3 May-June 1972
Volume 37 : Number 3
Coden: JFDSAZ 37(3) 337-502(1972)

JOURNAL of FOOD SCIENCE

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IFT *A PUBLICATION OF THE INSTITUTE OF FOOD TECHNOLOGISTS*

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from front cover

May-June 1972
Volume 37 : Number 3

JOURNAL of FOOD SCIENCE

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 JOURNAL OF FOOD SCIENCE (formerly *Food Research*) is published 6 times a year (bimonthly) by Institute of Food Technologists, Suite 2120, 221 N. LaSalle Street, Chicago, Illinois 60601 USA. Printed in USA. Second class postage paid at Champaign, Ill. and at additional mailing offices.

ABSTRACTS:
IN THIS ISSUE

THE EFFECT OF PRE-RIGOR CHANGES ON MEAT TENDERNESS. A Review. R.P. NEWBOLD & P.V. HARRIS. *J. Food Sci.* 37, 337–340 (1972)–A comprehensive review of the effect of pre-rigor chemical changes (rate and extent of pH fall) and physical changes (postmortem shortening) on meat tenderness. Concludes that since tenderness is greatly influenced by the conditions prevailing during the period between slaughter and the full development of rigor mortis, processing methods should be aimed at minimizing postmortem shortening.

THE RELATIVE IMPORTANCE OF SOME DETERMINANTS OF BEEF TENDERNESS. R.W. PURCHAS. *J. Food Sci.* 37, 341–345 (1972)–The nature of the effects of sex, weight and breed on beef tenderness were studied by measuring the effect of these factors on the response of meat tenderness to aging and to different cooking temperatures. Friesian-Brahman cross bulls produced meat which was significantly tougher than that of Friesians, but it responded to aging significantly more. A difference of 100 kg in the live weight of the steers and a difference of approximately 7 months in the age at which cryptorchidism was induced did not appear to affect tenderness. The cryptorchid meat was significantly tougher than steer meat prior to aging but it responded significantly more to aging. Also, cryptorchid meat showed significantly less toughening as the cooking temperature was increased from 60°C to 75°C. These results are interpreted as supporting the contention that variation in the nonconnective tissue portion of meat accounts for most of the variation in meat tenderness. Some theoretical considerations involving the incorporation of a range of values in a simple model support this suggestion.

THE EFFECT OF AGING ON PHYSICOCHEMICAL PROPERTIES OF ACTOMYOSIN FROM CHICKEN BREAST AND LEG MUSCLE. J.D. HAY, R.W. CURRIE & F.H. WOLFE. *J. Food Sci.* 37, 346–350 (1972)–Actomyosin prepared from pre-rigor, rigor, 21 hr and 7 day post-rigor chicken breast and leg muscle was compared with regard to magnesium and calcium mediated adenosine triphosphatase activity, sulfhydryl group availability, reduced viscosity and sedimentation pattern as observed in the schlieren optics of the L2-65B ultracentrifuge. Magnesium ATPase activity showed a maximum in the actomyosin prepared from muscle in the rigor state, paralleling similar maxima in the reduced viscosity for both leg and breast preparations. Calcium ATPase showed a minimum in rigor actomyosin from breast muscle, coinciding with increases in sulfhydryl availability. Leg muscle actomyosin showed no differences in calcium ATPase or sulfhydryl availability with aging. Sedimentation patterns were slightly different for both types of muscle at each state of aging. The results demonstrate that post-mortem aging of chicken muscle produces minimal observed effects in the extracted actomyosin and that breast and leg muscle preparations of actomyosin from young chickens are quite similar in properties.

THE EFFECTS OF ULTIMATE pH ON OVINE MUSCLE: WATER-HOLDING CAPACITY. P.E. BOUTON, P.V. HARRIS & W.R. SHORTHOSE. *J. Food Sci.* 37, 351–355 (1972)–Ultimate pH values ranging from 5.4–7.0 were obtained in the muscles of young and old sheep using pre-slaughter injections of adrenaline. A high speed centrifugation method was used to measure changes in water-holding capacity attributable to the effect of ultimate pH, aging, cooking, animal age and fiber contraction state. Water-holding capacity values after cooking and/or centrifuging showed no significant aging effect but increased ($P < 0.001$) with increasing pH. Cooking at 60, 70 and 90°C produced ($P < 0.001$) effects due to temperature and to pH in both cooking loss (an increase) and in expressible juice (a decrease). Temperature-induced differences in residual bound water were small. Differences due to animal age were small. Contracted muscles had significantly reduced water-holding capacities compared with their stretched counterparts.

THE EFFECTS OF ULTIMATE pH ON OVINE MUSCLE: MECHANICAL PROPERTIES. P.E. BOUTON, P.V. HARRIS & W.R. SHORTHOSE. *J. Food Sci.* 37, 356–360 (1972)–Pre-slaughter injections of adrenaline were used to induce a range of ultimate pH, 5.4–7.2, in muscles from young and old sheep. Compression, shear and tensile testing methods were employed to assess the effects attributable to various structural components contributing to the large changes in tenderness associated with an increase in ultimate pH. The results obtained using muscles of different fiber contraction states were compared with the effects of aging and it was concluded that, although pH affected the adhesion between the fibers, it was the fibers per se which were primarily affected and influenced mechanical properties most.

CHARACTERIZATION OF THE RED PIGMENTS PRODUCED FROM FERRIMYOGLOBIN BY IONIZING RADIATION. G.G. GIDDINGS & P. MARKAKIS. *J. Food Sci.* 37, 361–364 (1972)–The red pigment produced by gamma irradiation of ferrimyoglobin (+3 oxidation state) is ferrylmyoglobin (+4 oxidation state), when ample oxygen is present. When the oxygen is at a very low concentration or absent prior to irradiation, oxymyoglobin (+2 oxidation state) is formed.

PENTOSANS IN SORGHUM GRAIN. A. KARIM & L.W. ROONEY. *J. Food Sci.* 37, 365–368 (1972)–Sorghum grain with wide differences in endosperm characteristics differed significantly in pentosan content. The range for grain from 31 sorghum varieties was 2.51–5.57%. The pericarp was the richest in pentosans (21%) followed by the germ (6%) and endosperm (1%). Most of the pentosans were located in cell walls of the kernel. Environment significantly influenced the pentosan content. Pentosan content of sorghum was not potentially useful for predicting processing properties of sorghum grain.

CHARACTERIZATION OF PENTOSANS IN SORGHUM GRAIN. A. KARIM & L.W. ROONEY. *J. Food Sci.* 37, 369–371 (1972)–Water-soluble and alkali-soluble pentosans in the whole kernel, pericarp and endosperm of sorghum grain were characterized and compared. The major fraction of pentosans in whole grain and endosperm of sorghum kernel is water soluble, while pentosans in sorghum pericarp consist almost entirely of the alkali-soluble fraction. Both water-soluble and alkali-soluble pentosans from whole kernel, endosperm and pericarp of sorghum grain contain galactose, glucose, xylose and arabinose. Water-soluble pentosans have a higher arabinose/xylose ratio than the alkali-soluble pentosans. Both water-soluble and alkali-soluble pentosans from the sorghum pericarp contain larger quantities of pentose sugars than those from the whole kernel and endosperm. The composition and properties of sorghum pentosans are comparable with those of other cereal grains.

QUANTITATIVE DETERMINATION OF THE OLIGOSACCHARIDES IN DEFATTED SOYBEAN MEAL BY GAS-LIQUID CHROMATOGRAPHY. J. DELENTE & K. LADENBURG. *J. Food Sci.* 37, 372–374 (1972)–A simple and rapid method for the determination of soybean meal oligosaccharides was developed. The soybean meal is extracted with cold water, deproteinized and lyophilized. The dry residue is silylated and the solution is injected into a gas chromatograph. The resulting peaks are identified and measured by comparison with similar chromatograms obtained from known quantities of pure sugars. A typical sample contained the following oligosaccharides: galactose 1%, sucrose 8.9%, raffinose 0.9% and stachyose 4.2%. Trace amounts of glucose and manninotriose were also found.

AMINO ACID COMPOSITION OF SELECTED POTATO VARIETIES. M.S. KALDY & P. MARKAKIS. *J. Food Sci.* 37, 375–377 (1972)–The quality of proteins in one cultivar and five clonal selections of the potato (*Solanum tuberosum* L.) was evaluated by amino acid analysis. Seventeen amino acids were determined by means of a Beckman Model 120C amino acid analyzer. Methionine and cystine + cysteine were oxidized with performic acid to methionine sulfone and cysteic acid, respectively, prior to acid hydrolysis. Tryptophan was determined colorimetrically after hydrolysis with pronase. Protein scores for the cultivar Russet Burbank, and the five clones #58, #321-65, #322-6, #709 and #711-3 were 73, 78, 60, 62, 73 and 68 respectively, and averaged 69. Methionine was the limiting amino acid in all six samples.

EFFECT OF AMINO ACID SUPPLEMENTATION OF DEHYDRATED POTATO FLAKES ON PROTEIN NUTRITIVE VALUE FOR HUMAN ADULTS. C. KIES & H.M. FOX. *J. Food Sci.* 37, 378–380 (1972)–The objective of the study was to determine the first limiting amino acid in dehydrated potato flakes for maintenance of nitrogen retention in human adults. Mean nitrogen balances of seven human adults fed 4.0g N/day from potatoes plus no amino acid supplement, plus L-methionine, plus L-leucine, plus L-phenylalanine or plus a combination of the listed amino acids were −1.18, −0.27, −0.83, −0.91 and −0.30g N/day, respectively. The significant improvement in nitrogen retention resultant from methionine supplementation of the potato diets indicates methionine to be the first limiting amino acid in potato protein (dehydrated potato flakes) for protein nutriture of the adult human. The protein value of the evaluated dehydrated potatoes could be significantly improved via .37% L-methionine supplementation.

PEROXIDASE LOCALIZATION AND LIGNIN FORMATION IN DEVELOPING PEAR FRUIT. A.S. RANADIVE & N.F. HAARD. *J. Food Sci.* 37, 381–383 (1972)–The data reported here support the thesis that localization of peroxidase on the cellular particulate fraction is associated with sclereid development. Total peroxidase activity was consistently higher in developing Bartlett fruit than in "Yuzuhada fruit" which contained large and numerous stone cells. However, bound peroxidase, released from cell macerates at high ionic strength, was present at higher levels in developing fruit which contained excessive stone cells. The pear variety containing abundant sclereids also had lower levels of endogenous calcium during early development. The results suggest that mineral nutrition in pear fruit may profoundly alter lignin metabolism by affecting the localization and, hence, the metabolic control of peroxidase.

THE APPLICATION OF AMINO ACID COMPOSITION TO THE CHARACTERIZATION OF CITRUS JUICES. C.E. VANDERCOOK & R.L. PRICE. *J. Food Sci.* 37, 384–386 (1972)–The individual amino acids of commercial orange and lemon juices were quantitatively determined by ion-exchange chromatography. Several of the major amino acids were correlated with other constituents of the juice. The correlations of the total amino acids with the mol percentage of γ-aminobutyric acid and citric acid with the mol percentage of serine and the sum of aspartic acid plus asparagine were particularly high for orange juice. It appears that these relationships might be useful in estimating juice content or determining the authenticity of a sample.

FATTY ACID DISTRIBUTION IN ORANGE JUICE PHOSPHOLIPIDS. R.J. BRADDOCK. *J. Food Sci.* 37, 387–388 (1972)–The fatty acids of 'Valencia' orange juice phosphatidyls-ethanolamine and -choline showed a preponderance of palmitic and stearic acids at the 1-position, with polyunsaturated acids at the 2-position. A comparison of fresh and processed orange juice indicated that more lipid phosphorus could be extracted from processed than from fresh juice. However, the percentages of phosphatidyl-ethanolamine and phosphatidyl-inositol decreased and the phosphatidic acid fraction increased upon processing.

COLORIMETRY OF FOODS: ORANGE JUICE. E.A. GULLETT, F.J. FRANCIS & F.M. CLYDESDALE. *J. Food Sci.* 37, 389–393 (1972)–Orange juice samples containing small increments of carotenoid pigment were ranked for color differences. A similar series where pulp content varied was included. Samples of varying thickness, presented against white and black backgrounds, were evaluated visually under 7400°K light and instrumentally with a GE spectrophotometer and a Hunterlab D25 colorimeter. Calculation of absorption and scattering coefficients using the Kubelka-Munk treatment suggested the possibility of characterizing a food optically for predicting success of a color measurement technique. Large color differences observed for 2 mm cells, white background presentation with Hunterlab D25 colorimeter, resulted from disproportionately greater scattering for these cells. The Hunter Citrus colorimeter was shown to be as good as the thin-layer presentation method for measuring color of orange juice.

ODOR THRESHOLDS AND RELATIVE INTENSITIES OF VOLATILE AROMA COMPONENTS IN AN ARTIFICIAL BEVERAGE IMITATING WHISKY. P. SALO, L. NYKÄNEN & H. SUOMALAINEN. *J. Food Sci.* 37, 394–398 (1972)–A synthetic beverage imitating whisky was made of 13 alcohols, 21 acids, 24 esters, 9 carbonyl compounds and rectified grain spirit and water. The odor threshold levels were determined for compounds, some mixtures, aroma fractions and the whole beverage. The relative contribution of the components to the total odor intensity was calculated. The core of the aroma is composed of some esters (ethyl acetate, ethyl caproate, ethyl caprylate, ethyl caprate, ethyl laurate and isoamyl acetate) and a few carbonyl compounds (butyr-, isobutyr-, valer- and isovaleraldehyde and diacetyl). This core is supplemented by several minor components: esters and acids, the last of which also showed a suppression effect. The elimination of the alcohol fraction made no detectable difference to the aroma.

BIOCHEMISTRY OF TEA FERMENTATION: PRODUCTS OF THE OXIDATION OF TEA FLAVANOLS IN A MODEL TEA FERMENTATION SYSTEM. G.W. SANDERSON, J.E. BERKOWITZ, H. CO & H.N. GRAHAM. *J. Food Sci.* 37, 399–404 (1972)–The products formed on oxidation of tea flavanols in a model tea fermentation system were studied by paper chromatography in order to determine more fully the relationship between these flavanols and the polyphenolic constituents of black tea. Specific relationships between the tea flavanols (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, (−)-epigallocatechin gallate and (+)-catechin and the known black tea constituents theaflavin, theaflavin gallates, bisflavanols (A, B and C), epitheaflavic acid and 3-galloyl epitheaflavic acid were verified. In addition, it was shown that thearubigins are formed as a result of oxidizing any of the tea flavanols; there was no indication of a specific precursor or intermediate. Further, the thearubigins underwent continuous change as the tea fermentation process progressed. These results support the contention that thearubigins are polymeric proanthocyanidins.

THE VOLATILE CONSTITUENTS OF OIL OF THYME. G.F. RUSSELL & K.V. OLSON. *J. Food Sci.* 37, 405–407 (1972)–Volatile components of oil of thyme were separated by gas-liquid chromatography. Individual components were characterized using chemical and spectroscopic methods such as infrared (IR) spectroscopy, ultraviolet (UV) spectroscopy, mass spectrometry (MS), melting point determination, gas chromatographic (GC) retention times and chemical synthesis. Compounds identified were: tricyclene, α-pinene, camphene, β-pinene, myrcene, α-terpinene, limonene, 1,8 cineole, γ-terpinene, p-cymene, trans-sabinene hydrate, linalool, fenchyl alcohol, pinocarvone, 1-terpinen-4-ol, carvacrol methyl ether, caryophyllene, trans-pinocarveol, α-terpineol, borneol, geranyl acetate, thymol and carvacrol.

A PRECISE METHOD FOR THE DETERMINATION OF DIMETHYL SULFIDE IN PROCESSED FOODS. M.P. WILLIAMS, J.E. HOFF & P.E. NELSON. *J. Food Sci.* 37, 408–410 (1972)–Equal volumes of a product solution and helium gas are equilibrated in a 50 ml hypodermic syringe and the gaseous phase is analyzed for dimethyl sulfide by gas chromatography. Henry's law applies to the system in the syringe and Henry's constant is essentially identical for all processed food samples. It is demonstrated that adsorption of dimethyl sulfide onto solid food particles dispersed in the aqueous phase does not occur. The standard errors for five replicate samples prepared from each of three processed foods with mean dimethyl sulfide concentrations of 3.0, 6.1 and 15.1 ppm are ± .10, ± .24 and ± .29 ppm, respectively. Concentrations in commercially processed vegetable juice, kraut juice, peas, tomato juice, yellow sweet corn and beets are reported.

