research Note

USE OF THE PENETROMETER FOR DEFORMATION TESTING OF FOODS

INTRODUCTION

THE DEFORMABILITY of a food is a physical characteristic that is widely used as a measure of food quality. Szczesniak and Bourne (1969) have shown that deformability of foods is frequently used as a sensory method for evaluation of food firmness. Bourne (1967) has shown that in order to obtain the best resolution between samples of similar deformability it is desirable to use small deforming forces and in addition, it was shown that it is desirable to introduce a small preloading force in a deformation test in order to eliminate the error introduced by irregularities in the surface of the article of food. Bourne used the Instron in this work because it gives accurate deformation values for both rigid foods and soft foods.

The penetrometer is an instrument that is widely used, particularly for measuring the firmness of edible fats and similar products. It consists basically of a cone attached to a vertical shaft that can be locked in any given position. In operation the tip of the penetrometer cone is placed on the surface of the food to be tested. The shaft is unlocked and the cone and shaft assembly is allowed to sink into the food under the force of gravity for 5 sec after which it is locked again and the depth of penetration is measured to the nearest 0.1 mm on the dial gage. Penetrometers cost approximately $200–$600 each depending on the design and whether an automatic timing device is part of the assemblage or not. The low cost of the penetrometer renders it an attractive candidate for routine-testing of foods. We have found that the penetrometer can be adapted quite easily to perform deformation tests provided that a fairly soft commodity is being tested.

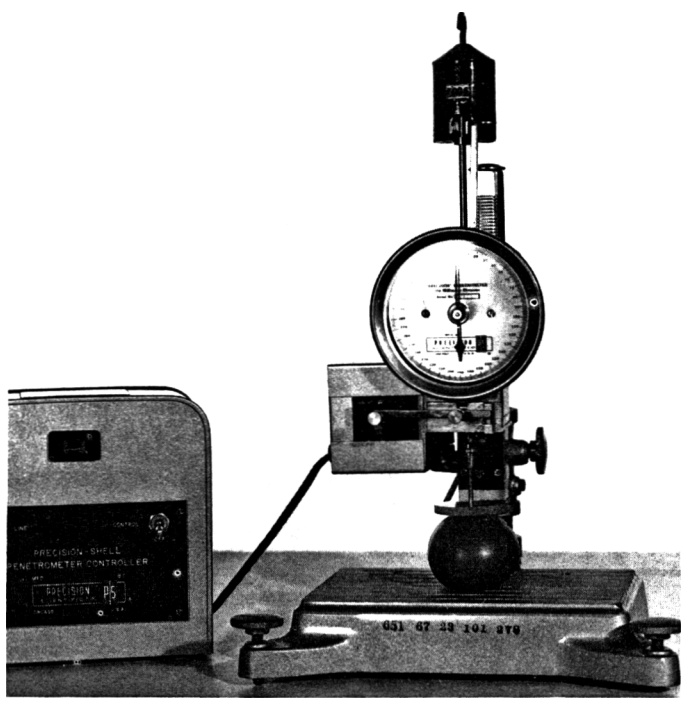

Fig. 1–Penetrometer being used for a deformation test. Note the flat disc that replaces the cone and compresses the tomato, and the weight at the top of the shaft.

EXPERIMENTAL

A 5 CM DIAM DISC, 0.5 cm thick, was cut from a piece of hard plastic and a 1/8 in. diam brass rod was inserted in the center of the disc, normal to the plane of the disc. One end of the brass shaft was level with the lower side of the disc and the other end extended approximately 3 cm above the upper side of the disc. This attachment was inserted into the penetrometer in place of one of the usual cones. The disc and unloaded shaft weighed 64g. The article of food is placed in position (see Fig. 1) and the plastic disc is brought down close to the surface of the article by means of a rack and pinion. The penetrometer is turned on and the disc and shaft are allowed to fall freely for 5 sec and then locked. The distance that the shaft and disc have fallen is measured on the dial gage. A selected weight (preferably as small as possible) is then placed on the upper end of the shaft and the weighted cone and shaft assembly is allowed to fall freely for 5 sec and then again locked and a second reading is taken from the dial gage. The difference between the two dial readings gives the deformation of the article of food in units of 0.1 mm.

In normal penetrometer testing with a cone, it is critical that the point of the cone be placed exactly at the surface of the food. In the deformation test the initial placement is not critical, since this is a test by difference. The compressing disc should be brought close to the article of food because the readings may be affected if there is an extensive distance of free-fall; however, the initial placement is not critical. The 64-g weight of the unloaded disc and shaft assembly is sufficient to give the small preliminary compression that eliminates those errors that might be caused by intrinsic irregularities in the surface of the food piece.

RESULTS & DISCUSSION

FIGURE 2 shows the deformation of two tomatoes that were picked in the green-ripe stage and held in a ripening room at 65°F. The X on the graph denotes the day on which the first sign of pink appeared on the blossom end of the tomato. Figure 3 shows the change in deformation of a single jumbo-sized marshmallow that was allowed to age on the bench. One advantage of the deformation test is that it is nondestructive and one article of food can be used repeatedly.

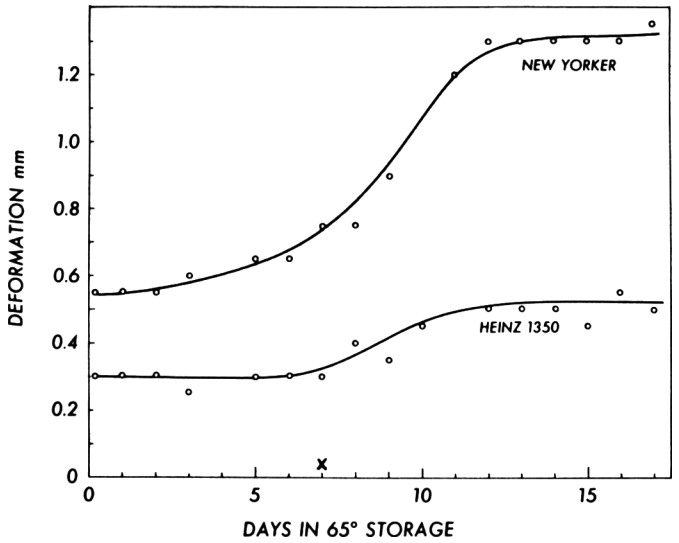

Fig. 2–Change in deformation of two tomatoes as they ripen. New Yorker is a medium soft variety, Heinz 1350 is a relatively firm variety. Deforming force 500g.