ABSTRACTS:
IN THIS ISSUE

ECONOMIC APPLICATIONS OF SWEETNESS SCALES. H.R. MOSKOWITZ & T. WEHRLY. *J. Food Sci.* 37, 411–415 (1972)–A computer simulation of sweetness mixtures was made based upon empirical sweetness scales and experimentally derived models of sweetness additivity. Two problems were investigated: total cost subject to maintaining overall mixture sweetness and its dual, total sweetness subject to maintaining the mixture's cost. Pairwise, mixtures of sucrose, glucose, fructose, sorbitol, Na saccharin and Na cyclamate were subject to analysis at a number of overall sweetness and cost levels. The results suggest that a computer scan of mixtures may yield specific mixtures satisfying cost or other types of constraints.

IMMERSION FREEZING OF FISH IN DICHLORODIFLUOROMETHANE. S.B. BUCHOLZ & G.M. PIGOTT. *J. Food Sci.* 37, 416–419 (1972)–Salmon frozen by immersion in dichlorodifluoromethane (DDM) were compared with salmon frozen by air blast and liquid nitrogen-boosted air-blast methods. Assessment was made by taste panel, 2-thiobarbituric acid and peroxide rancidity determinations and drip losses. Results indicate that DDM-frozen salmon are comparable in quality to salmon frozen by conventional means. Freezing times for packaged and unpackaged fish fillets were measured and presented in nomographic form as a function of thickness. Decline of DDM residues after freezing was studied.

DEVELOPMENT OF IRRADIATION STERILIZED CODFISH CAKES. F. HEILIGMAN & L.J. RICE. *J. Food Sci.* 37, 420–422 (1972)–Codfish cakes made from cod fillets, white corn meal, gelatin and salt were irradiated under a variety of conditions and stored at different temperatures. The products were evaluated periodically for preference and changes in sensory characteristics by consumer and technological panels. Fish cakes that were given a 12-D sterilizing dose (3.2 Mrad at −30°C) were scored high in the acceptable range and comparable in quality to the nonirradiated controls shortly after irradiation and after 25 months storage at 21°C. Storage at higher temperatures (38°C) caused a brown discoloration which became evident after 4 months and was so pronounced after 9 months that the fish cakes were considered unacceptable. Lowering the radiation dose and irradiating at subzero temperatures improved product quality; however, benefits by lowering the irradiation temperature from −30°C to −80°C were marginal, if at all. No differences could be noted in fish cakes irradiated with gamma rays from a Cobalt-60 source or electrons generated by a linear accelerator.

EFFECT OF HYDROGEN PEROXIDE ON THE COLOR, COMPOSITION AND NUTRITIVE QUALITY OF FPC (FISH PROTEIN CONCENTRATE). J. RASEKH, B.R. STILLINGS & V. SIDWELL. *J. Food Sci.* 37, 423–425 (1972)–Studies were conducted to determine the effect of hydrogen peroxide on lightening the color of FPC (fish protein concentrate) made from whole Atlantic menhaden (*Brevoortia tyrannus*) by isopropyl alcohol extraction. FPC samples were treated with 0.30, 0.60, 1.25, 2.50 and 5.00% hydrogen peroxide (based on dry weight of the FPC) and then spray dried. Results showed that treatment of the FPC with 0.60% or more of hydrogen peroxide significantly lightened the color. No changes in the amino acid composition of the samples were noted, except for a slight decrease in the amounts of methionine and tyrosine in samples with 2.50 and 5.00% hydrogen peroxide. The use of 0.30 and 0.60% hydrogen peroxide had no effect on nutritive quality; however, the use of 1.25% or more lowered the nutritive quality slightly. This was possibly due to the breakdown of some amino acids, such as methionine, tyrosine and cystine by hydrogen peroxide.

PHOSPHOLIPID CHANGES IN MUSCLE FROM FROZEN STORED LAKE MICHIGAN COHO SALMON. R.J. BRADDOCK & L.R. DUGAN JR. *J. Food Sci.* 37, 426–429 (1972)–Phospholipid hydrolysis was shown to occur in dorsal muscle from whole frozen stored salmon as evidenced by losses of specific phospholipids. Comparisons were made between the fatty acids in the total phospholipids and the triglycerides of fresh frozen and frozen stored Coho salmon. A preferential hydrolysis of phosphatidylethanolamine containing $C_{16:0}$, $C_{18:1}$ and $C_{22:6}$ fatty acids was implied from fatty acid analyses which showed that the $C_{16:0}$ and $C_{22:6}$ acids were more concentrated and the $C_{18:1}$ acid less concentrated in the remaining lyso-phosphatidylethanolamine following 6 months storage at −20°C.

PRESSURE FREEZING-AIR DRYING: A NEW TECHNIQUE TO REDUCE DETERIORATION IN DRYING TISSUE. G.J. HAAS, H.E. PRESCOTT JR. & J. D'INTINO. *J. Food Sci.* 37, 430–433 (1972)–Freezing of cellular materials under gaseous pressure prior to regular air drying prevents shrivelling and aids rehydration of many tissues; in some cases an appearance similar to freeze-dried products can be achieved. This method is applicable to a wide range of foods, but only certain gases can be used. There appears to be some reduction in flavor loss in the dried product obtained by this technique. The mechanism of obtaining unshrivelled structure is based on the presence of gas during drying. The gas which has been dissolved, due to the elevated pressure, is pushed into the tissue during freezing and leaves gradually during drying, meanwhile preventing shrivelling and collapse. No determinable chemical changes could be observed. Pressure freezing followed by freeze drying often yields a more expanded product which dehydrates and rehydrates more rapidly than that obtainable by regular freeze drying.

REFRIGERATED APPLE SLICES: PRESERVATIVE EFFECTS OF ASCORBIC ACID, CALCIUM AND SULFITES. J.D. PONTING, R. JACKSON & G. WATTERS. *J. Food Sci.* 37, 434–436 (1972)–Apple slices of two varieties were dipped in solutions at pH 3.5 and 7.0 containing ascorbic acid, calcium chloride and sulfur dioxide in various combinations. The slices after dipping and draining were stored at 34°F in plastic bags. At intervals of 1 or 2 wk the samples were evaluated for color and general quality and light reflectance was measured. Ascorbic acid alone or calcium alone did not protect color satisfactorily in the concentrations used, but a combination ascorbic acid-calcium dip maintained the natural color for more than 2 months in refrigerated storage. Calcium also increased the effectiveness of low concentrations of sulfur dioxide in protecting color. Three-way combinations of ascorbic acid, calcium and sulfur dioxide were no more effective than two-way combinations containing calcium.

DEHYDRATED CELERY: EFFECTS OF PREDRYING TREATMENTS AND REHYDRATION PROCEDURES ON RECONSTITUTION. H.J. NEUMANN. *J. Food Sci.* 37, 437–441 (1972)–Dehydrated celery treated before air drying with either sucrose, dextrose, sorbitol, or glycerol had improved reconstitution characteristics, such as increased weight, larger size, fuller shape and a more tender, crisper texture, compared to dried untreated celery. Dried treated celery, prepared from frozen unblanched celery treated after thawing, and also from fresh raw material, rehydrated 43–218% and 21–26%, respectively, more than dried untreated celery. When rehydrated samples (sucrose-treated and untreated) were redried and again rehydrated, treated celery rehydrated no better than untreated. However, water temperature used to originally rehydrate significantly affected successive rehydrations. Predrying treatments of water leaching, 1.5% Na_2CO_3, 0.5% $NaHSO_3$ and/or 60% sucrose are also evaluated.

INFLUENCE OF ALUM ON THE FIRMNESS OF FRESH-PACK DILL PICKLES. J.L. ETCHELLS, T.A. BELL & L.J. TURNEY. *J. Food Sci.* 37, 442–445 (1972)–The acidic properties were determined for alum salts (aluminum potassium sulfate, aluminum ammonium sulfate, aluminum sodium sulfate, and aluminum sulfate) that have been used as "firming agents" in the manufacture of pickled food products. Aluminum sulfate, the alum salt most commonly used today by the pickle industry, when added to fresh-pack (pasteurized) whole, dill pickles, caused a noticeable reduction in firmness of the stored pickle as compared to nonalum controls. Pickles prepared with lactic acid, either with or without alum, led to a greater loss in firmness during storage than those prepared with acetic acid. The addition of alum to fresh-pack pickles, prepared under good manufacturing procedures, particularly as to proper acidification and effective pasteurization, is a highly questionable practice.

INFLUENCE OF DIFFERENT ORGANIC ACIDS ON THE FIRMNESS OF FRESH-PACK PICKLES. T.A. BELL, L.J. TURNEY & J.L. ETCHELLS. *J. Food Sci.* 37, 446–449 (1972)–Acetic, lactic, citric, malic and oxalic acids were studied as to their ability to cause texture changes in fresh-pack (pasteurized) cucumber pickles. All of these except malic were evaluated for their equilibration rates with whole cucumbers and found to follow an exponential curve. Oxalic acid equilibrated at the fastest rate, followed by acetic, lactic and citric acids. Acetic acid treatments showed the least effect on cucumber firmness, followed by lactic, citric, malic and oxalic acids. From a texture standpoint, it would be better not to have the acids fully equilibrated with the cucumbers at the time of pasteurization. During storage, most of the acidification treatments, although apparently free of microbial growth, increased in titratable acidity and decreased in pH values. This unexplained change in acidic condition was accompanied by a loss in pickle firmness. Acetic acid proved superior to the other acids tested in retaining cucumber firmness. The use of lactic, citric, malic, or oxalic acids in the manufacture of fresh-pack pickles would not be recommended. Acetic acid (vinegar) should remain the acidulant of choice to give the best texture to pickle products.

PREPARATION AND EVALUATION OF SOYBEAN CURD WITH REDUCED BEANY FLAVOR. D.J. SCHRODER & H. JACKSON. *J. Food Sci.* 37, 450–451 (1972)–A process is described for the production of a bland soybean curd. Whole soybeans were blended with hot water (85–95°C) and the resulting soybean milk strained and steamed for 30 min. Precipitation of curd was effected by the addition of calcium sulfate. The curd was allowed to drain freely and then placed in small cheese hoops and pressed overnight. Taste panel evaluations of the pressed curd indicated that the beany flavor was reduced significantly. Meat product substitutes made from the curd by the addition of emulsifiers and flavoring agents were found to be very acceptable by a taste panel.

WHIPPING PROPERTIES OF SPRAY-DRIED COMPLEXES FROM WHEY PROTEIN AND CARBOXYMETHYLCELLULOSE. P.M.T. HANSEN & D.H. BLACK. *J. Food Sci.* 37, 452–456 (1972)–A study was made of the whipping properties of whey protein/hydrocolloid complexes resulting from treatment of diluted whey at pH 3.2 with NaCMC and H_2O_2. The washed, neutralized, and spray-dried products contained approximately 60% protein, 30% CMC, 10% water and no detectable H_2O_2. The powders were soluble and possessed whipping qualities in water and skim milk comparable to egg white. Studies revealed that the treatment of the whey or the neutralized complex prior to spray drying with 0.02–0.10% H_2O_2 was required for satisfactory foam development. At a concentration of 4% complex in water, stiff foams were produced after 15 min of whipping which remained stable for more than 30 min. A blend of the complex with an equal amount of sodium caseinate improved the volume of the foam and decreased the whipping time but did not increase foam stability. Fully stable foams were produced by addition to the mixture of $Ca(OH)_2$ to pH 9.5 and sucrose in an amount equal to twice the weight of water. This high-density sugar whip was suitable for use as a frosting and for the production of shaped meringues by drying at 100–150°C for 3–4 hr.

VACUUM PACKAGING OF LAMB: EFFECTS OF STORAGE, STORAGE TIME AND STORAGE TEMPERATURE. L.E. JEREMIAH, G.C. SMITH & Z.L. CARPENTER. *J. Food Sci.* 37, 457–462 (1972)–A total of 788 lamb cuts were used to determine the effects of vacuum packaged storage, storage time and storage temperature on the subsequent case-life and palatability of retail cuts. Chops from vacuum packaged sirloins and racks were significantly less desirable in odor upon removal from vacuum, but the color of these chops did not deteriorate at a faster rate than that of fresh chops. Storage in vacuum packages shortened the effective retail case-life of loin chops, especially from the standpoint of odor and such chops were significantly less desirable than fresh chops in flavor and overall satisfaction after 3 and 5 days of retail display, respectively. Leg roasts stored under vacuum for 7 days were significantly more desirable in palatability than roasts stored for longer periods. Storage of vacuum packaged loins at 0°C rather than 7°C was associated with lower bacterial counts, a lower incidence of off-odors and higher flavor and overall palatability ratings. Even short periods of vacuum packaged storage at low temperatures had detrimental effects on the subsequent retail case-life and palatability of lamb cuts.

VACUUM PACKAGING OF LAMB: EFFECT OF CERTAIN FACTORS ON RETAIL CASE-LIFE AND PALATABILITY. L.E. JEREMIAH, G.C. SMITH & Z.L. CARPENTER. *J. Food Sci.* 37, 463–468 (1972)–A total of 610 wholesale lamb cuts were used to study the effects of subcutaneous fat thickness, boning and trimming, lighting, packaging procedure, sanitation level and time postmortem on the acceptability of retail cuts during retail display and their subsequent palatability. Retail case-life was limited primarily by color and in no instance did chops from vacuum packaged loins have a case-life greater than 2½ days. Trimming prior to packaging did not affect color or odor of subsequent chops; boning prior to packaging increased shrinkage; and lighting during storage decreased color and odor desirability. Fresh cuts were significantly more desirable in color than those which were vacuum packaged. The optimum time to vacuum package cuts for subsequent refrigerated storage appears to be the 4th day postmortem.

COMPARISON OF PRE-COOKED IRRADIATED CHICKEN AND LAMB WITH AND WITHOUT PARTIAL DEHYDRATION. S.R. AGARWAL, F. HEILIGMAN & E.M. POWERS. *J. Food Sci.* 37, 469–472 (1972)–Studies were conducted to determine the individual and combined effect of partial dehydration (to 45% moisture), addition of parabens (0.07%) and sub-sterilizing doses of gamma irradiation (0.5–2.0 Mrad at ambient temperature) on precooked chicken and lamb. Samples were stored at 21°C and evaluated periodically for microbial, sensory and chemical parameters. Precooked nondehydrated samples treated with 4.5 Mrad at −30°C were used as standard references. Partial dehydration of chicken and lamb irrespective of parabens treatment had no effect on preference, odor and flavor but resulted in a deterioration of texture and appearance; whereas an increase of irradiation dose to 1.0 Mrad in chicken with parabens caused significant deterioration in preference, odor and flavor. The nondehydrated samples of chicken, with or without parabens, treated with 1.0 or 2.0 Mrad at ambient temperature were comparable in sensory attributes with samples irradiated with 4.5 Mrad at −30°C. The nondehydrated lamb, with or without parabens, irradiated with 1.0 Mrad at ambient temperature was preferred to the samples irradiated with 4.5 Mrad at −30°C. All the samples had total plate counts, anaerobic and mold counts less than 10/g and were free from *Cl. botulinum* toxin. During storage H_2 and CO_2 in the headspace of the samples increased, whereas CO, CH_4 and N_2 remained constant or decreased. Storage had no effect on NPN, pH and FFA.

EMULSION STABILITY AND PROTEIN EXTRACTABILITY OF OVINE MUSCLE AS A FUNCTION OF TIME POSTMORTEM. J.P. VAN EERD. *J. Food Sci.* 37, 473–475 (1972)–A large intermuscular variation was observed in the time taken for rigor mortis to develop in ovine muscle at room temperature; average times for semitendinosus and semimembranosus muscles were 8 and 14 hr respectively. The development of rigor mortis was characterized by a drop in protein extractability of 45% for semitendinosus muscle and 33% for longissimus dorsi muscle. Column chromatography on Sepharose 4B showed that this was due to a drop in the extractability of myofibrillar proteins. The decrease in protein extractability coincided with a decreased emulsifying efficiency. Whereas myosin and actomyosin were both found to be effective emulsifiers, the latter was much more efficient.

ABSTRACTS:
IN THIS ISSUE

BEEF COLOR AS RELATED TO CONSUMER ACCEPTANCE AND PALATABILITY. L.E. JEREMIAH, Z.L. CARPENTER & G.C. SMITH. *J. Food Sci.* 37, 476–479 (1972)–A system for evaluation of beef muscle color was developed, defined and tested for consumer acceptance. The colors in the scale developed did not differ significantly in hue, but differed significantly ($P < 0.05$) in both value and chroma. Steaks that were visually scored as two or three in this system possessed significantly less total pigment ($P < 0.05$) than steaks with visual scores of five or six. Consumers preferred steaks which were neither very dark nor very pale and discriminated heavily against very dark steaks. Consumers appeared to prefer steaks which were paler than the "cherry red" formerly suggested as "ideal." Measures of color did not appear to be related to any of the palatability traits. The Macbeth-Munsell Disk Colorimeter, Gardner Color Difference Meter and Bausch and Lomb Spectronic-20 Spectrophotometer equipped with a reflectance attachment accounted for approximately the same amount of variation in visual color scores, with the Photovolt-610 Reflectance Meter accounting for considerably less of the variation in visual color. Objective color measurements accounted for considerably more of the variation in visual color than a combination of pH and total pigment concentration.

DEVELOPMENT OF A PROTOTYPE SAUSAGE EMULSION PREPARATION SYSTEM. A. HAQ, N.B. WEBB, J.K. WHITFIELD & G.S. MORRISON. *J. Food Sci.* 37, 480–484 (1972)–An efficient and a highly controllable prototype sausage emulsion preparation system incorporating features of: (1) scrapers, (2) cooling coils, (3) oil delivery tube, (4) AC/DC measuring electrodes, (5) temperature thermocouple and (6) variable speed motor base was developed. The system resulted in very acceptable cook stability and physical properties of emulsions. A small quantity of materials (150–160g) and a short preparation time (160 sec) were required for the system and one person could satisfactorily perform the operation.

POST-IRRADIATION EVALUATION OF PATHOGENS AND INDICATOR BACTERIA. N.P. TIWARI & R.B. MAXCY. *J. Food Sci.* 37, 485–487 (1972)–The impact of radiation and post-irradiation recovery of cells of *Salmonella* species, *Staphylococcus aureus, Escherichia coli* and *Streptococcus faecalis* was studied using nonselective and selective media. The death of cells during 24 hr after radiation was attributed primarily to injured cells, defined as those capable of growth on plate count agar but unable to grow on selective media. More injured cells died at 37°C than at lower temperatures to 5°C. The nature of the selective medium influenced the number of injured salmonellae cells capable of recovery and growth. The proportion of injured cells to normal cells was approximately the same for dose levels from 34–136 Krad. *E. coli* and *S. aureus*, however, showed an increased proportion of injured cells with an increased dose up to 170 and 272 Krad, respectively. Cells injured by irradiation were not as infective as unirradiated cells when judged by egg yolk challenge.

A SIMPLIFIED METHOD FOR THE ANALYSIS OF GLUTAMINE. Y.D. LIN, F.M. CLYDESDALE & F.J. FRANCIS. *J. Food Sci.* 37, 488–489 (1972)–Spinach puree was analyzed for pyrrolidone-carboxylic acid (PCA) using an Automatic Organic Acid Analyzer (AOAA). Puree from the same batch was then buffered to pH 6.8 with a buffer solution of potassium dihydrogen phosphate and sodium tetraborate and final adjustment with concentrated hydrochloric acid. This puree was then subjected to hydrolysis for 2 hr in a boiling water bath. After cooling to room temperature, PCA was analyzed by the AOAA. The quantity of PCA formed from glutamine during hydrolysis was calculated by subtracting the amount of PCA in the fresh puree from that in the hydrolyzed puree. Since one mole of glutamine quantitatively converts to one mole of PCA, the amount of glutamine present in the fresh sample is equivalent to the PCA formed during hydrolysis.

TECHNIQUE FOR DETERMINING OXYGEN CONCENTRATION INSIDE PACKAGES. D.G. QUAST & M. KAREL. *J. Food Sci.* 37, 490–491 (1972)–A simple and inexpensive technique for determining oxygen concentration inside food packages is described. This technique can be used to evaluate the effectiveness of inert gas packaging processes as well as to determine the rate of oxygen uptake of food products and oxygen permeabilities of packaging films.

TEMPERATURE GRADIENT FREEZE-DRYING MICROSCOPE STAGE. M. FREEDMAN, J.H. WHITTAM & H.L. ROSANO. *J. Food Sci.* 37, 492–493 (1972)–A versatile temperature gradient freeze-drying stage has been designed. The major parts are a wettable (sand blasted) microscope slide inserted on a cold plate which is connected to a vacuum chamber. Rates of freezing have been varied from 5mm/min to as slow as 5mm/60 min. A variable vacuum, as low as 50μ of mercury can be applied to the cell. Visual microscopic observations and time-lapsed photography are used to record the conditions. This cell is an inexpensive, simply constructed apparatus which can be utilized in freeze-drying research and as an engineering aid to help investigate the dynamics of many industrial freeze-drying processes.

INFLUENCE OF PROCESSING PROCEDURES ON TOTAL COUNT, PRESUMPTIVE COLIFORMS, AND Clostridium perfringens IN BEEF FOR FURTHER COOKING. B.F. DENNIS, L.E. KUNKLE, H.W. OCKERMAN, R.J. BORTON & V.R. CAHILL. *J. Food Sci.* 37, 494–495 (1972)–A chopped beef product vacuum packed in a fibrous casing was sufficiently heated to coagulate the protein on the surface after which it was cooled and frozen. There were no significant changes in the number of total aerobes, presumptive coliforms or *Clostridium perfringens* during the processing of this product indicating it is no more of a public health hazard than the fresh beef from which it was manufactured.

TOCOPHEROL SUPPLEMENTATION AND LIPID STABILITY IN THE TURKEY. R.W. WEBB, W.W. MARION & P.L. HAYSE. *J. Food Sci.* 37, 496 (1972)–Experiments were conducted to establish the effect of method (oral or injection) and level of tocopherol supplementation in stabilizing turkey lipids during the cooking process. Significant effects ($p < 0.01$) were found due to tocopherol treatment, meat type (breast or thigh) or cooking, but not to sex. Treatment × Cooking interaction was also significant ($p < 0.01$). Tocopherol supplementation, oral or injection, to the turkey offers considerable potential in reducing rate of oxidative deterioration of lipids during meat cookery.

REDUCTION OF ANTITHIAMINE ACTIVITY IN CRAYFISH BY HEAT TREATMENT. J.E. RUTLEDGE & L.C. YING. *J. Food Sci.* 37, 497–498 (1972)–Thiaminase activity was found to be restricted to the cephalothorax region of crayfish. The flesh material in this region was capable of destroying 3.7 μg of thiamine hydrochloride per minute per gram of tissue on a dry weight basis. The digestive glands located in this region constituted 87% of the total activity. Prolonged heating and boiling failed to destroy all activity. Thus, it appears that at least two factors were responsible for the destruction of thiamine, one heat sensitive and the other heat stable or possibly the assay procedure was inadequate.

A SIMPLE METHOD TO DETERMINE THE WATER-HOLDING CAPACITY OF MUSCLE FOODS. B. DAGBJARTSSON & M. SOLBERG. *J. Food Sci.* 37, 499–500 (1972)–A technique utilizing a blender, dry ice, polycarbonate centrifuge tubes, molecular sieve packing, filter paper, analytical balance, a high speed centrifuge and a 1.0-g sample of muscle food was developed for measuring water-holding capacity. The standard deviation of values within a sample was approximately 0.01g liquid expressed per gram sample which was approximately 2.0% of the mean value.

THE POSSIBLE IDENTIFICATION OF AN IRRADIATION TREATMENT OF FISH BY MEANS OF ELECTRICAL (ac) RESISTANCE MEASUREMENT. D. EHLERMANN. *J. Food Sci.* 37, 501 (1972)–Measurement of tissue impedance of trout at the frequencies of 16 and 100 kHz allows the estimation of storage time regardless of whether the fish have been irradiated or not. Additional measurement at 1 kHz permits approximate estimation of the radiation dose if a dose of 200 krad or above was used.

EFFECT OF PRE- AND POST-MORTEM HANDLING ON REFLECTANCE CHARACTERISTICS OF CANNED SKIPJACK TUNA. A.C. LITTLE *J. Food Sci.* 37, 502 (1972)–Reflectance data on samples representing 34 combinations of pre- and post-mortem handling conditions, rested or stressed before death, held at various temperatures for specified time intervals postmortem and canned immediately or after freezing showed that on the basis of Y value alone the samples showed remarkable consistency in color evaluation: over-all mean 28; range 22–33 with 82% between 26–30. None of the handling practices prior to canning was sufficiently severe to affect systematically and significantly the reflectance values of the canned fish; therefore, differences must be ascribed to individual variation. A systematic decrease in magnitude of chromaticity shift, particularly in Δx, was observed on increasing holding temperature and finally on increasing time at the highest temperature to which fish were subjected.

Letters TO THE SCIENTIFIC EDITOR

Letter to the Editor

February 24, 1972

Dear Sir:

I would like to comment on the letter by Sutton and Carpenter about the paper entitled "Computer-aided predictions of food storage stability: Oxidative deterioration of a shrimp product" by I.B. Simon, T.R. Labuza and M. Karel, J. Food Sci. 36(2): 280. This letter and a reply by my co-author Dr. Labuza were printed in the J. Food Sci. 37(1): ii. In addition to the points made by Labuza, with which I agree, let me point out the following:

(1) The specific case of rapid steady state attainment has in fact been recognized by us, and we have published several papers on simple graphical techniques for calculation of steady state concentrations.

Jurin and Karel, Food Technol. 17(6): 104
Karel and Go, Modern Packaging 37(6): 123
Veeraju and Karel, Modern Packaging 40(2): 168
Karel, Food in Canada 27(4): 43

(2) Dietrich Quast and I have submitted to the J. Food Sci. several papers dealing with an analysis of storage behavior of dehydrated foods which show that, unfortunately, the steady state case, while simpler in calculation requirements, does not adequately describe the situation and a more complex approach is required for adequate description of the storage behavior of these foods.

I would, however, like to commend Drs. Sutton and Carpenter for pointing out that in many cases a simplified approach will yield adequate solutions.

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Errata Notice

- J. Food Sci. 37(1): 4–7 (1972), R. Hagenmaier, C.M. Cater and K.F. Mattil: "A characterization of two chromatographically separated fractions of coconut protein." On page 4, Introduction, line 14 and following, change to: "produced 7 billion coconuts, which are estimated to contain enough protein for 10g per Filipino per day."

- J. Food Sci. 37(1): 81–85 (1972). R.L. Buchanan and M. Solberg: "Interaction of sodium nitrate, oxygen and pH on growth of Staphylococcus aureus." On page 81, change title to: "Interaction of sodium nitrite, oxygen and pH on growth of Staphylococcus aureus."

- J. Food Sci. 37(1): 108–112 (1972), M.K. Veldhuis, R.E. Berry, C.J. Wagner Jr., E.D. Lund and W.L. Bryan: "Oil- and water-soluble aromatics distilled from citrus fruit and processing waste." On page 108, Figures 1 and 2 are reversed. The line drawing with the caption "Cold-pressed orange peel oil mill" is Figure 1–Typical flow diagram of cold-pressed peel oil mill; the line drawing of the condensers, recovery units, etc., is Figure 2–Distilled oil and aroma recovery unit.

JFS BASIC SCIENCE

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THE EFFECT OF PRE-RIGOR CHANGES ON MEAT TENDERNESS. A Review

INTRODUCTION

THE TENDERNESS of meat is notoriously variable. It varies not only among anatomically different muscles but also among corresponding muscles from animals of the same or different species, and it is influenced by both pre-slaughter and post-slaughter factors. Over the years a great deal of research has gone into establishing the effects of pre-slaughter factors such as species, breed, age, sex, nutrition and exercise, and of post-slaughter treatments such as aging (i.e., prolonged storage at temperatures above freezing) and freezing.

Structurally, striated muscle can be regarded as being made up of a fibrillar component which is responsible for the contraction and relaxation of the muscle and a connective tissue component which holds the fibers together as well as attaching the muscle to the skeletal framework. In the earlier part of the century it was believed that the quantity and strength of the connective tissue determined the toughness of the meat (Lehmann, 1907; Mitchell et al., 1926; Mackintosh et al., 1936). However, there is now ample evidence that changes in the myofibrillar component pre-rigor (i.e., during the period between slaughter and the full development of rigor mortis) can markedly influence the tenderness of the resulting meat.

One of the earliest observations indicating that tenderness was influenced by pre-rigor changes was that muscle cut or excised soon after slaughter was tougher when rigor mortis had developed than uncut muscle which had gone into rigor mortis on the bone (Lowe and Stewart, 1946; Ramsbottom and Strandine, 1949; Koonz et al., 1954; Paul and Bratzler, 1955; de Fremery and Pool, 1960; Locker, 1960; Herring et al., 1965a; Cagle and Henrickson, 1970; McCrae et al., 1971). Another was that meat cooked soon after slaughter was more tender than meat cooked soon after the development of rigor mortis (Moran and Smith, 1929; Ramsbottom and Strandine, 1949; Paul et al., 1952; de Fremery and Pool, 1963; Marsh, 1964; Weidemann et al., 1967).

Pre-rigor changes known to influence tenderness, and factors which influence these changes are discussed below.

CHEMICAL CHANGES

USUALLY several hours elapse before rigor mortis is fully developed. During this period glycogen, the carbohydrate reserve in muscle, is converted to lactic acid and as a result the pH of the muscle falls. (The final pH value reached is referred to as the ultimate pH–Callow, 1937.) At the same time, the concentration of adenosine triphosphate (ATP), the immediate source of chemical energy for muscle contraction, falls. These two events are closely interrelated.

Both the rate and the extent of the postmortem fall in pH have been related to the tenderness of the resulting meat. For example, it has been shown with chicken muscle that toughness is increased by treatments which increase the rate of pH (and ATP) fall (de Fremery and Pool, 1960; Khan and Nakamura, 1970). It has also been reported that beef is least tender when the ultimate pH is about 6.0 and increases in tenderness as the ultimate pH increases above or decreases below this value (Bouton et al., 1957). In contrast, the tenderness of rabbit (Miles and Lawrie, 1970), sheep (Bouton et al., 1971) and fish (Kelly et al., 1966) has been shown to be greater with higher ultimate pH.

Factors affecting rate of pH fall

The rate of fall of pH depends on temperature (Bate-Smith and Bendall, 1949; Bendall, 1951; Marsh, 1954; Marsh and Thompson, 1958; Bendall, 1960; de Fremery and Pool, 1960; Cook and Langsworth, 1966; Cassens and Newbold, 1967) but the effect of temperature is not the same with all muscles. For example, with rabbit psoas muscle, the lower the temperature in the range 0–37°C the more slowly the pH falls (Bendall, 1960). In ox sternomandibularis (neck) muscle, however, although the pH falls more slowly the lower the temperature in the range 5–37°C, it falls more rapidly at 1°C than at 5°C during the first few hours postmortem (Cassens and Newbold, 1967; Newbold and Scopes, 1967).

The rate of pH fall can also vary among different muscles from the same animal, and among corresponding muscles from individuals of the same or different species (Lawrie, 1966a). The variability among corresponding muscles is particularly great with pigs (Briskey, 1964) and may be related to the intensity of the nervous stimulus reaching the muscle before and during slaughter (Bendall, 1966; McLoughlin, 1970).

Pre-slaughter injection of muscle relaxants such as magnesium salts (Howard and Lawrie, 1956; Sair et al., 1970; Campion et al., 1971), myanesin (Bate-Smith and Bendall, 1949; Bendall, 1966), or curare (Bendall, 1966; Sair et al., 1970; McLoughlin, 1970) has been reported to decrease the rate at which the pH falls postmortem, while pre-slaughter injection of calcium salts has been found to increase it (Howard and Lawrie, 1956; Campion et al., 1971). In the experiments of Lister and Ratcliffe (1970), however, pre-slaughter injection of magnesium had no effect on the rate of pH fall.

Factors affecting extent of pH fall

In the living animal the pH of resting muscle is about 7.3. Since the postmortem fall in pH is the result of the production of lactic acid from glycogen, it is clear that the extent of the pH fall may depend on the amount of glycogen present in the muscle at the time of slaughter. The glycogen content can be reduced by starvation, exhausting exercise, the imposition of pre-slaughter stresses of various sorts, or by struggling at the time of slaughter (Lawrie, 1966b). It can also be reduced by the pre-slaughter injection of insulin (Bate-Smith and Bendall, 1947, 1949; Howard and Lawrie, 1956, 1957), or adrenaline (Radoucco-Thomas et al., 1959; de Fremery and Pool, 1963; Penny et al., 1963; Klose et al., 1970; Khan and Nakamura, 1970; Bouton et al., 1971). Ultimate pH values of greater than 7.0 have been obtained by reducing the glycogen reserves before slaughter. Even when there is an adequate supply of glycogen in the muscle at the time of slaughter the ultimate pH is rarely less than 5.4–5.5 and, for reasons which are not yet clear, it is sometimes appreciably higher than this. Abnormally low values, down to 4.8, have been recorded in pig muscle (Lawrie et al., 1958).