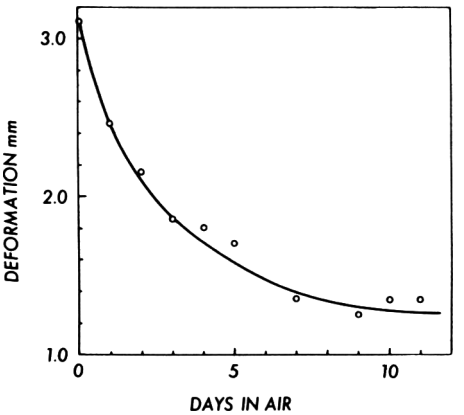

Fig. 3–Change in deformation of a single jumbo marshmallow exposed to air without packaging. Deforming force 100g.

Figures 2 and 3 show that the penetrometer can be a useful instrument for measuring deformability of soft foods such as tomatoes and marshmallows. Although the weight that is used for deformation can be varied according to the need, the previous work of Bourne, (1967), showed that it is desirable to make this as small as possible consistent with obtaining measurable readings on the dial of the penetrometer. The dial readings become so small with rigid foods that the precision of measurement is lost.

The technique has the advantage that the penetrometer is an inexpensive instrument that is easily adapted to the deformation test. Provided that the food being tested is soft and of reasonable size this should prove a simple and effective method for measuring the deformability of foods. It is not as accurate as the Intron method, but it could be used for routine quality control tests and in other applications where low cost and high testing speed are more important than extreme accuracy. The manually operated model can be operated outside the laboratory and would probably be useful to plant breeders and others who are interested in field testing.

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A Research Note

A SIMPLE SHEAR PRESS FOR MEASURING TENDERNESS OF WHOLE SOYBEANS

INTRODUCTION

THE PREPARATION of canned and dried food products from whole, full-fat soybeans was described by Nelson et al. (1971). An important quality characteristic of these products is the tenderness of the soybean. A number of fine instruments are available for measuring tenderness; the L.E.E. Kramer Shear Press (Kramer et al., 1951) is one example. This shear press simulates the action of teeth by first compressing and then shearing the food (Kramer and Twigg, 1959). However, such instruments are generally quite sophisticated and consequently, expensive. The purpose of this communication is to describe a reliable instrument that is inexpensive, portable and manually operated.

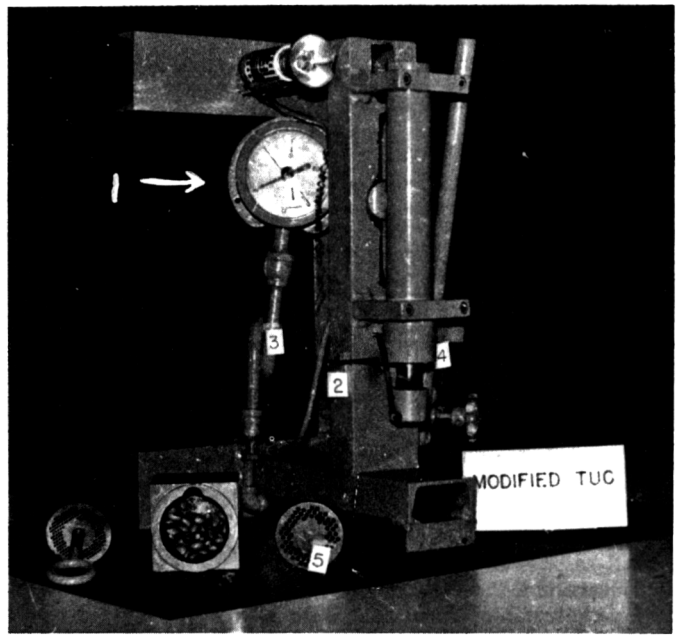
MATERIALS & METHODS

FIGURE 1 shows a Succulometer (The United Co., Westminister, Md.) modified to operate as a shear press. The Succulometer was turned 90° so the piston entered the cup from above. A maximum-reading needle was inserted in the face of the pressure gauge (1, Fig. 1). A battery operated light was added to indicate when the jack piston had extended a constant distance into the cup (2, Fig. 1). A pigtail connection (3, Fig. 1) was used between the gauge and the hydraulic cylinder. A stop (4, Fig. 1) limiting the length of stroke of the pump handle was added. The final change was the substitution of the solid block with a 3/8 in. thick copper plate (5, Fig. 1) with 72 holes, 1/8 in. diam.

A determination can be performed simply and quickly. A weighed sample was placed in the sample chamber and the copper plate placed on it. The chamber was inserted into position under the piston. The hydraulic piston was manually pumped until the light went on, indicating a constant penetration of the perforated plate through the soybean sample. The maximum pressure developed was related to the tenderness of the bean.

Sample preparation

Instrument evaluation was done with Wayne variety soybeans at 11% moisture taken from the same lot. Three methods of processing were used to produce samples of beans that ranged from very tough to very tender. Relatively tough samples were prepared by boiling 300g of soybean in 2000 ml of tap water for various times. The soybeans were then cooled in tap water, drained and weighed. The second method was to soak 300g of soybeans in 2000 ml of



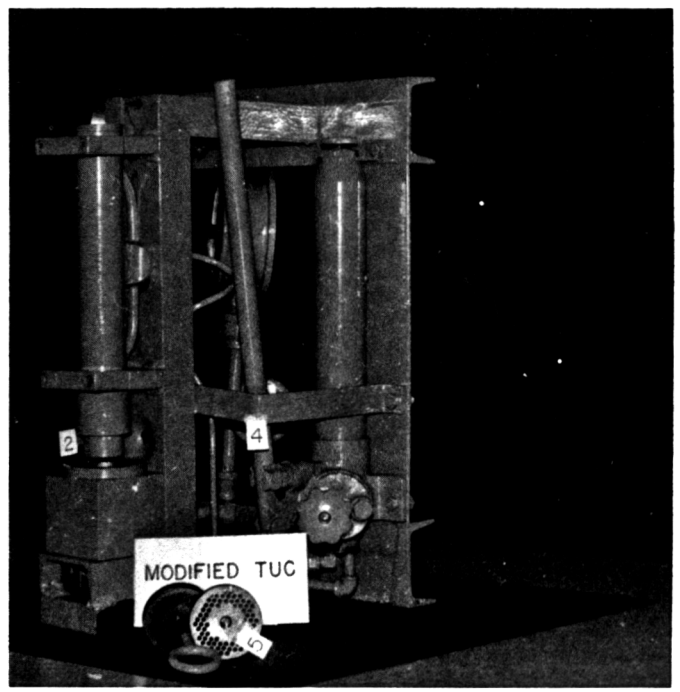


Fig. 1—Left and right views of the simple shear press.