The temperature at which the muscle is stored postmortem has only a small effect on the ultimate pH (Cassens and Newbold, 1967).

PHYSICAL CHANGES

AT THE TIME of slaughter, muscle is plastic and highly extensible; in full rigor it is firm and

relatively inextensible. In addition to losing its extensibility, unrestrained muscle shortens during the development of rigor mortis. Shortening can occur only while ATP is present. When all the ATP is lost the rigor process is complete, and the muscle is fixed in whatever state of contraction it happens to be at the time.

In 1960, Locker concluded that there was a relationship between postmortem shortening and tenderness. This relationship has been the subject of several investigations in recent years (Marsh, 1964; Herring et al., 1965a, b, 1967; Marsh and Leet, 1966a, b; Buck and Black, 1967; Buck et al., 1970; Davey et al., 1967; Weidemann et al., 1967; Cooper et al., 1968; Howard and Judge, 1968; Marsh et al., 1968; Welbourn et al., 1968; Smith et al., 1969; McCrae et al., 1971). According to Herring et al. (1967) the length of the basic myofibrillar subunit, or sarcomere, gives a measure of the contraction state of the muscle. Using ox muscles excised soon after slaughter, Marsh and Leet (1966a, b) and Davey et al. (1967) have shown that shortening by up to 20% of the excised length produces relatively small changes in tenderness, whereas further shortening from 20–40% produces a several-fold increase in shear value. With still further shortening there is a progressive decrease in toughness until at 60% shortening shear values are of the same order as those obtained at 20% shortening or less. According to Davey et al. (1967) the sarcomeres are about 2.5 μm long in freshly excised ox neck muscle, so that 40% shortening corresponds to a sarcomere length of about 1.5 μm, which is the approximate length of the myosin filaments. Recently, McCrae et al. (1971) have shown the relationship between postmortem shortening and tenderness for lamb muscles closely resembles that previously found for ox neck muscles.

It has also been shown (Herring et al., 1967; Davey et al., 1967) that stretching muscle and allowing it to go into rigor in this condition has little effect on tenderness. The toughness of unshortened muscle has been referred to as "background" toughness and is believed to represent the situation where the contribution of the connective tissue to toughness is predominant. The increase in toughness with shortening is believed to be due to changes in the myofibrillar structure (Marsh and Leet, 1966b).

Postmortem shortening could be a factor in the relative increases in tenderness that occur during subsequent aging or conditioning of the meat. Davey et al. (1967) have demonstrated that when the amount of shortening of ox neck muscle increases above 20% the tenderness improvement with aging becomes smaller. At 40% shortening or greater there is no improvement. However, Herring et al. (1967), using ox semitendinosus muscle, showed that during aging muscle which had shortened to a sarcomere length of about 1.5 μm improved in tenderness.

Factors affecting postmortem shortening

Postmortem shortening is dependent on temperature but not all muscles show the same temperature-dependence. For example, at 37°C, rabbit psoas muscle excised soon after slaughter shortens by more than 30% of its excised length, and the amount of shortening decreases steadily to about 9% at 2°C (Locker and Hagyard, 1963). On the other hand, with ox neck muscle, the amount of shortening decreases from about 30% at 37°C to 10–15% at 15°C, but increases with further reduction in storage temperature–a phenomenon known as cold-shortening (Locker and Hagyard, 1963). At 0°C this muscle shortens by up to 50% of its excised length. Ovine (Cook and Langsworth, 1966), porcine (Galloway and Goll, 1967; Hendricks et al., 1971) and avian (Smith et al., 1969) muscles have also been shown to cold shorten.

Even greater shortening can occur in muscles frozen pre-rigor and then thawed. Thaw-shortening in excess of 70% has been reported in rabbit psoas (Lawrie, 1968), ox neck muscle (Marsh and Leet, 1966b; Scopes and Newbold, 1968) and sheep longissimus dorsi (Marsh and Thompson, 1958). When it exceeds 40–50% it is accompanied by the exudation of large amounts of fluid (Marsh and Thompson, 1957, 1958; Marsh and Leet, 1966b).

The amounts of cold shortening and thaw-shortening decrease as the period between slaughter and exposure to cold or freezing conditions, respectively, is extended (Marsh and Thompson, 1958; Locker and Hagyard, 1963; Marsh and Leet, 1966b).

In view of its contribution to meat toughness, it is clearly desirable to minimize or prevent postmortem shortening. This can be accomplished by allowing rigor mortis to develop at a temperature at which postmortem shortening is minimal, or by physically restraining the muscle from shortening. Muscle restrained from shortening until the rigor process is complete does not shorten when released (Marsh and Thompson, 1958) and is not made tougher by pre-rigor exposure to cold-shortening or thaw-shortening conditions (Marsh, 1964; Marsh and Leet, 1966b). Thaw shortening in muscle frozen pre-rigor can also be prevented by keeping the muscle at a temperature just below its freezing point for several days before allowing it to thaw. Under these conditions the chemical changes associated with the development of rigor mortis are completed while there is sufficient ice in the muscle to prevent shortening (Moran, 1930; Marsh and Thompson, 1958).

On the carcass not all muscles are restrained to the same extent. Some are firmly attached to the skeleton at both ends, others at one end only. In addition, the amount by which a muscle can shorten depends on the position of the carcass during the development of rigor mortis. For example, it has been shown (Herring et al., 1965b) that some muscles from a side of beef suspended by the hind leg are more tender than the corresponding muscles from a side positioned horizontally; other muscles are less tender and others again are unaffected. Hostetler et al. (1970a), Arango et al. (1970) and Harris and Macfarlane (1971) have investigated the effects of hanging sides of beef in different ways and have found that, compared with the conventional method of hanging by the Achilles tendon, hanging by the obturator foramen (aitch bone) improves the tenderness of some important cuts, notably the loin, inside round and rump. In their study, Harris and Macfarlane showed that some muscles from sides suspended by the aitch bone were as tender, 2 days postmortem, as were those removed from conventionally suspended sides 2 days postmortem and then aged for 2 wk at 0–1°C. Bouton and Harris (1972) have shown that the tenderness of sheep biceps femoris, semimembranosus and longissimus dorsi muscles can be improved by hanging the whole carcass from the pelvis instead of from the Achilles tendon.

The time-temperature history of a carcass during the pre-rigor period can also have a marked effect on the tenderness of the resulting meat. The longissimus dorsi muscles from lamb carcasses held for various periods at 20°C before being exposed to freezing conditions have been shown by Marsh et al. (1968) to be more tender when the holding period exceeds 16 hr than when it is shorter than this. McCrae et al. (1971) have shown that lamb muscles vary widely in their response to pre-rigor freezing of the carcass and that increasing the holding period at 18°C from 10–16 hr before freezer-entry greatly improves the tenderness of some muscles. However other muscles are very tender despite early freezing.

From studies of the effects of physical and mechanical treatments on the tenderness of the ox longissimus dorsi muscle, Smith et al. (1971) concluded that chilling the carcass in a cooler at 16°C for more than the first 16–20 hr postmortem produces as big an improvement in the tenderness of this muscle as can be brought about by changing the method of carcass suspension.

Schmidt and Gilbert (1970) have compared the tenderness of beef muscles removed pre-rigor and stored at 15°C for 24 or 48 hr with the corresponding muscles (controls) left on the carcass and chilled at 9°C for 24 hr. The biceps femoris and longissimus dorsi muscles stored at 15°C for 24 hr were of equivalent tenderness to their controls while those stored at 15°C for 48 hr were significantly more tender. The semimembranosus showed no treatment effect while the excised semitendinosus muscles were significantly tougher than their controls.

The mechanism of postmortem shortening is believed to be the same as that of muscular contraction in vivo. Hence an understanding of the factors involved in the physiological control of muscular contraction could lead to the development of methods for preventing postmortem shortening which do not involve temperature control or physical restraint.

Throughout muscle there is a very fine anastamosing network of tubules (the sarcoplasmic reticulum) which have the ability to store calcium. For contraction to occur, not only must ATP be present, but calcium must be released from this network and diffuse to active sites on the contractile proteins. Weiner and Pearson (1966, 1969) have shown that intravenous injections of calcium chelating agents into rabbits and pigs before slaughter inhibit postmortem shortening of the semitendinosus muscle, and result in a significant improvement in the tenderness of the pig muscle but not of the rabbit muscle. These results indicate that postmortem shortening can be reduced by chemical as well as by physical means. However, an acceptable chemical method has not yet been devised. The development of such a method is an area of research which merits a great deal of attention.

SUMMARY & CONCLUSIONS

THE PRODUCTION of tender meat is not entirely the responsibility of the meat producer, for tenderness is greatly influenced by the conditions prevailing during the period between slaughter and the full development of rigor mortis. Thus the meat processor, by using unsuitable proc-

essing methods, can make potentially tender meat tough.

It has been established that tenderness is related to postmortem shortening and processing methods should therefore be aimed at minimizing this shortening.

Experiments with lamb and beef have shown that one way of reducing "processing toughness" is to hold the carcass for about 20 hr in a room at 15–20°C before placing it in a freezer. A disadvantage of this method is that bacteria can grow well at this temperature and a distinct health risk may be introduced. If the humidity is reduced to a level which discourages the growth of salmonellae, evaporative losses from the carcass can be high.

Another approach to the prevention of shortening is to change the method of hanging the carcass (or side) during the development of rigor mortis. The change in posture brought about by hanging from the Achilles tendon leads to considerable shortening of some of the major muscles of the hind-leg and back. Much of this shortening can be avoided by suspending the carcass from the aitch bone. Although such a method improves the tenderness of these muscles it produces a change in carcass conformation which makes both 'boning out' and commercial handling more difficult. These disadvantages may possibly be overcome by further modifications to the method of suspending the carcass.

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Ms received 10/13/71; revised 1/10/72; accepted 1/16/72.

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THE RELATIVE IMPORTANCE OF SOME DETERMINANTS OF BEEF TENDERNESS

INTRODUCTION

DETERMINANTS of meat tenderness may be either direct or indirect depending on whether they affect tenderness directly or through an effect on another determinant. It follows that direct determinants will be intrinsic properties such as meat composition or the degree of contraction of the muscle fibers, while indirect determinants may be either external characteristics, such as nutrition, or intrinsic characteristics, such as animal age or genotype.

A number of techniques have been used to assess the relative importance of several possible direct determinants. Howard and Judge (1968) measured several characteristics and assessed their importance by multiple regression techniques with the stepwise removal of variables. Cover et al. (1962) were able to make inferences based on the way in which taste panelists answered certain carefully selected questions about different components of tenderness.

In this study sex, weight and breed type were considered as possible indirect determinants of beef tenderness. The aim was to acquire some information on the direct determinants involved by measuring the effects of sex, weight and breed type on the response of tenderness to different cooking temperatures and to different aging times. Also, a theoretical quantitative assessment was made of the relative effects on overall tenderness of changes in composition and of changes in the tenderness of individual components. A simple model was considered under a range of conditions.

MATERIALS & METHODS

STEAKS were obtained from animals used in experiments designed primarily for purposes other than the assessment of tenderness. However, the animals were raised in the same place and under similar conditions. Table 1 lists some characteristics of these animals together with references which give more details. Two or three days following slaughter in a commercial abattoir, cuts containing ribs 8, 9, 10, 11 and 12 from both sides were brought to the laboratory for physical dissection. Outside slices of at least 3 mm from the dissected M. longissimus from the 10-11-12 rib cut were discarded and then four 2.5 cm thick steaks were cut for all groups except S_{500} where only three steaks were cut, since only ribs 10 and 11 were dissected for that group. Two steaks were allocated to each of the four treatments described in Table 2 except that in the case of group S_{500} no steaks were allocated to treatment C. Steaks were stored at 4° ± 1°C in polyethylene bags. As much air as possible was excluded from the bags but they were not evacuated or sealed. Cooking was accomplished by suspending the plastic bags over a water bath so that the portion containing the steak was immersed (Marsh et al., 1966; Bouton et al., 1971). Following cooking, the steaks were cooled overnight at 4°C in the plastic bags. The tenderness of six 14 × 14 mm cores was then assessed by shearing perpendicular to the fibers with a Warner-Bratzler shear device. Care was taken to cut the cores parallel to the muscle fibers.

The statistical significance of specific comparisons was tested by means of orthogonal contrasts (Sokal and Rohlf, 1969).

RESULTS & DISCUSSION

MEAN SHEAR values are shown in Figure 1, while the changes in shear values with 14 days aging and with a change of 15°C in the cooking temperature are shown in Figure 2. Table 3 lists the statistical significance of the orthogonal comparisons that were made.

Effects of sex, weight and breed

Meat from Friesian-Brahman cross bulls (FBB) was significantly tougher than that from Friesian bulls (FB) for measurements A, B and C (Table 3), and it responded to aging (A–D) to a significantly greater extent than the FB meat (Fig. 2, Table 3). Brahmans and Brahman crosses have been shown previously to produce tougher meat than British breeds (Palmer, 1963; Ramsey et al., 1963), although Howard (1963) noted that animals from Brahman sires produced more tender meat than those from British sires. The tenderness characteristic differing most between the two genotypes was the response to 2 wk aging which implies that the meat from the two groups differed in the characteristics which are responsible for tenderness changes during aging. There is evidence that some of the most important of these changes are associated with the myofibrils, particularly in the Z line region (Davey

Table 1—Characteristics of the groups of animals

Group designation	Breed	Sex	No. of animals	Age at castration[a] (month)	Approx age at slaughter (month)	Approx live weight at slaughter (kg)	Feed immediately pre-slaughter	Reference for further details
S_{500}	Friesian	Steer	12	3	18	500	Grain	Davies and Faichney (1971)
S_{400}	Friesian	Steer	10	3	17–20	400	Grain	Kellaway (1971)
EC	Friesian	Cryptorchid	10	3	17–20	400	Grain	Kellaway (1971)
LC	Friesian	Cryptorchid	10	10	17–20	400	Grain	Kellaway (1971)
FB	Friesian	Bull	13	–	10	300	Pasture	Kellaway and Colditz (1971)
FBB	Friesian × Brahman	Bull	11	–	10	300	Pasture	Kellaway and Colditz (1971)

[a]In the case of the cryptorchids, their testes were forced into the body cavity rather than removed (Hudson et al., 1968).

and Gilbert, 1967; Davey and Dickson, 1970), thus suggesting that the Brahman-Friesian bulls had less stable Z lines than the Friesian bulls. Alternatively, the greater effect of aging shown on Brahman-Friesian cross meat may have resulted from the Friesian bull meat having tenderized mainly before the first tenderness measurement at approximately 5 days, in which case it could be argued that the Brahman-Friesian Z lines structure was more stable.

In this study neither the weight of the steers at slaughter (S_{400} vs. S_{500}) nor the age at which the cryptorchids were operated on (EC vs. LC) had any significant effect on any of the measures of tenderness considered (Table 3). Zinn et al. (1970) reported significantly higher shear values for samples of M. longissimus from Hereford steers and heifers at 427 kg than for similar animals at 339 kg. However, the former group was 120 days older. Murray (1970), on the other hand, showed a significant negative relationship between shear values of M. longissimus of Angus steers and live weight over the range 300–400 kg. No differences were detected by Suess et al. (1966) in the tenderness of steaks from Angus steers slaughtered at 386, 424 and 455 kg live weight. Thus, the relationship between live weight and tenderness of beef in this live-weight range seems inconsistent.

The decrease in the significance of the differences in tenderness between cryptorchids and steers with increased postmortem aging is consistent with the significantly greater response to aging shown by meat from cryptorchids (Table 3). This greater response to aging may again be interpreted in terms of myofibrillar or Z line stability. If the degree of breakdown of Z line structure is dependent to some extent on the activity of lysosomal proteases (Valin, 1970), then stability of lysosomal membranes may be important. This may partially explain the greater aging effect on tenderness of cryptorchid meat as cryptorchids have more circulating testosterone than steers (Kellaway et al., 1971) and testosterone has a labelizing effect on lysosomal membranes (Weissmann and Thomas, 1964).

The response of tenderness to an increase in cooking temperature from 60°C to 75°C was significantly greater for steer meat than for cryptorchid meat suggesting that steer meat differs significantly in the components which cause changes in tenderness as meat is heated over this range. According to Hamm (1966), the decrease in tenderness normally encountered as meat is heated from 60°C to 75°C is due to the coagulation of myofibrillar proteins and shrinkage of collagen. The concomitant partial solubilization of collagen (Goll et al., 1964) would increase tenderness. Therefore, the results in Table 3 imply that steer meat either has more proteins which coagulate over this temperature range, has more collagen or collagen that shrinks to a greater extent, or has collagen which is less soluble than that of cryptorchids.

Care should be taken in the interpretation of the steers vs. cryptorchids comparison as the physiological differences are confounded with a difference in mean weight. However, the absence of any significant differences between the two groups of steers would suggest that differences between the steers and cryptorchids were not due to weight effects.

Confounding effects cannot be separated so easily in the comparisons between bulls and steers plus cryptorchids. The significant differences shown in Table 3 may be due to a sex

Table 2–The four treatments applied to different steaks from each animal to give the four tenderness measurements A, B, C and D

Tenderness measurement	Storage time (days post mortem)	Storage temp (°C ± 1)	Cooking time (min)	Temp of the water bath (°C ± 1)
A	5 ± 1	4	60	75
B	12 ± 1	4	60	75
C	12 ± 1	4	60	60
D	19 ± 1	4	60	75

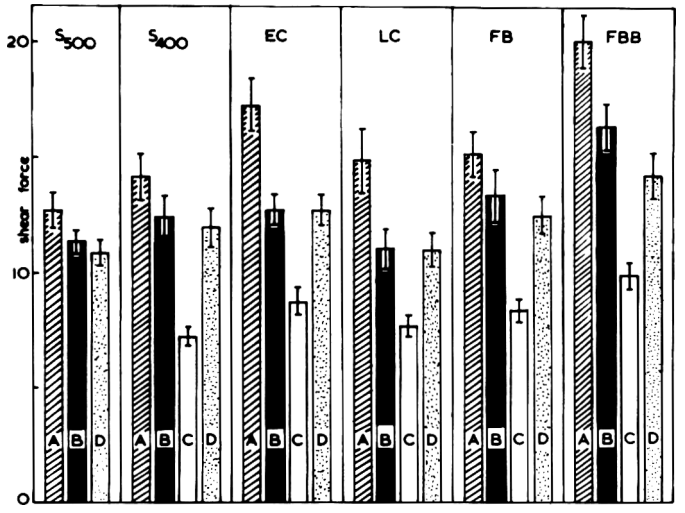

Fig. 1–Mean shear values with standard errors for the six groups of animals (Table 1) and for the four measurements of tenderness (Table 2).

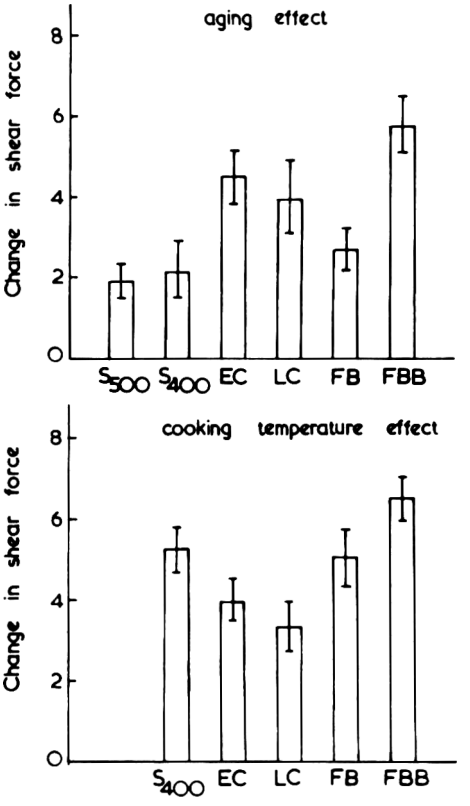

Fig. 2–Means and standard errors of the changes in shear force as meat was aged from 5–19 days and as the cooking temperature was increased from 60° C to 75° C. Values are given within each of the experimental groups (Table 1).

Table 3–Levels of statistical significance of the five orthogonal comparisons for each measurement of meat tenderness

Comparison[a]	Tenderness measurement[b]					
	A	B	C	D	A–D	B–C
FB vs. FBB	**	*	*	NS	***	NS
S_{400} vs. S_{500}	NS	NS	NS	NS	NS	–
EC vs. LC	NS	NS	NS	NS	NS	NS
(S_{400} + S_{500}) vs. (EC + LC)	*	NS	NS	NS	**	*
(S_{400} + S_{500} + EC + LC) vs. (FB + FBB)	**	***	*	*	NS	**

[a]Explanations of the abbreviations used are given in Table 1.
[b]Explanations of the postmortem treatments are given in Table 2.
***P < 0.001; **P < 0.01; *P < 0.05; NS P > 0.05

effect, a weight effect, a breed effect or an effect of immediate pre-slaughter nutrition. The results in Figures 1 and 2 would suggest that the breed differences discussed previously make an important contribution. It has already been pointed out that slaughter weight effects on meat tenderness are inconsistent. Field (1971) has recently reviewed studies involving comparisons between the tenderness of meat from bulls and steers and although bull meat is usually less tender, the differences are frequently not statistically significant.

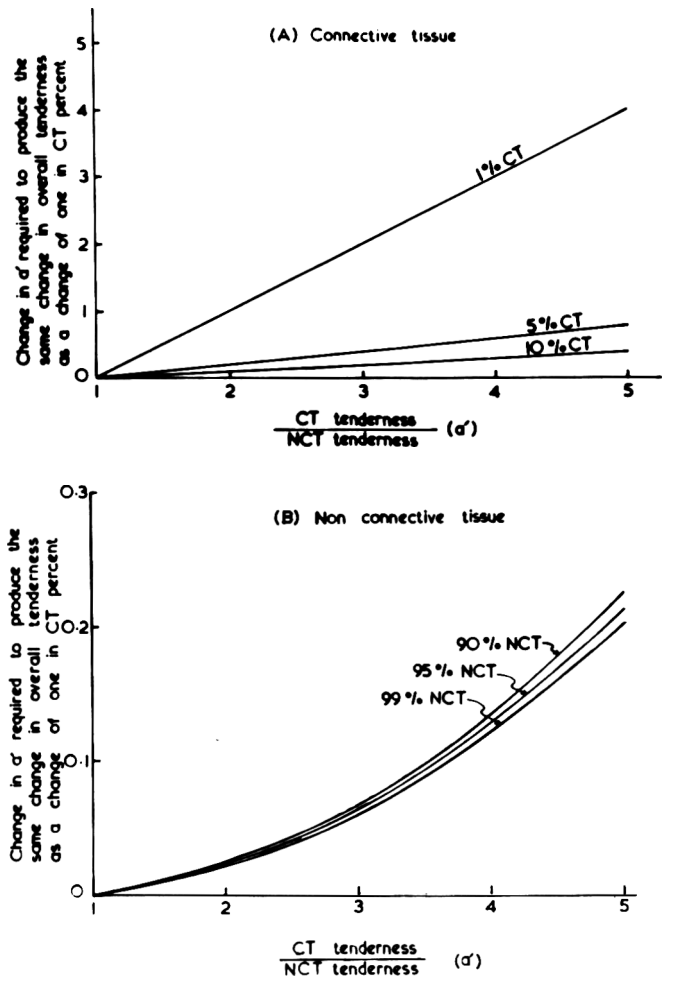

Fig. 3–Changes in the tenderness of connective tissue (A) and nonconnective tissue (B) that would give rise to the same change in overall tenderness as a change of 1 in CT percent. Values are plotted for ratios of connective tissue tenderness to nonconnective tissue tenderness from 1–5 and for 1, 5 and 10% connective tissue.

Consideration of data from all the animals regardless of treatment revealed close relationships and significant correlation coefficients between initial shear force (A, Table 2) and the response of tenderness to aging ($r_{A.(A-D)} = 0.761$, $P < 0.01$) and between shear force at a cooking temperature of 75°C (B) and the response of tenderness to a change in cooking temperature from 60°C to 75°C ($r_{B.(B-C)} = 0.856$, $P < 0.01$). That is, there was a tendency for aging and for changing the cooking temperature from 75°C to 60°C to become less effective in terms of tenderness improvement as the initial tenderness decreased. The first of the relationships is consistent with the previous suggestion that the main meat tenderness difference between Brahman-Friesian and Friesian bulls and between cryptorchids and steers was associated with the characteristics of meat that change during aging. However, the second relationship, which indicates that greater effects from cooking temperature may be expected with higher initial shear values, does not fit in with the group differences shown previously (Table 3), except possibly with the apparently greater response of bulls than cryptorchids to the cooking temperature change (Fig. 2).

As noted above, changes in tenderness with aging and with an increase in cooking temperature from 60°C to 75°C appear to be largely due to changes in the properties of the myofibrillar proteins. Therefore, the demonstration of significant effects of indirect tenderness determinants (sex, weight, breed) on these characteristics (Table 3) supports the suggestion that most of the variation in overall tenderness can be attributed to variation in the tenderness of the nonconnective tissue portion of meat (Davey and Gilbert, 1967; Szczesniak and Torgeson, 1965). Further support for this contention may be obtained from simple theoretical considerations as follows.

Theoretical effects of composition

For simplicity, meat is considered to be a two-component system comprising connective tissue (CT) and nonconnective tissue (NCT). It is assumed that the tenderness of these two components contribute additively and independently to overall tenderness so that:

$$T = ax + b(100 - x) \quad (1)$$

where T = overall tenderness [(force/area) x 100]; x = percent CT in a cross sectional area; a = tenderness of CT (force/area); and b = tenderness of NCT (force/area).

It is apparent from equation (1) that changes in T may result either from changes in the connective tissue percent (x) or from changes in the tenderness of connective tissue or nonconnective tissue (a or b). The relative importance of these three sources of variation are considered below.

If all tenderness measurements are expressed in terms of NCT tenderness (b), equation (1) becomes:

$$T_b = a'x + 100 - x \quad (2)$$

where $T_b = T/b$ and $a' = a/b$.

By differentiation of equation (2) with respect to x,

$$\frac{dT_b}{dx} = a' - 1$$

or

$$\frac{\Delta T_b}{\Delta x} = a' - 1 \quad (3)$$

since dT_b/dx is independent of x.

The results of Field et al. (1969) suggest that a′ would have a value between 1 and 2 for rib steaks from cattle of less than 2 yr cooked to an internal temperature of 71.1°C. However, this was based on the tenderness of epimysium which may not be the same as that for perimysium. In this connection, Mohr and Bendall (1969) have shown that intramuscular connective tissue is less soluble, and, therefore, probably less tender (Herring et al., 1967) than tendon connective tissue, while Field et al. (1970b) demonstrated differences in the thermal transitions for intramuscular and epimysial connective tissue of beef.

The change in T_b resulting from a change of one in the CT percent is equal to $a' - 1$ [equation (3)]. Thus, the relative effect of changes in CT percent and CT or NCT tenderness may be assessed by estimating the required changes in CT or NCT tenderness that would produce the same change in T_b as a change of one in CT percent.

First, for changes in CT tenderness, equation (2) is differentiated with respect to a′ to give

$$\frac{dT_b}{da'} = x$$

and

$$\Delta a' = \frac{\Delta T_b}{x} \quad (4)$$

From equation (4) the changes in CT tenderness in terms of a′ that produce the same change in overall tenderness as a change of one in CT percent may be calculated for different values of x and a′. Figure 3a plots such values for ranges of a′ from 1–5 and for 1, 5 and 10% CT.

The effect of changes in NCT tenderness (b) can be assessed similarly by expressing all tenderness values in terms of CT tenderness (a) and then differentiating the resulting equation with respect to b′:

$$\frac{dT_a}{db'} = 100 - x \quad (5)$$

where $T_a = T/a$ and $b' = b/a$. From equation (5):

$$\Delta b' = \frac{\Delta T_a}{100\text{-}x}$$

$$= \frac{b' \Delta T_b}{100 - x} \text{ (since } T_a = T_b b').$$

But the required changes in NCT tenderness estimated in this way are expressed in terms of b′. These may be converted to a′ units by multiplying by $(a')^2$, so as to make them directly comparable with changes in CT tenderness producing the same overall effect. Values comparable to those for CT are plotted for NCT in Figure 3b.

It can be seen from Figure 3, for example, that if there is 5% CT, and if the ratio of CT tenderness to NCT is 2.0, then an equivalent change in overall tenderness will be produced by (i) a change of 1 in CT percent, (ii) a change of 0.2 in a′ when the change is due to a change in CT tenderness only, or (iii) a change of 0.0208 in a′ when the change is due to a change in NCT tenderness only.

The main value of an analysis such as this is probably in the prediction of relative rather than absolute values for the three sources of variation. The most notable result in this regard is the much smaller change in NCT tenderness that is required to produce the same effect as relatively large changes in CT percent and CT tenderness. In the above example a 1% increase in NCT tenderness produced the same result as a 10% increase in CT tenderness. Thus, it seems hardly surprising that although some studies have shown CT content (Mackintosh et al., 1936; Ramsbottom et al., 1945; Hiner et al., 1955) and CT characteristics such as solubility (Herring et al., 1967) to be related to meat tenderness, many other studies have not (Wilson et al., 1954; Goll et al., 1963; McClain et al., 1965; Field et al., 1969, 1970a).

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Ms received 11/4/71; revised 1/25/72; accepted 1/26/72.

This work was supported by the Australian Meat Research Committee. The skilled technical assistance of Miss Eileen Stubenrauch is gratefully acknowledged.

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THE EFFECT OF AGING ON PHYSICOCHEMICAL PROPERTIES OF ACTOMYOSIN FROM CHICKEN BREAST AND LEG MUSCLE

INTRODUCTION

THE NATURE of the changes in muscle meat which occur during post-mortem aging and which bring about tenderness is not fully understood, but it must be mainly the result of changes in the interaction of the muscle proteins. These proteins have been the subject of a review by Goll et al. (1970), who summarize and discuss post-mortem alterations in the sarcoplasmic, stroma and myofibrillar proteins. Sarcoplasmic proteins undergo degradation and denaturation during post-mortem aging but no direct link has been shown to exist between these changes and the development of tenderness. As well, most workers agree that little alteration of the stroma protein occurs during post-mortem aging. Only the myofibrillar proteins appear to undergo structural changes which may be related to development of tenderness in meat. These changes are manifested by a loss of Z-line and occasionally M-line structure as seen in electron micrographs and as alterations in the interaction between actin and myosin.

Actomyosin extracted from muscle following post-mortem storage has exhibited a number of physical and chemical changes. Firstly, there is an increase in Mg^{++} mediated adenosine triphosphatase (ATPase) activity, which is most pronounced at rigor mortis, while there is little change in Ca^{++} mediated ATPase activity. This has been noted in rabbit (Fujimaki et al., 1965a and b) and bovine (Herring et al., 1969a; Robson et al., 1967) actomyosin, and in rabbit (Penny, 1968; Yang et al., 1970), bovine (Goll and Robson, 1967) and porcine (Greaser et al., 1969) myofibrils. Secondly, there is an increased sensitivity of actomyosin to dissociation by ATP during post-mortem storage (Fujimaki et al., 1965b, c; Herring et al., 1969b; Arakawa et al., 1970), and lastly, an increase in viscosity during rigor mortis (Fujimaki et al., 1965b; Herring et al., 1969b). These changes may be related to the progressive lengthening of the sarcomere following rigor mortis (Gothard et al., 1966; Stromer et al., 1967; Takahashi et al., 1967). These observations suggest that there is a close interaction between actin and myosin during rigor mortis which diminishes with aging. In fact, Fujimaki et al. (1965a) and Herring et al. (1969b) have specifically demonstrated a larger amount of actin bound to myosin during rigor mortis.

To date little attention has been directed towards changes in the actin-myosin interaction during post-mortem aging of chicken muscle. Chicken muscle is special in that it undergoes rigor mortis very rapidly, starting within 3 hr of death and being completed within 12–24 hr (deFremery and Pool, 1960). The present study examines some of the chemical and physicochemical properties of chicken muscle actomyosin extracted at different post-mortem times in order to delineate changes occurring through rigor mortis and to examine differences in the myofibrillar proteins of white breast muscle and red thigh muscle.

MATERIALS & METHODS

Reagents

Disodium adenosine triphosphate (ATP) with a low Ca^{++} content was obtained from Sigma Chemical Co., St. Louis, Mo. All other reagents were analytical grade.

Muscle source

Eight-to-ten week old commercial Peel strain broilers, 5–6 lb in weight, hatched and raised at the University of Alberta Lab. Animal Centre were used in this study. The birds were sacrificed by severing the carotid arteries, and then scalded at 60°C for 90 sec, plucked, eviscerated and placed in an ice-water bath. Muscle samples were removed in pre-rigor at 30 min, in rigor at 3 hr, in post-rigor at 21 hr and 7 days. To prevent bacterial contamination, the carcasses to be aged were stored at 2°C, and wrapped in paper towels which had been soaked in 10 mM sodium azide. Swabs taken from the carcasses at each period of sampling showed no observable bacterial growth.