Table 1–A comparison of pressure readings of the L.E.E. Kramer Shear Press and the Simple Shear Press on whole soybeans treated in various ways

Treatments	L.E.E. Kramer 1000 range max. point readings in psi Avg. of duplicate readings	Simple Shear Press Readings in psi Avg. of triplicate readings
Boiling time in tap water (min)		
30	280.0	890.0
45	225.0	781.6
60	210.0	683.0
75	205.0	590.0
90	190.0	577.0
	Correlation coefficient r = 0.9981	
Soaked 15 hr in 0.5% $NaHCO_3$ and boiled in 0.5% $NaHCO_3$ (min)		
30	152.2*	375.0
45	106.5*	248.3
60	63.0*	133.3
75	47.2*	109.3
90	39.0*	108.3
	Correlation coefficient r = 0.9985	
Retorted at 250° F (min)		
30	76.5*	171.6
60	40.5*	81.6
90	27.7*	61.6
	Correlation coefficient r = 0.9991	

* Points taken at 300 range and converted to 1000 range

tap water containing 0.5% $NaHCO_3$. After 15 hr of soaking, the soybeans were drained and boiled for various times in 0.5% $NaHCO_3$ solution. The soybean sample was then drained, cooled in tap water, drained again and weighed. The third method was to seal 100g of soybeans with 200 ml of tap water in a #2 can and retort at 250° F for various times. After the heat treatment the cans were cooled and the contents of three cans of the same treatment were combined. The free liquid was drained and the samples were weighed.

The drained weight of beans obtained from these treatments ranged from 608 to 703g; one-tenth of the total weight of the beans was placed into the Simple Shear Press chamber for testing. The object was to obtain the same amount of soybean solid matter with every test. Determinations were made in triplicate. One-sixth of the total weight of the beans was also tested, in duplicate, with the L.E.E. Kramer Shear Press (Matz, 1962).

RESULTS & DISCUSSION

TABLE 1 shows a comparison of whole soybeans treated in various ways and tested with both the L.E.E. Kramer machine and the simple shear press. The maximum pressure in psi shown by the pressure gauge was recorded from each instrument. The average reading from each instrument was compared to that from the other. Samples that were boiled in tap water for various times and tested by both machines had a correlation coefficient of 0.9981. Samples that were soaked 15 hr in 0.5% $NaHCO_3$ and then boiled for various times in a similar solution had a correlation coefficient of 0.9985. The correlation coefficient of retorted samples was 0.9991. The overall coefficient of correlation among all 13 treatments was 0.9933. The coefficient of variation of all 13 samples ranged from 0 to 12.75% and the average was 4.61%.

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A Research Note

FLAVOR DETECTION THRESHOLD VALUES FOR ETHYL CAPRYLATE AND PHENYL ETHYL ALCOHOL AND ESTIMATES OF THE PERCENT POPULATION HAVING GREATER SENSITIVITY

INTRODUCTION

ONE PURPOSE of this note is to present data relative to threshold values for ethyl caprylate and phenyl ethyl alcohol in water, 10% ethanol and corn oil. The second is to make two suggestions as to means of estimating the percent of the population whose thresholds will be even lower than the lowest value observed.

There appears to be only four threshold values reported for ethyl caprylate (Stahl, 1973): two for taste and two for odor. There is one value reported for phenyl ethyl alcohol, as odor. There are very few comparisons of the thresholds of individuals for the same substance in different solvents such as that of Wasserman (1966) who determined threshold levels for guaiacol, 4-methyl guaiacol and 2,6-dimethoxyphenol in water and mineral oil. Honkanen et al. (1964), Keith and Powers (1968), Salo (1970a, b) and Salo et al. (1972) have reported threshold values for ethyl caprylate and phenyl ethyl alcohol. Interactions of taste substances with alcoholic solutions have been studied by Berg et al. (1955), Pangborn et al. (1964) and Martin and Pangborn (1970).

EXPERIMENTAL

IN EARLY 1971, 10 randomly-selected judges were used to establish threshold values for ethyl caprylate in water, 10% ethanol and corn oil. In mid-1972, the trials were repeated with a new panel (17 different judges). Phenyl ethyl alcohol was then also included as a test substance.

Each panelist was asked to state whether two samples of a pair were alike or different. Like pairs consisted of two samples of solvent or solvent plus the test substance. Unlike pairs consisted of a particular concentration of the test substance and plain solvent. At each session, 18 pairs were evaluated. To bracket the threshold, the solution was initially changed in strength by 1/10 or 10, according to whether the solution was above or below the panelist's threshold. Once bracketing had been accomplished, solution strengths were changed by 1/2 or 2 to narrow the range. When each panelist was approximately at this threshold, sets of 18 pairs were presented to him in order of increasing concentrations, i.e., within any one set the concentration was the same, but each set was increased in concentration until the panelist designated correctly 13 out of 18 pairs as being either the same or different. Plotting of the percent matching answers versus concentration on probability paper yielded a straight line near the threshold concentration. The threshold was graphically selected (ASTM, 1968) except that the matching percentage used was 70 instead of the 75% recommended by the ASTM. The 75% matching level is defined as the "difference" threshold. The absolute threshold is defined as the concentration yielding 50% matching responses, but the 50% level is plain nonsense for paired comparisons. By chance alone, the panelists should be correct about 50% of the time. The 70% matching level was chosen because it is close to the 0.05 statistical probability level (13 out of 18 pairs being 72.2%).

RESULTS

TABLE 1 shows the panel means and the ranges for the nine sets of trials. The means and the upper levels for the 1972 trials were higher because a greater portion of the panel members was less sensitive than the 1971 panel members. The most sensitive members of both panels had about the same degree of sensitivity.

Sensitivity to ethyl caprylate and to phenyl ethyl alcohol was lower when the solvent was 10% ethanol than when water was it. For both chemicals, the slopes of their regression lines were much greater when water was the solvent. There was no significant difference between the slopes when oil and 10% ethanol were the solvents. Figure 1 shows the regression of thresholds on concentration of ethyl caprylate for the 1971 and 1972 data combined.

Correlation coefficients were calculated for the threshold of each judge to each chemical between each possible pair of solvents. For the 27 pairs of threshold values for ethyl caprylate in water and 10% ethanol, for example, the correlation coefficient was 0.803, which is significant at $P = 0.001$, but this still accounts for only 64.5% of the relationship. The same was true for the other combinations of solvent and of chemical. General trends existed, but individuals with low thresholds for the test substance in one solvent were not always the ones with the lowest thresholds for the same substance in the other two solvents. At the extreme, one panelist who was the most sensitive to ethyl caprylate in 10% ethanol was least sensitive to it in corn oil.

When the panelists were asked to taste samples while holding their noses, the thresholds were generally much higher. The thresholds observed agreed fairly well with prior studies for these compounds for either odor or flavor (Honkanen et al., 1964; Keith and Powers, 1968; Salo, 1970a, b; Salo et al., 1972).

The results of the 1972 trials were compared with the extreme-value regression lines and the lower 95% confidence limits for the 1971 data using the procedure employed by Powers et al. (1971). When alcohol was the solvent, two threshold values for the 1972 trials, i.e., 2.5 and 1.43 ppm, were lower than the lowest of the 1971 trials. Based upon the 1971 regression line, 8% and 2.2% of the population would be predicted as having threshold levels as low as 2.5 and 1.43 ppm, or, at the lower 95% confidence limit, 23.5 and 9.3% of the population might have thresholds as low as 2.5 and 1.43 ppm. Based upon 17 judges, the observed percentages were 8 and 4%, well within the lower 95% confidence limit.

Table 1—Flavor detection threshold values for ethyl caprylate and phenyl ethyl alcohol

No. of judges	Solvent	Threshold, mg/liter: Panel mean	Range
	Ethyl caprylate, 1971 trials		
10	Water	1.78	0.15 – 7.0
10	10% ethanol	6.07	3.0 – 14.5
10	Corn oil	146.9	25.0 – 676.0
	Ethyl caprylate, 1972 trials		
17	Water	15.7	0.25 – 50.0
17	10% ethanol	15.2	1.43 – 66.0
17	Corn oil	224.5	50.0 – 1,000.0
	Phenyl ethyl alcohol, 1972 trials		
17	Water	41.1	0.175 – 400
17	10% ethanol	33.4	6.3 – 150
17	Corn oil	174.4	25.0 – 600

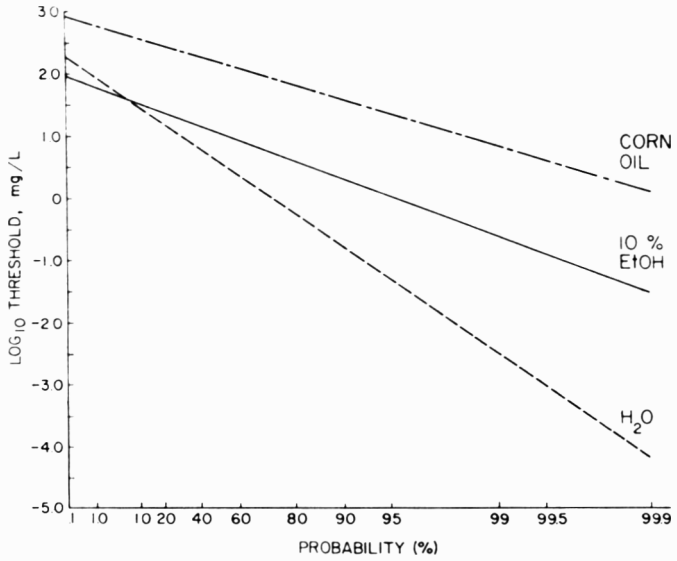

Fig. 1—Extreme-value regression of thresholds for ethyl caprylate on concentration. The regression curves are based on the thresholds of 27 judges. The three solvents were distilled water, 10% ethanol and corn oil. To simplify graph confidence limits are not shown.

The 1972 set of trials did not conform as closely to the 1971 set as did the two trials Powers et al. (1971) made with quinine sulfate, but extreme-value analysis seems to have merit. In air and water pollution especially, it would be desirable to be able to estimate the percentage of the population which might be more sensitive to a compound than panel tests indicate. Quite obviously for such purposes, the estimate should be made from results of a larger panel than the 10–27 individuals used here. An alternate procedure would be to use nonparametric estimates such as were published by Powers et al. (1962). Table 2 shows estimates of the percent of the population expected to have thresholds at least as low as the levels observed in our trials. The advantage to the extreme-value method is that one can calculate the percent of the population likely to be above (or below) any one of several concentrations (Fig. 1) rather than merely the percentage below the lowest value observed. Not only in air and water pollution but in the development of off-flavor in foods or in the formulation of pharmaceuticals one may wish to estimate concentration levels likely to induce adverse response on the part of different percentages of the consuming public.

Estimates were also made of the number of molecules involved at the threshold level. (Sips of solution were determined to average 2.44 ml.) For ethyl caprylate in water, 10% alcohol and corn oil, the respective values were 1.3×10^{18}, 1.2×10^{19} and 2.2×10^{19} molecules. For phenyl ethyl alcohol, the corresponding values were 2.1×10^{18}, 7.7×10^{19} and 3.1×10^{20}. The lowest threshold value for quinine sulfate which Powers et al. (1971) encountered was 2.4×10^{-12} M (1.8 parts per trillion). This threshold is much lower than reports in the literature, but it probably is real and not statistical chance as the judge was able to repeat himself. For the 70 judges, the panel average was 2.1×10^{-9} M which is not too much different for the panel average observed by Kouwenhoven (1970). The average for his panel apparently was 7.5×10^{-7} M. For a threshold at 1.8 parts per trillion and a sip of 2.44 ml, the number of molecules necessary to induce a response would be 3.6×10^{15}.