Actomyosin extraction

Samples of whole breast muscle from six individual birds and leg muscle from two individual birds were removed from the carcasses and trimmed free of visible fat and connective tissue. 50g of muscle were excised for each experiment, homogenized in 150 ml of water for 1 min in a Waring Blendor and the resulting supernatant discarded. The actomyosin was extracted from the residue by adding four volumes of Weber-Edsall solution following the procedure of Haga et al. (1965) and Mihalyi and Rowe (1966). Collagenous material was removed from the extract by centrifugation. The crude actomyosin was further purified by diluting the extract to 0.15M KCl and centrifugation at 7,000 × G for 10 min to separate the suspended actomyosin, which was then redissolved by the addition of 3M KCl to a final concentration of 0.6M KCl. This purification was repeated twice. The extract was dialyzed overnight against 15 vol of 0.6M KCl and finally clarified at 20,000 × G for ½ hr. The actomyosin solution was stored at 0°C and used within 3 days, although preparations stored for up to 2 wk showed no discernable alterations in SH availability, reduced viscosity or sedimentation velocity. Reported results are the averages of all determinations on all birds for each treatment group. Each individual measurement was done at least in duplicate.

Protein concentrations

Protein concentrations were measured using the Folin method of Lowry et al. (1951) as modified by Oyama and Eagle (1956), standardized against crystalline bovine serum albumin.

Measurement of ATPase activity

The Ca^{++} and Mg^{++} mediated ATPase activities of actomyosin were determined by measuring the release of inorganic phosphate at 25°C. The reaction mixture consisted of 20 mM Tris acetate buffer (pH 6.8), 1 mM $CaCl_2$ or $MgCl_2$ and 0.02M KCl. The protein solution was adjusted to give a concentration of exactly 0.25 mg/ml. 30 sec after the addition of protein 1 mM ATP was added and 2 ml aliquots were removed at 60-sec intervals and transferred to a tube containing 1 ml of cold 15% trichloroacetic acid. Denatured protein was removed by centrifugation and the inorganic phosphate liberated during the reaction was determined according to the method of Quass and Briskey (1968). Results were expressed as μmoles Pi/min/mg protein.

Sulfhydryl determinations

The SH groups in natural actomyosin were determined by the method of Sedlak and Lindsay (1968), a technique based upon original observations by Ellman (1958) who reported that 5,5'-dithiobis-2(-nitrobenzoic acid) (DTNB) is reduced by SH groups to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH.

0.75 ml of 0.2M Tris buffer and 0.2M disodium ethylenediamine tetracetic acid pH 8.2, was added to 0.5 ml of 0.2M KCl actomyosin solution (protein concentration = 2.5–4 mg/ml). To this, 0.05 ml 0.1M DTNB dissolved in methanol was added and the volumes made up to 5 ml with H_2O or 8M urea in duplicate samples and blanks. The tubes were spun at 3,000 × G for 15 min, and the absorbance of the supernatant was read at 412 mμ after complete color development.

Viscosity

The viscosity of the actomyosin solutions was determined at 25°C in a Cannon-Fenske viscometer of 5 ml capacity with an outflow time of 63.5 sec for water. The same viscometer

Table 1–ATPase activity of natural actomyosin isolated from chicken breast muscle[a]

Activator	Time of post-mortem storage			
	0 hr[b]	3 hr[b]	24 hr[c]	168 hr[b]
1 mM Ca^{++}	.14 ± .01	.10 ± .01	.14 ± .01	.14 ± .02
1 mM Mg^{++}	.12 ± .01	.16 ± .02	.16 ± .01	.10 ± .01

[a]Conditions of assay: 0.25 mg actomyosin/ml; 1 mM ATP; 20 mM KCl; 20 mM Tris-acetate pH 6.8, 25°C

[b]Figures are μmoles Pi/min/mg expressed as means plus or minus the standard deviation from determinations on separate muscle protein preparations from six different birds.

[c]Figures are μmoles Pi/min/mg expressed as mean plus or minus the standard deviation from determinations on separate muscle protein preparations from four different birds.

was used throughout the entire series of studies. Because of the thixotropic nature of the actomyosin solutions, the average of the first three readings was taken. The reduced viscosity was calculated from $\eta(\text{red}) = \frac{\eta(\text{rel})-1}{c}$, where c = g/dl.

Ultracentrifugal analyses

A model L2-65B preparative ultracentrifuge, equipped with the schlieren optical system was used for all analyses. An An-D rotor with a 12 mm center piece was operated at 40,000 rpm at 20°C. Sedimenting boundaries of the 5 mg/ml runs were photographed at 15 min intervals and of the 3 mg/ml runs every 2 min. Measurements of the schlieren patterns were made with a Bausch and Lomb measuring magnifier. Sedimentation coefficients were calculated according to the method of Schachman (1957), with no corrections being made for the Johnston-Ogston effect or for radial dilution of the protein.

RESULTS

ATPase activities

Mg^{++} ATPase activity for both leg and breast muscle actomyosin increased during rigor mortis, as shown in Tables 1 and 2, although the activities are much lower in the breast muscle than in the leg muscle for each aging period. These results corroborate previous studies of rabbit (Fujimaki et al., 1965b) and bovine (Herring et al., 1969a; Robson et al., 1967) actomyosin and rabbit (Penny, 1968), bovine and porcine myofibrils (Herring, et al., 1969a).

With the exception of breast actomyosin during rigor mortis, the Ca^{++} ATPase activities for breast and leg muscle do not alter during postmortem aging. Wu and Sayre (1971) and Khan and van den Berg (1964) in studies of the myosin and actomyosin respectively from chicken breast and leg muscle reported no differences in the Ca^{++} ATPase activities. However, in our analysis of breast actomyosin there is a marked reduction in Ca^{++} ATPase activity during rigor mortis. This observation is not easily reconciled with results reported for rabbit (Fujimaki et al., 1965b) and bovine (Herring et al., 1969a; Robson et al., 1967) actomyosin and bovine myofibrils (Goll and Robson, 1967) which showed increases in Ca^{++} ATPase activity during rigor mortis. However, Penny (1968) also noted a decrease in activity in studies with rabbit myofibrils during rigor though he attached no significance to these results.

ATPase activities mediated by Ca^{++} are marginally higher for breast (white) than for leg (red) muscle. Wu and Sayre (1971) and Wu (1969) reported the Ca^{++} ATPase activity of myosin from leg muscle to be 82–86% that of breast myosin but myosin from the red and white muscle of pigeons (Maddox and Perry, 1966) and rabbit myosin (Bárány et al., 1965; Seidel et al., 1964; and Sreter et al., 1966), actomyosin and myofibrils (Seidel et al., 1964) exhibit more pronounced differences in Ca^{++} ATPase activity.

Sulfhydryl analysis

Tables 3 and 4 illustrate that SH groups exposed after denaturation with 8M urea do not change appreciably with aging for both breast and leg muscle actomyosin. The number of groups exposed in the leg actomyosin is marginally less than in the breast muscle. Chajuss and Spencer (1962) using the nitroferricyanide technique for determining sulfhydryl content and Gawronski et al. (1967) using polarographic analyses showed a decrease in thiol concentration of chicken breast muscle with aging. Recently Caldwell and Lineweaver (1969), applying the DTNB method for determining sulfhydryls showed no change in SH concentration with aging.

The sulfhydryls exposed in the 20 mM KCl solution show little variation with length of storage for leg muscle, but in the breast muscle a pronounced increase in sulfhydryls is observed at rigor mortis.

Viscosity

The reduced viscosity of leg and breast actomyosin is markedly dependent on protein concentration as shown in Figures 1 and 2, a characteristic observed in other studies (Fujimaki et al., 1965b; Herring et al., 1969b).

The reduced viscosities for both leg and breast actomyosin increased to a maximum at rigor mortis and then declined slowly during post-rigor aging. The values for breast actomyosin were markedly depressed after 7 days aging. A similar trend has been observed in rabbit (Fujimaki et al, 1965b) and bovine actomyosin (Herring et al., 1969b) though Chaudry et al. (1969) showed little variation in his studies.

Ultracentrifugal analyses

Sedimentation diagrams for breast and leg actomyosin are shown in Figure 3. Duplicate or triplicate peaks of F-actomyosin (I in photograph A of the breast series and I in photograph E of the leg series) can be observed in each photograph. At 3.0 mg/ml the $S_{20,w}$ values for

Table 2–ATPase activity of natural actomyosin isolated from chicken leg muscle[a]

Activator	Time of post-mortem storage			
	0 hr[b]	3 hr[b]	24 hr[b]	168 hr[b]
1 mM Ca^{++}	.12	.12	.12	.11
1 mM Mg^{++}	.17	.20	.18	.16

[a]Conditions of assay: 0.25 mg actomyosin/ml; 1 mM ATP; 20 mM KCl; 20 mM Tris-acetate pH 6.8, 25°C

[b]Figures are μmoles Pi/min/mg protein expressed as the mean of determinations on separate muscle protein preparations from two different birds.

Table 3–Sulfhydryl group analyses of natural actomyosin isolated from breast muscle[a]

Diluent	Time of post-mortem storage			
	0 hr[b]	3 hr[b]	24 hr[c]	168 hr[c]
H_2O	3.5 ± .4	4.6 ± .8	3.8 ± .6	3.7 ± .2
8M Urea	9.8 ± .7	10.2 ± .6	10.4 ± .5	10.4 ± .5

[a]Conditions of analysis are as described in text in Methods.

[b]Figures are moles $SH/10^5$g protein expressed as means plus or minus the standard deviation from six separate muscle protein preparations from six different birds.

[c]Figures are moles $SH/10^5$g protein expressed as means plus or minus the standard deviation from four separate muscle protein preparations from four different birds.

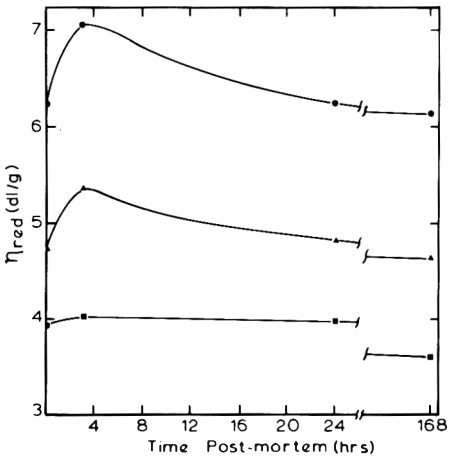

Fig. 1—Post-mortem changes in reduced viscosity of natural actomyosin isolated from chicken breast muscle. Final concentrations: 0.6M KCl; 20 mM Tris-acetate pH 6.8; concentrations of protein as shown. -●-● 3 mg/ml; -▲-▲ 2 mg/ml; -■-■ 1 mg/ml.

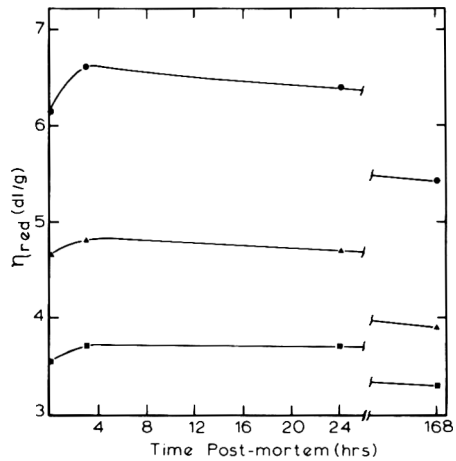

Fig. 2—Post-mortem changes in reduced viscosity of natural actomyosin isolated from chicken leg muscle. Final concentrations: 0.6M KCl; 20 mM Tris-acetate pH 6.8; concentrations of protein as shown. -●-● 3 mg/ml; -▲-▲ 2 mg/ml; -■-■ 1 mg/ml.

this peak varied between 35–50S. These are within the range of values reported for rabbit skeletal muscle actomyosin (Haga et al., 1965 and Johnson and Rowe, 1964) and cod muscle actomyosin (King, 1966). Fujimaki et al. (1965b) and Herring et al. (1969b) have reported slightly lower values at this concentration. Myosin A and/or G-actomyosin (Johnson and Rowe, 1964; King, 1966; Scharpf et al., 1966) were not observed in any of the preparations and this would be expected in view of the long extraction times which were used in the preparation of the natural actomyosin.

The gel component (II in photograph A of the breast series and photograph E of the leg series) regarded as a three dimensional network of gel actin and myosin is clearly discernable in each photograph. Rough quantitative measurements of the area of the gel component with time of aging were made and these suggest an increase in the gel-fraction with aging of both breast and leg preparations, in agreement with Okitani et al. (1965).

No noticeable differences in $S_{20,w}$ values were recorded with time of aging for either breast or leg although much variation was experienced with duplicate tests, a situation somewhat reminiscent of the viscosity studies. Although differences with aging can be observed in the general profile of the aggregated F-actomyosin species, interpretations of such changes must be considered beyond the scope of even the most fertile imagination!

A peak (III in photograph D) giving an $S_{20,w}$ value of between 13 and 14 appeared consistently in 7-day chicken breast preparations and occasionally in the 24-hr preparations but was absent in all stages of aging for leg preparations.

DISCUSSION

ATPase activities

From the results and discussions of other workers, the increase in Mg^{++} ATPase activity we observed during rigor mortis could be explained by three possible factors: the degree of contraction, the presence of α-actinin or the actin content of actomyosin. Increases in Mg^{++} ATPase activity resulting from shortening of muscle fiber is not acceptable, as Robson et al. (1967) and Goll and Robson (1967) extracted actomyosin and myofibrils from muscle fibers stored at 16°C and observed an increase in Mg^{++} ATPase activity without discernible shortening of the fibers. α-actinin, which is principally located in the Z-line, has been shown to induce Mg^{++} ATPase activity in actomyosin by Arakawa et al. (1970) and Briskey et al. (1967), but since degradation of the Z-line and release of α-actinin continues after completion of rigor mortis, one would expect the Mg^{++} ATPase to increase accordingly. As this does not occur, the possibility that α-actinin release is responsible for the increase in ATPase activity during rigor seems unlikely. There does appear to be a close association between the content of actin in actomyosin and increased Mg^{++} ATPase. Fujimaki et al. (1965a) have shown an increase in actin content to occur during rigor mortis while observing a parallel increase in Mg^{++} ATPase activity during the same period. This has been corroborated by Herring et al. (1969a) who, in addition, were unsuccessful in demon-

Table 4—Sulfhydryl group analyses of natural actomyosin isolated from leg muscle[a]

	Time of post-mortem storage			
Diluent	0 hr[b]	3 hr[b]	24 hr[b]	168 hr[b]
H_2O	3.8	3.9	3.8	3.5
8M Urea	9.6	8.7	9.4	8.7

[a]Conditions of analysis are as described in text in Methods.

[b]Figures are moles SH/10^5g protein expressed as the mean of two separate muscle protein preparations from two different birds.

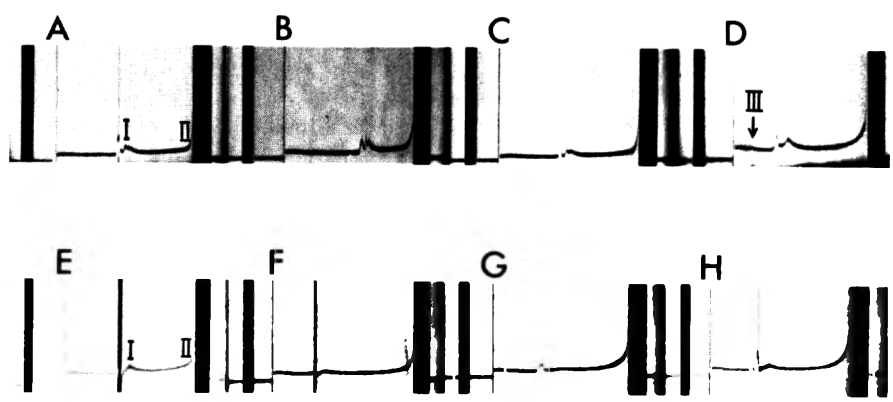

Fig. 3—Sedimentation patterns of natural actomyosin isolated from chicken breast and leg muscle. All samples were run at 40,000 rpm and a protein concentration of 5.0 mg/ml. Photographs were taken on Polaroid Land Roll Film Black and White 3,000 ASA, approximately 30 min after reaching operating speed. Photographs A, B, C and D are breast muscle extracts at 30 min, 3 hr, 24 hr and 7 day post-mortem. Photographs E, F, G and H are leg muscle extracts at 30 min, 3 hr, 24 hr and 7 day post-mortem.

strating a relationship between Mg^{++} ATPase activity and degree of tenderness. These workers develop strong arguments that the actin appears to be a major factor influencing the Mg^{++} ATPase activity in actomyosin. It has already been observed that an increased content of actin in actomyosin brings about an increase in reduced viscosity (Weber, 1950). In our results it is notable how closely the observed changes in Mg^{++} ATPase activity parallel the changes in reduced viscosity in both leg and breast actomyosin. Haga et al. (1965) also demonstrated this close relationship between Mg^{++} ATPase activities and reduced viscosities in their studies of the extraction of actomyosin with Weber-Edsall solution. Despite these arguments in favor of a close relationship between the content of actin in actomyosin and Mg^{++} ATPase activity, our findings cannot be totally reconciled with those of Perry and Leadbetter (1964) who showed that the heavy meromyosin-actin complex treated with phenylmercuric acetate to destroy the actin structure, can still be activated with Mg^{++}, even though their viscosimetric measurements showed dissociation of the complex.

Sulfhydryl analysis

One of the most interesting features of the sulfhydryl analyses is the pronounced increase in SH groups during development of rigor mortis in breast muscle. Since Ca^{++} ATPase activity is reduced at the same period of aging, one can suggest that a positive relationship exists between the exposure of SH groups and the reduction of Ca^{++} ATPase activity. Although this phenomenon has not been previously described, a reversal of this situation has been observed. Kielley and Bradley (1956) have shown that blocking of the SH groups with the sulfhydryl reagents, p-mercuribenzoate, or N-ethylmaleimide results in an increase in Ca^{++} ATPase activity. Recently Seidel (1969) accomplished a specific blocking of the rapidly reacting sulfhydryls of heavy meromyosin with DTNB; this modification resulted in a marked increase in Ca^{++} ATPase activity. It seems clear then, that the exposure of the sulfhydryls during rigor mortis in breast muscle is accompanied by a conformational change or some other subtle alteration which results in the observed reduction in Ca^{++} ATPase activity.

Though no losses of SH groups or conversion to disulfides were observed throughout the aging of chicken breast or leg muscle actomyosin, reductions in SH groups concomitant with an increase in disulfides have been reported by Buttkus (1970) for trout and rabbit myosins. It is feasible that actin may exert a protective masking effect over the SH groups of myosin preventing oxidation. Alternatively, chicken myosin may be unique in its ability to preserve its SH groups. Wu and Sayre (1971) have observed no reduction in the numbers of SH groups of red and white chicken muscle following aging for 24 hr.

Reduced viscosity

The changes in reduced viscosity of actomyosin in relation to Mg^{++} ATPase activity have already been discussed. A further explanation has been proposed by Herring et al. (1969b) and Fujimaki et al. (1965b), who suggest that rigor mortis causes a greater degree of asymmetry or higher axial ratio of the actomyosin particles. This infers that rigor mortis brings about a lesser degree of side-by-side aggregation or alternatively the occurrence of an end-to-end association of the actomyosin particles. The first suggestion is not compatible with our ultracentrifugal findings, as reduced S values were not observed during rigor. Furthermore, the suggestion of Herring et al. (1969b) that increases in the amount of gel component are associated with the viscosity changes is not corroborated by our ultracentrifugal data where the gel component continues to increase during aging rather than showing an increase during rigor followed by a decrease thereafter. In view of our findings, caution must be exercised in interpretations of such data.

The use of viscosity measurements as an index of tenderness is a debatable issue. Koonz et al. (1954) and deFremery and Pool (1960) have shown that chicken muscle begins to develop tenderness after 6 hr postmortem and the process appears to be almost complete within 24 hr according to Stadelman and Wise (1961). This, to some extent, appears to correlate with changes observed in our reduced viscosity studies up to 24 hr. However, beyond this time, a further decrease in reduced viscosity occurs and this is apparently not accompanied by a further increase in tenderness. Also, the marked changes recorded in the shear test determinations of Stadelman and Wise (1961) are not consistent with the small changes observed in reduced viscosity measurements. Other factors must be involved and it is likely that degradation of Z-line, which follows most closely the development of tenderness in a variety of species, is the chief factor. Observations by Takahashi et al. (1967b) using phase contrast microscopy have shown that maximum contraction occurs in chicken myofibrils after 2 hr. This is followed by a fragmentation which begins to occur at 9 hr and after 40 hr the myofibrils consist only of small fragments composed of 1–4 sarcomeres. Nevertheless, these studies did show a relaxation in chicken pectoral muscle which had been prepared in a similar fashion to our own preparations. This relaxed state could in turn relate to a drop in the reduced viscosity.

Ultracentrifugal analysis

While the results obtained in the L2-65B schlieren optics are not as precise or accurate as comparable data from the more sophisticated model E, certain characteristics in the sedimentation diagrams can be noted. The most distinguishing feature of the ultracentrifugal patterns is peak III, clearly seen in photograph D, Figure 3. Herring et al. (1969b) reported a similar peak in actomyosin prepared from tender bovine muscle aged 24 hr and from tough bovine muscle aged 5 days. His suggestion that the peak may have represented a dimer of myosin is compatible with our observation, based on Mg^{++} ATPase activities and reduced viscosities as an index of dissociation, that breast muscle myosin becomes more detached from actin after 7 days aging than from preparations at shorter periods of storage. Haga et al. (1966) revealed a peak with a sedimentation coefficient of 11S following Z-line degradation. Their proposal that this protein was derived from the Z-line itself does not entirely agree with our findings since the component was noticeably absent in leg preparations, where Z-line degradation must have occurred. In a study of the effect of aging on myosin derived from chicken breast and leg muscle, Wu and Sayre (1971) isolated a protein which they referred to as the T-component, which had a sedimentation constant of 14.5S. Interestingly enough, this component was absent in leg muscle and appeared in breast muscle only after 48 hr aging. Absence of ATPase activity failed to confirm the suggestion that it was a dimer of myosin, but this T-component does correspond closely to our unknown peak.

In conclusion, the results of this study indicate that only minor differences exist between the actomyosins of leg and breast muscle of young chickens, except for the reduced Ca^{++} ATPase activity of 3 hr postmortem breast preparations. Since leg and breast are totally different anatomical sites, with differing degrees of vascularity, one might expect wider variations in these parameters. In fact, previous workers have shown marked differences in the ATPase activities of myofibrillar proteins of red and white muscle. Changes in the measured parameters during aging parellel results found for other species, but are not nearly so pronounced.

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Ms received 12/28/71; revised 3/3/72; accepted 3/4/72.

The authors gratefully acknowledge the financial support of the National Research Council of Canada (grant #NRC A-5751) and the many helpful suggestions of Dr. C.M. Kay and Dr. W.D. McCubbin of the Dept. of Biochemistry.

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THE EFFECTS OF ULTIMATE pH ON OVINE MUSCLE: WATER-HOLDING CAPACITY

INTRODUCTION

WATER-HOLDING CAPACITY (WHC) has been defined by Hamm (1960) as the ability of meat to retain its own or added water during the application of some external force or treatment. Hamm (1960) reviewed many of the methods which have been used to measure WHC, and the most commonly used was the press method of Grau et al. (1953) and Grau and Hamm (1953; 1957). Other methods reported included the sedimentation method of Mohler and Kiermeier (1953) and centrifugation methods (Marsh, 1952; Wierbicki and Deatherage, 1958; Hamm, 1958; Penny et al., 1963; Miller et al., 1968). Some changes of hydration were detected by measuring cooking losses or by measuring weight losses during storage, freezing, etc. (Siemers and Hanning, 1953; Hamm and Grau, 1955).

Because of the important influence of protein charge on the hydration of muscle proteins, the influence of pH on the WHC of meat has been investigated by Hamm (1960) who added acid or alkali to the homogenized meat samples to achieve the desired pH. WHC was then determined using the press method. With these techniques he investigated the effects of many treatments including aging (Hamm, 1959a), freeze-drying (Hamm and Deatherage, 1960a) and heating (Hamm and Deatherage, 1960b). However, these measurements were all on muscle homogenates containing added water and Hamm (1959a) has shown that the amount of added water could have a profound influence on the measured WHC of these muscle homogenates. It has also been shown (Hamm, 1959b) that the minimum of the pH-hydration curve of ground beef muscle was shifted from pH 4.5 to about 5.5 by increasing the quantity of added water from 20 to 80%. This apparent effect on the WHC was explained as due to the decrease in the ionic strength of the medium surrounding the muscle proteins.

In the present work some sheep were injected with adrenaline pre-slaughter to produce meat with ultimate pH values covering the range from 5.4–7.0. The high speed centrifugation method due to Akroyd, described by Bouton et al. (1971) has been used to investigate the effects on moisture retention due to aging, animal age, fiber contraction state and heating as well as ultimate pH.

MATERIALS & METHODS

Animals, pre-slaughter and slaughter procedures

Two groups of Merino wethers were used in these experiments: a group of 24, aged 1 yr with a mean liveweight of 31.1 ± 0.6 kg and a group of 20, aged 3 yr with a mean liveweight of 43.4 ± 0.9 kg. The sheep were penned individually and fed a ration of chaffed alfalfa hay and milo, 1:1, for periods of 2–8 wk until slaughter.

To produce muscles with a high ultimate pH, subcutaneous injections of 1:500 adrenaline (w/v) in a 0.3% solution of ascorbic acid were administered in two doses. In the younger sheep the total amount of adrenaline given in the two doses varied from 0.24 to 1.02 mg/kg of liveweight. The first dose was given 23.8–19.0 hr before slaughter and the second 1.5–6 hr before. The total amount administered to the older sheep varied from 0.31 to 1.06 mg/kg liveweight given 21.6–29.5 hr and 1–6 hr before slaughter.

Several methods were used to produce muscles with a low ultimate pH. Some control animals were stunned with a captive-bolt pistol and some were shot in the head from a distance, with a 0.22-in. calibre rifle with a telescopic sight. Other animals were anaesthetized with a rapid intravenous injection of pentobarbitone sodium BP. The muscle relaxant 'Myanesin' (B.D.H. Ltd., London) [3-(2-methylphenoxy) propane-1:2-diol] was administered intravenously to some animals. Such treatments were carried out while the sheep were in their normal pens. Adrenaline-treated sheep were stunned with a captive-bolt pistol after they had been handled individually about 20m to a slaughter facility.

Animals were bled approximately 1 min after stunning. The cadavers were dressed and a semitendinosus (ST), a psoas major (PM) and a deep pectoral (DP) muscle were removed within 25 min of stunning. The ST and PM muscles were used for other experiments reported elsewhere. The dressed carcasses were placed in a chiller (0–1°C) 35 min after stunning.

Measurement of pH

pH values were measured directly on raw and cooked meat at 20°C using a Radiometer pH meter (Model 29b, Radiometer Inc., Copenhagen) with a Phillips probe-type combined electrode (C64/1). The pH values for the raw meat were measured after the carcasses had been hung for 3 days at 0–1°C, or after aging for 17 days at 0–1°C. Ten pH readings were made on each sample and the mean used as a measure of the pH.

Measurement of WHC

The method used was a further modification of the Akroyd high speed centrifugation

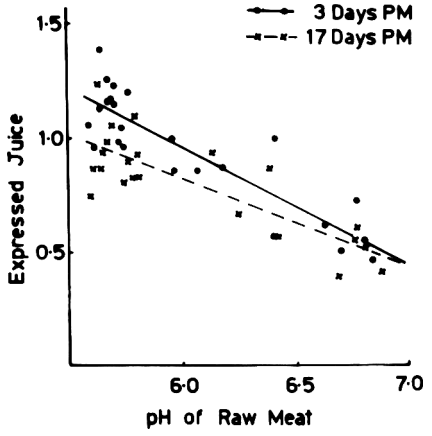

Fig. 1—The effect of ultimate pH on the expressed juice (EJ) losses, g water/g fat-free dried meat residue, obtained for raw fresh (3 days postmortem) and aged (17 days postmortem) longissimus dorsi muscles (1-yr-old sheep).

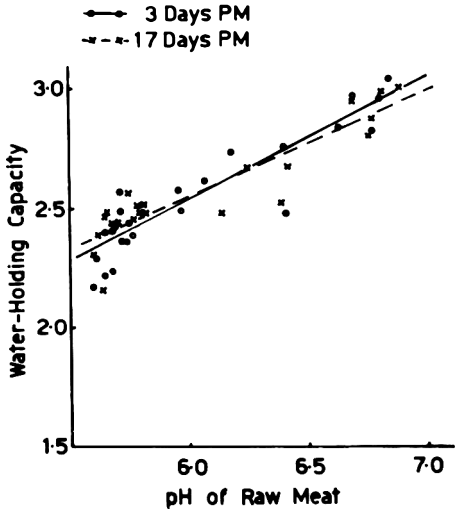

Fig. 2—The effect of ultimate pH on the water-holding capacity (WHC), g water/g fat-free dried meat residue, obtained for raw fresh (3 days postmortem) and aged (17 days postmortem) longissimus dorsi muscles (1-yr-old sheep).

method described by Bouton et al. (1971). Accurately weighed meat samples 3–4g were centrifuged at 36,000 rpm (100,000G) for 1 hr in stainless steel tubes using a type 50 rotor in a Model L Beckman Spinco Preparative Centrifuge (Spinco Div. of Beckman Instruments Inc., Palo Alto, Calif.). No water was added to the samples and after centrifuging, the juice expressed from the meat was decanted off and retained. The raw and cooked meat samples were removed from the tubes with forceps, carefully dried with paper tissues and then reweighed to determine liquid loss. The water contents of the meat samples when raw and after cooking or centrifuging were determined by measuring the weight loss produced in samples of known weight after heating in an oven at 105°C for 18–24 hr. The water contents of the centrifugally expressed juices were also determined using similar methods. Fat content was determined on oven dried samples by the AOAC (1960) method. Four replicates were centrifuged for each sample and the coefficient of variation was about 5%, as previously reported by Bouton et al. (1971).

In an earlier paper by Bouton et al. (1971) the WHC results were expressed as the fraction of total water content still retained by the meat after centrifuging. That definition was considered to be unsatisfactory when appreciable quantities of moisture were lost in weep or drip. Water-holding capacity (WHC) was redefined as the amount of water (g) remaining bound, to each g of fat-free, dried meat residue, after centrifuging. The water retained after cooking (WRC), which was a measure of cooking loss, was expressed as g of water retained by each g of fat-free, dried meat residue. Similarly the juice which could be centrifuged from either the raw or cooked meat (EJ) was expressed as g of water lost per g of fat-free, dried meat residue. The water left after cooking and then centrifuging the cooked meat was defined as residual bound water (RBW) and was similarly related to fat-free, dried meat residue.

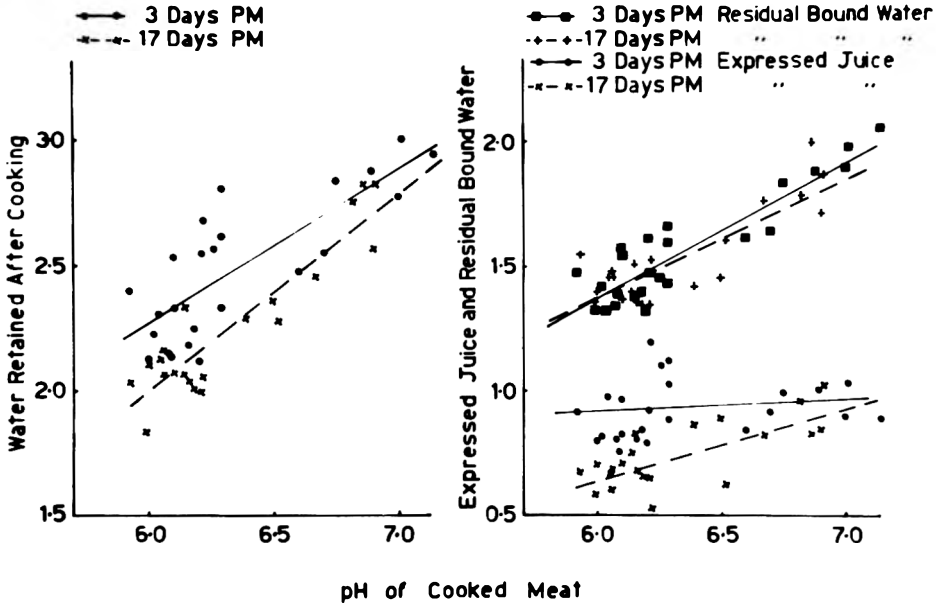

Fig. 3—Water retained after cooking (WRC), expressed juice (EJ) and residual bound water (RBW), g water/g fat-free meat residue, obtained for fresh (3 days postmortem) and aged (17 days postmortem) longissimus dorsi muscles (1-yr-old sheep) cooked at 70°C for 1 hr and plotted as a function of the pH of the cooked meat.