Table 2—Estimates of percent of population likely to have thresholds as low or lower than observed ones

Solvent	Lowest threshold observed, mg/liter	% of Population estimated to have thresholds as low or lower: From regression line	From lower 95% confidence limit of regression line	Nonparametric estimate 95% confidence limit
		Ethyl caprylate[a]		
Water	0.15	7.5	34.0	10.5
10% ethanol	1.43	6.5	16.0	10.5
Corn oil	25.0	5.5	14.0	10.5
		Phenyl ethyl alcohol[a]		
Water	0.18	9.5	23.0	16.2
10% ethanol	6.3	5.0	14.5	16.2
Corn oil	25.0	4.9	15.5	16.2

[a]Estimates for ethyl caprylate based on 27 panelists; for phenyl ethyl alcohol, 17 panelists.

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A Research Note

REFINEMENT AND EXTENSION OF f_h/U:g PARAMETERS FOR PROCESS CALCULATION

INTRODUCTION

BALL (1923, 1928) developed extensive mathematical procedures for estimating the sterilizing values of heat processes applied to canned foods and for estimating time-temperature specifications that would result in heat processes having any designated sterilizing values. In developing these procedures, he found that for any value of the ratio f_h/U there was always a corresponding value of g (see "Nomenclature" for definition of terms). He also found that this relationship depended upon a number of microbial resistance and thermal process variables. In consequence, he developed tables of the f_h/U:g relationships to implement application of his mathematical procedures.

In recognition that the relationships presented by Ball did not adequately account for variations in the lag (j factor) of cooling, Stumbo and Longley (1966) presented limited tables—for z values of 12 to 22 Fahrenheit degrees (F°)—which accounted for the magnitude of the j of the cooling curve. Values for these tables were determined by use of the improved General Method of process evaluation (Schultz and Olson, 1940). Beyond the fact that these were very limited in coverage, they did not adequately account for the influence of temperature gradient at the start of cooling on the magnitude of the j value of the cooling curve. Also, they were subject to some error due to the method used to establish hypothetical heating and cooling curves.

Jen et al. (1971) devised a computer program for the General Method and through it developed f_h/U:g tables for z values covering a range of 8–80. This procedure accounted for the influence of temperature gradient only for g_c values up to 10. Also, it was based on certain approximations that have since been found unnecessary, and which no doubt introduced some error. Further, since the development of these tables, the need for f_h/U:g tables for z values up to 200 has been realized.

In view of the importance of highly accurate f_h/U:g relationships for evaluating heat processes not only for foods that are conventionally processed in cylindrical containers, but for processes for foods that are batch processed in vats, in continuous flow heat exchangers or by any method wherein the heating and cooling curves can be well defined, a procedure was devised which resulted in notable refinement of the f_h/U:g tables as well as permitting the extension of these tables to cover a range of z values from 8 to 200. This communication details this procedure along with noting wherein major improvements have been made compared to the procedure of Jen et al. (1971).

It may be noted that a number of other related works have been presented recently by a number of investigators involving the use of computer-derived tables for thermal process evaluation (cf. Herndon et al., 1968; Hayakawa, 1968, 1969; Hayakawa and Ball, 1969; Griffin et al., 1969, 1971; and, Cohen and Wall, 1971). It is felt that, though these works are of signal importance to the overall subject, they are not sufficiently relevant to this presentation to justify their review or comparison here.

PROCEDURE

PROGRAM CHECKER[1] was written to generate f_h/U:g tables for z values ranging from 8 to 200. The following is a description of the working of CHECKER, which includes modification of the approach employed by Jen et al. (1971).

(a) Jen et al. used a 10 × 10 finite difference mesh. Time-temperature histories for heating and cooling were computed using Δt = 0.125 min, but the lethal rate curves were computed using Δt = 0.25 min (Teixeira et al., 1969).

CHECKER employed a 20 × 20 finite difference mesh. A time increment of Δt = 0.1 min was used for the numerical computation of time-temperature histories (Teixeira et al., 1969) and the integration of lethal rate curves, both of which were done simultaneously. The time-temperature histories for all 20 nodes along the radius were recorded on magnetic tape.

(b) Jen et al. did not use cooling curves to compute j_c values for all nodes along the radius of the container (only for the node at the geometric center for each f_h/U value). The nonuniform temperature distributions, at the beginning of cooling, were neglected for $g_c > 10$ F°.

CHECKER monitored 65 different g values (from 60 F° to 0.00001 F°) at each one of the 20 nodes along the radius of the container. A total of 1,300 distinct and independent sets of heating and cooling time-temperature histories were computed and recorded on magnetic tape.

(c) Jen et al. simulated progressively decreasing g_c values independently and separately. Each simulation completely repeated the preceding computation for heating.

CHECKER incorporated a scanning and

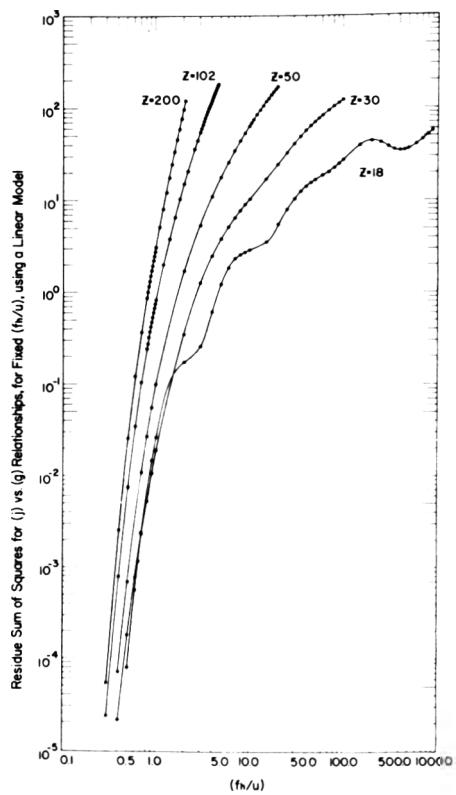

Fig. 1—Error due to assumption of linearity between g and j (cf. Stumbo and Longley, 1966).

[1] Arrangements to obtain Program CHECKER may be made by contacting Dr. K.S. Purohit.

Table 1–Influence of node location and temperature distribution at beginning[a] of cooling on cooling j values

g value at node F°	node #1 from wall	node #5 from wall	node #10 from wall	node #15 from wall	node #20 from wall
40	0.4846	0.7203	1.3432	1.9849	2.2216
30	0.5083	0.7037	1.3317	1.9278	2.1491
20	0.4710	0.6959	1.3192	1.8774	2.0828
10	0.4537	0.6925	1.3073	1.8314	2.0245
1	0.4466	0.6946	1.2964	1.7936	1.9765
10^{-1}	0.4494	0.6944	1.2953	1.7897	1.9721
10^{-5}	0.4497	0.6946	1.2952	1.7892	1.9715

[a]Any cooling j value given is that which would be observed if cooling were started when the g value at that node were as stated.

restart mechanism to determine, every 0.1 min, if any one of the g values had been reached by any one of the nodes along the radius. When such a node and g values were found, the temperature distribution in the container and the f_h/U value of the node were saved in memory locations. At the end of the cooling phase, the temperature distribution and f_h/U value were restored. Thus real time simulated in CHECKER was equal to the time required to reach the lowest g value (0.00001 F°) at the slowest heating point in the container (geometric center). By such time all g values at all other nodes had been ascertained.

(d) Jen et al. computed lethal rate curves, corresponding to different z values, one at a time, even though the same sets of time-temperature histories were used for all z values.

CHECKER simultaneously performed numerical computations for temperature and integrations of lethal rate curves corresponding to 20 different z values. Five passes thus provided the f_h/U values corresponding to 100 different z values, with four passes merely involving reading the 1,300 sets of time-temperature histories from magnetic tape.

(e) Jen et al. assumed linear relatiohship between adjacent log (g) and log (f_h/U) at any given node along the container radius. Therefore, g values were obtained corresponding to f_h/U values ranging from 0.1 to 1,000, for z values ranging from 8–80.

CHECKER fitted polynomials of degree 20 to obtain functions of the type,

$$j = \phi(g) \qquad (1)$$

for each node along the radius. For each z value, CHECKER also fitted polynomials of degree 20 to obtain functions of the type,

$$\log(g) = \epsilon\,[\log(f_h/U)] \qquad (2)$$

for each node along the radius. The lowest and highest f_h/U values, for which g values were obtained from Eq. (2), were governed by the lowest (0.00001 F°) and highest (60 F°) g values originally used in computing 1,300 sets of time-temperature histories.

(f) CHECKER computed new g (Eq. 2) and j (Eq. 1) values for all nodes along the radius, for fixed values of z and f_h/U. A linear equation was then fitted to obtain a function of the type,

$$g = \eta(j) \qquad (3)$$

for fixed z and f_h/U values. Residual sum of squares or error, due to this approximation was recorded. Finally, g values corresponding to j_c values ranging from 0.4–2.0 (Eq. 3) were computed for fixed z and f_h/U values.

RESULTS & DISCUSSION

f_h/U:g TABLES for z values ranging from 8–200 were obtained. They are being published by Stumbo (1973). Sample process calculations employing z values in the range of 12–22 indicated that answers obtained when using these tables agreed within ± 3% of those obtained when using tables presented by Stumbo and Longley (1966).

Residue sum of squares or error, due to the assumption of linearity between g and j_c (Stumbo and Longley, 1966) for several z values over their respective ranges of f_h/U is presented in Figure 1. The maximum average error (over the range of j) for these tables was found to be ± 2.5%. Considering other errors inherent in process determination, an error of this magnitude appears to be insignificant and confirms the earlier findings of Stumbo and Longley (1966).

Table 1 demonstrates variations in j values along the radius, for different g values, on which cooling curves were evaluated. Both position and temperature distribution (in the container when the g value of interest is reached at that point) influence the cooling j. It should be pointed out that Jen et al. (1971), using a 10 × 10 mesh and Δt = 0.125 min, obtained a cooling j of 0.3202, at the node closest to the container wall. Table 1 shows that CHECKER, using a 20 × 20 mesh and Δt = 0.1 min, ascertained cooling j values ranging from 0.4846–0.4497 at the node closest to the container wall. This difference can be reconciled only in view of the differences enumerated in part (b) of the Procedure, that is, Jen et al. did not use cooling curves to compute j_c values for this node and did not account for nonuniform temperature distribution for g_c values greater than 10, whereas CHECKER did.

NOMENCLATURE

- f– Time, in minutes, required for straight-line portion of semilog heating or cooling curve traverse one log cycle.
- f_h– f of heating curve when it can be represented by one straight line. Also, f of first straight-line portion of a broken heating curve.
- g– Difference, in Fahrenheit degrees, between retort temperature and the maximum temperature reached by the food at the point of concern.
- g_c– g when the point of concern is the geometrical center of the container.
- j_c– A cooling curve lag factor. A factor that, when multiplied by the difference between retort temperature and food temperature when cooling starts, locates the intersection of an extension of the straight-line portion of the cooling curve and a vertical line representing the beginning of cooling.
- t– Time, in minutes.
- U– The equivalent, in minutes at retort temperature, of all lethal heat received by some designated point in the container during process.
- z– Number of Fahrenheit degrees required for the thermal destruction curve to traverse one log cycle. Mathematically equal to the reciprocal of the slope of the thermal destruction curve.
- Δt– Size of time increment, in minutes, for numerical computation.
- η– Functional relationship denoting linear equation obtained to correlate g and j values for fixed values of z and f_h/U.
- ϵ– Functional relationship denoting 20th degree polynomial obtained to correlate log (g) and log (f_h/U) for each node along the radius and for fixed values of z.
- ϕ– Functional relationship denoting 20th degree polynomial obtained to correlate j and g values for each node along the radius.

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Mercury in Food

A Scientific Status Summary by the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition

Mercury is one of the naturally occurring elements. It is found in soils, rocks, and in the waters of lakes, streams, and oceans. It is found in the waters of lakes remote from man's industrial activities. It can be found in varying amounts in air, higher concentrations being found in air over ore deposits and in the vicinity of volcanoes from which mercury is vaporized.

Since mercury is ubiquitous, it is to be expected that trace amounts can be found in all foods. Foods growing in areas having higher concentrations of mercury in soil can be expected to have higher concentrations in the food than foods grown in lower mercury-in-soil regions.

Mercury can leach out of soils into water, and the water run-off carries the mercury into streams to the ocean. Mercury in water that passes through soils having the capacity to absorb and bind mercury can be lost to the soil.

Mercury vaporizes from soil and water into air, gets washed out of the air by rain and snow, and is returned to the soil and to streams, lakes, rivers, and oceans. It is logical to assume that this vaporizing and washing out of mercury has been taking place since the earth was formed. Plants and animals, including man, evolved in this earth environment containing mercury.

Mercury Converted from One Form into Another

Some plants appear to concentrate mercury; small droplets of elemental mercury can be seen in tissues of such plants, according to Shacklette (1970). Some microorganisms convert inorganic mercury to methyl and dimethyl mercury; Wood, et al. (1968) first published evidence of such conversion by extracts of a methanogenic bacterium. The alkyl mercury compounds may be absorbed by fish or by other animals. Some microorganisms and animals have the ability to convert organic mercury compounds back to inorganic salts of mercury and elemental mercury. Chapter 11, "Puri-

fication of Industrial Wastes by Microbial Methods," in the book *Biochemical and Industrial Aspects of Fermentation* (Ono et al., 1971) contains an excellent discussion of this cycle of inorganic mercury to organic mercury and back to inorganic mercury again.

Methyl mercury is soluble in water, but dimethyl mercury is quite insoluble and volatilizes from water into air. Dimethyl mercury is decomposed by ultraviolet light, so it would not be expected that large amounts of dimethyl mercury would accumulate in air. Mercury released from dimethyl mercury in air and elemental mercury vaporized into air can react with ozone, oxides of nitrogen, or other oxidants to form oxides, nitrates, or other salts of mercury; these compounds formed by reaction of gases may remain present in air until washed out by rain and snow or may agglomerate and settle out. Thus, there is the cycling of inorganic mercury as such and also via organic mercury between soil, water, and air.

Toxicity of Inorganic Mercury Studied

Since mercury is ubiquitous, everyone consumes traces of mercury in food and water; trace amounts of mercury can be found in urine samples from anyone.

Persons who work in industries that use mercury (manufacture of special instruments, calibration of scientific glassware, mercury cell chlor-alkali industry, certain types of battery manufacture, as examples) may absorb and excrete 10-20 times more mercury than persons who do not have such industrial exposure. The United States Department of Labor has established an allowable level of 0.1 mg of elemental mercury vapor or inorganic salts of mercury per cubic meter of air for industrial exposures. Industrial exposure at the allowable level can result in absorption of 1.0 mg of mercury per 8-hr work day, assuming the exposure to be limited to the working day, which is not necessarily true.

A study by Smith et al. (1970) of workers exposed to mercury in the mercury cell chlor-alkali industry did not show any significant effects from exposure when the estimated time-weighted average 8-hr exposure was 0.1 mg of mercury per cubic meter of air based on measurements in the general work environment. More recently, it has been shown by Henderson (1972) and by Danziger and Possick (1973) that contamination of skin and clothing with mercury can lead to a mercury concentration in the breathing zone of an individual that is much higher than the concentration in the general work environment. Therefore, it is highly probable that the total exposure of the workers studied by Smith et al. was higher than their estimates of exposure.

Henderson (1972) and Shandar and Simson (1971) have concluded that exposures to elemental mercury and inorganic salts of mercury that result in a urinary mercury concentration of 0.3 mg/l for periods up to 10 years did not result in any adverse signs or symptoms. Since mercury is excreted in feces, sweat, and exhaled breath as well as in urine, a daily intake of 1.0 mg per day of elemental mercury or inorganic salts of mercury appears safe.

According to Kolbye (1972), the United States Food and Drug Administration's "Mercury in Foods Survey" has shown that all of the foods sampled, except shrimp, had less than 0.02 parts per million (mg/kg) of mercury. Two of the shrimp samples had 0.03 ppm, one had 0.04 ppm, and one had 0.05 ppm. The foods sampled did not include fish. Thus, the average U.S. diet appears to contain less than one-fiftieth the amount of mercury that persons may absorb from industrial exposure without adverse effects.

Toxicity of Organic Mercury Studied

The short chain alkyl mercury compounds—methyl, dimethyl and ethyl mercury—are more toxic than elemental mercury, inorganic salts of mercury, and the aryl mercury compounds such as phenyl mercuric acetate. The U.S. Department of Labor has established a limit of 0.01 mg of alkyl mercury per cubic meter of air allowable for an 8-hr work day. Exposure at the allowable level could result in absorption of 0.1 mg of alkyl mercury.

A group of twenty workers employed in the manufacture of ethyl mercury compounds and agricultural formulations of the compounds was studied for a period of five years by Dinman et al. (1958). It was estimated that the men had an exposure of greater than 0.01 mg per cubic meter and less than 0.10 mg per cubic meter. That exposures did occur was evi-

denced by increased urinary mercury excretion; the urinary mercury concentrations for these workers were approximately 2-5 times the average concentration for the general population. Only a small fraction of alkyl mercury is excreted in urine, so these workers obviously were exposed and did absorb the alkyl mercury compounds. Physical examinations and clinical laboratory studies failed to reveal any evidence of pathological alterations among the men.

Minamata Disease Linked to Mercury

Unfortunately, the discharge of an industrial waste stream containing methyl mercury into Minamata Bay in Japan resulted in high concentrations of methyl mercury in fish and shellfish. Starting in 1953, humans and cats in the Minamata Bay area were observed to have developed neurological disorders, but it was several years before the relationship of the disorders to the high concentrations of methyl mercury was understood.

Kurland et al. published the first report on this incident in 1960. It is estimated that there were at least 116 people who were irreversibly damaged (43 died) between 1953 and 1960 in the Minamata Bay area. In 1958, shellfish in the area contained 27-102 ppm of mercury on a dry weight basis; these values may be low due to difficulties of analysis. In 1959, shellfish from the Minamata River were found to have 9-24 ppm of mercury on a wet weight basis.

Many Japanese depend much more than we do for seafoods as their main source of protein. Also, internal organs as well as the muscle tissue of fish are used in a number of Japanese dishes. The livers of fish that are accumulating methyl mercury from high concentrations in the water have a higher concentration of methyl mercury than the flesh.

Misuse of Mercury Treated Seeds Cited

There also have been several unfortunate instances of persons having eaten seed grains that had been treated with alkyl mercury fungicides or have fed such grains to animals, then eaten the animals. One such incident occurred in Guatemala and has been discussed by Ordonez et al. (1966). Another incident involved a family in Alamagordo, New Mexico, that in 1969 ate meat of hogs that had been fed treated grain. The most recent incident involved persons in Iraq in 1971; here again, seed grain treated with alkyl mercury fungicide was used as food by humans.

These cases obviously are from a misuse of treated seed grains. The U.S. Environmental Protection Agency has acted to eliminate the use of alkyl mercurial fungicides in order to prevent accidental poisonings from misuse of treated seed grains.

Several ornithologists in Sweden observed a decrease in the population of some birds in about 1955. Alkyl mercury compounds were used for seed treatment in Sweden. It was found in 1958 that the mercury content of birds found dead in that year was much higher than the mercury content of museum specimens. In the spring of 1966, Sweden issued regulations prohibiting the use of alkyl mercury compounds as seed-dressing agents. Predatory birds as well as seed-eating birds were found to have elevated mercury levels, and it also was found that fish from a number of locations had elevated mercury concentrations.

Johnels and Westermark (1969) have reviewed the development of information relative to mercury concentrations and sources of mercury in Sweden. With the banning of the agricultural uses of alkyl mercury compounds and a reduction of industrial discharges of mercury into the environment in Sweden, an increase in bird population and a decrease in mercury concentration in fish in some locations are being observed.

Sweden Limits Mercury in Fish

In 1967, Sweden established a limit of 1.0 ppm of mercury in commercial fish for use as human food. This did not prevent individuals from catching and eating fish that might have a higher mercury content, although Sweden did issue warnings that the consumption of fish caught in some areas should be restricted to once a week. Mercury concentrations greater than 3 ppm have been found in fish muscle tissue in Sweden. Approximately 50-90% of the mercury is in the form of methyl mercury. Fish consumption by the Swedish population on the average is greater than by

the United States population.

In 1972, Skerfving (1972) of the National Food Administration, Stockholm, Sweden, reported that no cases of clinical methyl mercury poisoning resulting from fish consumption have been found in either Sweden or Finland, although both countries have a continuing search for such cases.

Mercury Discharges in U.S. and Canada Studied

In early 1970, fish caught in Lake St. Clair in the state of Michigan in the United States were found to have elevated amounts of mercury; again, a high percentage of the mercury was methyl mercury. The elevated mercury levels were attributed to elemental mercury and inorganic salts of mercury discharged from an industrial plant in Sarnia, Ontario, Canada.

This finding led to an intensive study of mercury discharges into rivers and lakes in the United States and Canada. Even the discharges from municipal sewage treatment plants may elevate the mercury content in the water and bottom sediments of the receiving streams. Concentrations of mercury in fish in approximately 10 locations in the United States were found to be sufficiently high to cause temporary bans on fishing or warnings to limit consumption of fish taken from an area.

Since early 1970, there have been epidemiological studies in several locations of elevated mercury content, but no cases of clinical methyl mercury poisoning resulting from fish consumption in the United States have been reported. There will continue to be epidemiological studies to be sure that nothing has been overlooked.

Concentration in Fish Has Not Increased Significantly

In addition to elevated concentrations of mercury in fresh water fish caught in areas of elevated mercury content in water or bottom sediments, it has been found that swordfish and tuna appear to accumulate methyl mercury as they grow. The U.S. Food and Drug Administration does not allow the sale of fish containing more than 0.5 ppm (0.5 mg/kg) of mercury. Many commercially caught swordfish have been found to contain more than 0.5 mg of mercury per kilogram, and the swordfish industry has declined.

Canned tuna with more than 0.5 mg. of mercury per kilogram has been found in the United States and has been withdrawn from the market. It is possible, by sorting, for the canned tuna industry to select fish that meet the Food and Drug Administration requirement; the canned tuna industry now includes mercury content as one of their quality specifications.

It is doubtful that the concentration of mercury has increased significantly from man's use of mercury. Analyses of museum specimens of ocean fish indicate that fish caught several decades ago had mercury levels in the same ranges as presently found in swordfish and tuna. It is highly probably that for many years persons have consumed swordfish and tuna that had concentrations of mercury above the present guidelines of 0.5 mg/kg.

Recently, Ganther et al. (1972) have shown that selenium in the diet reduces the toxic effect of methyl mercury even at mercury concentrations of 10-20 ppm in the diet. They also have shown that the selenium content of tuna appears to increase as the fish grows in parallel with the increase in methyl mercury content. It appears that in the natural environment of the ocean, accumulation of selenium serves to reduce the toxicity of the accumulating methyl mercury.

Where high concentrations of methyl mercury are discharged into a bay such as at Minamata, methyl mercury might be expected to accumulate rapidly in fish without the parallel accumulation of selenium. This may help to account for the apparently different effects seen from supposedly equal intakes of fish having elevated concentrations of methyl mercury. Work is continuing on this point.

Guidelines Have Margin of Safety

The guideline of 0.5 mg of mercury per kilogram (2.2 lb) of fish has a considerable margin of safety. With the knowledge that selenium can offset the toxic effects of methyl mercury, the margin of safety may be even greater than had been believed previously, particularly for tuna and swordfish.

As stated earlier, the U.S. Food and Drug Administration has a continuing program of analyzing foods for mercury content. The foods have included

large numbers of tuna, swordfish, and other fish of commercial significance. All of the foods except some shrimp samples and fish samples, particularly large swordfish and tuna, have had mercury concentrations less than one twenty-fifth the Food and Drug Administration guideline of 0.5 mg/kg. It is possible, by sorting, to eliminate swordfish and tuna above the guideline.

Mercury in Food Not a Toxic Hazard

The naturally occurring element, mercury, does not appear to pose a toxic hazard in the food supply of the United States. There have been cases of poisoning where methyl mercury has been dumped into the environment or treated seed grains have been misused. There is no evidence that alkyl mercury formed by microorganisms in nature has led to methyl mercury poisoning.

This does not imply that there is no reason to control the release of elemental mercury and inorganic salts of mercury into the environment from industrial operations. It does indicate that the problem of localized situations where the rate of natural synthesis of methyl mercury exceeds the rate of recycling back to inorganic forms of mercury has been recognized before irreversible damage has occurred. The steps that have been taken to reduce discharges of elemental mercury and inorganic salts of mercury and the reduction or elimination of use of mercurials in agriculture provide an increasing margin of safety against the possibility of an excessive amount of mercury in any form finding its way into any portion of the food supply of the United States.

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