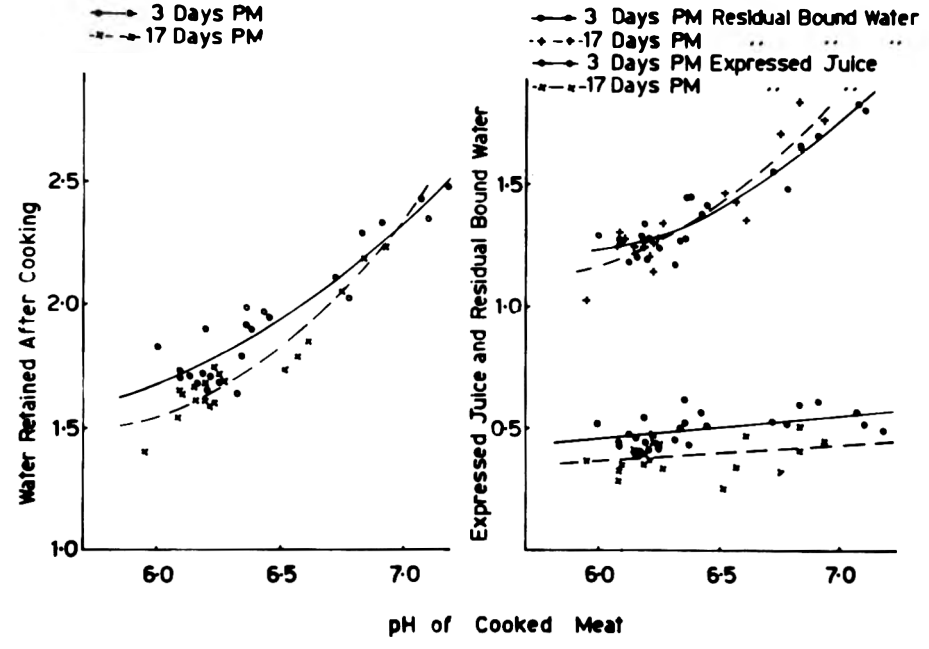

Fig. 4—Water retained after cooking (WRC), expressed juice (EJ) and residual bound water (RBW), g water/g fat-free meat residue, obtained for fresh (3 days postmortem) and aged (17 days postmortem) longissimus dorsi muscles (1-yr-old sheep) cooked at 90°C for 1 hr and plotted as a function of the pH of the cooked meat.

Muscle samples and treatments

The DP muscle was removed from one side of the carcass, within 25 min of stunning. Those from the young animals were allowed to cold shorten (sarcomere length 1.3–1.5 μm) at 0–1°C while those from the older animals were immersed in liquid nitrogen and frozen prerigor within 35 min of death.

The contralateral DP muscles served as controls and were removed from the carcasses after storage at 0–1°C for 3 days. The muscles from the younger animals were then studied immediately after removal from this storage while those from the older animals were frozen in liquid nitrogen and stored at −40°C with the pre-rigor frozen samples, until required. The muscles removed from frozen storage were cut into measured lengths and weighed before thawing at room temperature. After thawing, the muscles were dried with paper tissues, reweighed to determine thaw losses and the lengths remeasured to determine the extent of thaw rigor contraction.

The control (sarcomere length 2.8–3.0 μm) and cold-shortened DP muscles (sarcomere length 1.3–1.5 μm) from the younger animals were divided into two parts: one was minced while the other was cooked at 80°C for 1 hr. Part of the thawed pre- and post-rigor frozen samples were hand-cut into small pieces of a few mm square for WHC measurements.

Both longissimus dorsi (LD) muscles were removed from young and old animals after 3 days storage at 0–1°C, and the lumbar sections were used for WHC measurements. From the younger animals, one muscle was used immediately after 3 days storage and the other was vacuum sealed in Cryovac (gas impermeable) bags and used after aging, at 0–1°C, for a further 14 days. Samples were weighed before and after to determine 'weep losses'. Both the 3-day aged and 17-day aged samples were used for WHC measurements on both raw meat and on meat cooked at 70°C and 90°C for 1 hr. For the older animals only the effect of cooking temperature was investigated and 3-day aged samples were used. These were examined both raw and when cooked at 60, 70 and 90°C for 1 hr.

Cooking methods

The samples used for cooking were trimmed of all excess fat and superficial connective tissue. The cooking methods used have been described by Bouton et al. (1971).

Statistical methods

Animals of each age group were selected at random. A regression analysis was carried out on the data of each particular muscle of animals of the same age group to show the dependence of all WHC, WRC and EJ measurements on pH, to estimate the regression coefficients, and to establish the best fitting lines to the corresponding data. Regression coefficients for the different age groups were tested for homogeneity and analysis of covariance used to test the significance between adjusted treatment effects and between adjusted age groups. Reported values represent the mean of 4–6 observations per muscle per animal.

RESULTS & DISCUSSION

Effects of aging

The amount of juice which could be centrifugally expressed from the raw LD muscles after 3 and 17 days aging at 0–1°C is shown in Figure 1. The EJ from both the 3-day aged and 17-day aged meat was significantly ($P < 0.001$) linearly related to pH and, when the values were adjusted to the pH of the noninjected animals (pH 5.6), the values from the 3-day aged meat were significantly ($P < 0.001$) higher than the values obtained for the 17-day aged meat. This result could be interpreted as indicating that aging improved the water retention properties. However such an interpretation makes no allowance for the moisture lost in weep from the samples during storage. WHC results from the LD shown in Figure 2 thus represent the moisture remaining bound to this fat-free meat residue after centrifuging and/or storage. These results still show a highly significant ($P < 0.001$) relationship with pH, but there is no longer any difference in regression coefficient and no significant effects attributable to aging. The differences found in expressible (EJ) moisture were not significant when the moisture lost in weep from the samples during storage was taken into account.

The effects of cooking the 3-day and 17-day aged meat at temperatures of 70 and 90°C for 1 hr are illustrated in Figures 3 and 4. WRC results have a highly significant ($P < 0.001$) linear relationship with pH for both aging periods and at both temperatures. The samples cooked at 90°C have significant quadratic ($P < 0.05$) regression components for both aging periods. After adjusting for the pH effects the 17-day aged samples have significantly ($P < 0.001$) greater cooking losses at both cooking temperatures; however, the amount of moisture (EJ) which could be centrifuged from these cooked samples is significantly ($P < 0.001$) greater for the 3-day aged samples. These cooking and centrifuging losses thus counterbalance each other with the net results that the RBW results show no significant effect due to aging, although they are dependent ($P < 0.01$) on pH; the regression lines are shown in Figures 3 and 4. The increase in cooking loss with aging was associated with the increase in tenderness produced by aging (Bouton et al., 1972). It was thought likely that it was related to protein myofibrillar structural changes which produced the improved tenderness associated with the aging process.

Effects of cooking

WRC results at all cooking temperatures are significantly linearly related to pH ($P < 0.001$). However, for samples cooked at 90°C the regression of WRC on pH is significantly improved ($P < 0.01$) when a quadratic component is included (see Fig. 5). These results are similar to the published results of Hamm and Deatherage (1960b) for the muscle homogenates.

EJ results for the samples cooked at 60, 70 and 90°C are shown in Figure 6. EJ results for samples cooked at 60°C show no dependence on pH, but for the samples cooked at the higher temperatures significant ($P < 0.05$) though small increases in EJ with increases in pH are noted. Significantly ($P < 0.001$) more juice was released from the samples cooked at 60°C than from the samples cooked at 70°C. Similarly the samples cooked at 90°C released less juice than the samples cooked at lower temperatures.

RBW results for all three cooking

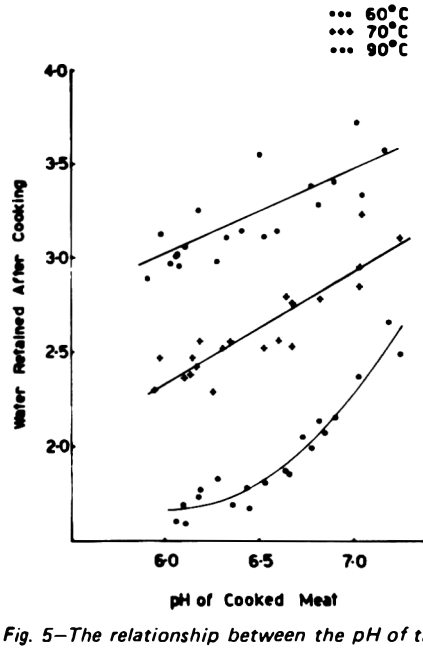

Fig. 5—The relationship between the pH of the cooked meat and the water retained after cooking (WRC), in g water/g fat-free dried meat residue, obtained at 3 days postmortem for longissimus dorsi muscles (3-yr-old sheep), cooked at 60, 70 and 90°C for 1 hr.

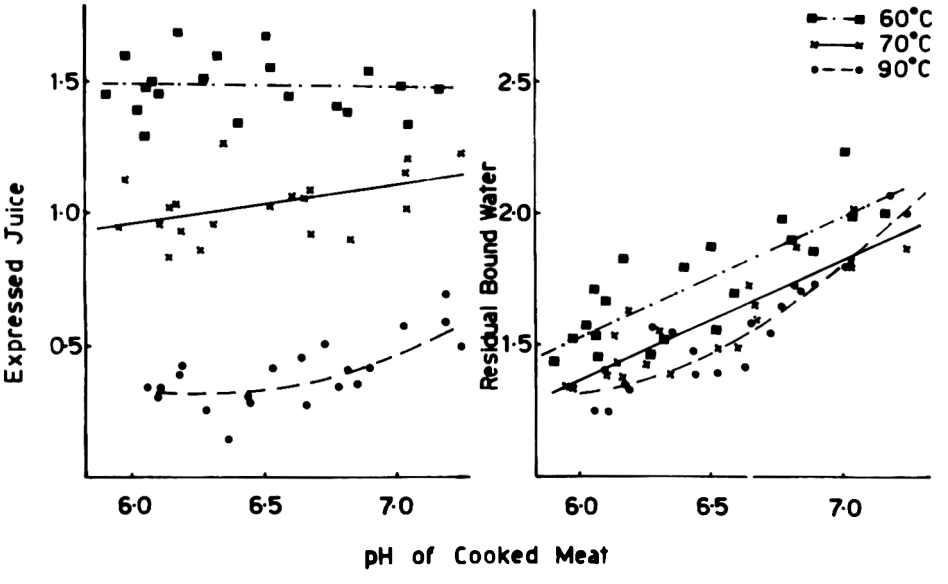

Fig. 6—The relationship between the pH of the cooked meat and the expressed juice (EJ) and residual bound water (RBW) from longissimus dorsi muscles (3-yr-old sheep) at 3 days postmortem, cooked at 60, 70 and 90°C for 1 hr. EJ and RBW are expressed in g water/g fat-free dried meat residue.

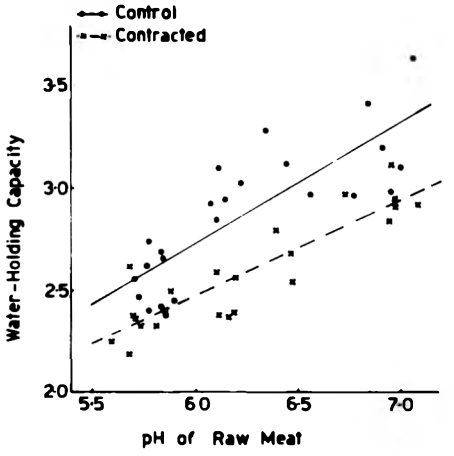

Fig. 7—The effect of ultimate pH on the water-holding capacity (WHC), g water/g fat-free meat residue, obtained at 3 days postmortem for control and cold-shortened deep pectoral muscles from 1-yr-old sheep.

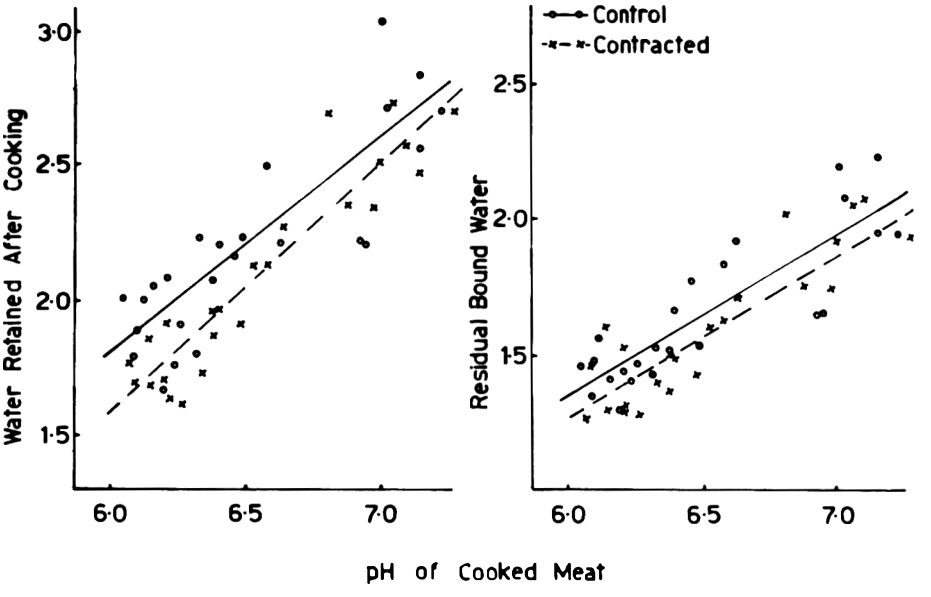

Fig. 8—Water retained after cooking (WRC) and residual bound water (RBW), g water/g fat-free dried meat residue, obtained 3 days postmortem for control and cold-shortened deep pectoral muscles from 1-yr-old sheep cooked at 80°C for 1 hr shown as a function of the pH of the cooked meat.

temperatures show only minor differences in value; only the 60°C results are significantly ($P < 0.05$) greater than those obtained at 70 and 90°C. All the results are significantly ($P < 0.001$) dependent upon pH.

It has been suggested by Hamm (1960) that is was the amount of moisture remaining bound to the tissues after cooking rather than the amount of juice expressible from the tissues which was related to the organoleptic assessment of juiciness. The present results indicate that both of these suggestions are compatible. The moisture remaining bound after cooking is equivalent to our WRC results and the expressible moisture is equivalent to the EJ results. The RBW results are equal to the differences between those WRC and EJ results, and it has been shown (Fig. 6) that there is little difference between the RBW results obtained at 60, 70 and 90°C. High WRC results are, therefore, accompanied by high EJ values.

Effects of animal age

The WHC, WRC and RBW values were all significantly dependent on pH so that differences due to animal age were determined on data adjusted to remove those pH effects using the respective regression equations. Values were adjusted to the mean pH of noninjected, control animals. Animal age had no significant effect on adjusted WHC values: 2.37 for 1-yr old and 2.48 for 3-yr old animals with an effective S.E. of ± 0.08. Neither the WRC values, 2.44 and 2.39 (± 0.07), nor the RBW values, 1.44 and 1.41 (± 0.03) for 1- and 3-yr-old sheep respectively, of samples cooked at 70°C were significantly different. The WRC values of samples cooked at 90°C were significantly lower for the older animals, 1.65 compared with 1.75 (± 0.03) and the RBW values from the older animals significantly greater, 1.33 compared with 1.26 (± 0.02).

The WHC results from the raw control DP muscles from the 1-yr-old sheep were significantly ($P < 0.001$) greater than the values obtained for the comparable DP muscles from older sheep. However, the sample treatments were not identical and differences could have been accentuated because of the extra freezing and thawing treatments received by samples from the older sheep.

Effect of fiber contraction state

The WHC of the raw DP muscles from the younger animals is shown in Figure 7. The WHC results are significantly ($P < 0.001$) greater for the control muscles than for the contracted ones. The results obtained for the muscles cooked at 80°C are shown in Figure 8. The WRC results obtained for the contracted muscles are significantly ($P < 0.01$) lower than those obtained for the noncontracted muscles. The RBW results for the contracted muscles are also significantly lower ($P < 0.05$) than those obtained for the control muscles (Fig. 8). WHC, WRC and RBW results for both the contracted and control muscles all have a significant ($P < 0.001$) linear relationship with pH.

The WHC results obtained from the thawed raw pre- and post-rigor frozen DP muscles from 3-yr-old sheep are shown in Figure 9. The WHC of muscles of both contraction states are both pH dependent at the ($P < 0.001$) level. The WHC values for the contracted muscles are significantly ($P < 0.001$) less than those obtained for the control muscles.

It is evident that a stretched muscle, such as the DP (sarcomere length 2.8–3.0 μm) has higher WHC than its cold-shortened counterpart (1.3–1.5 μm). Part of the difference in WHC between these muscles could be due to the contraction

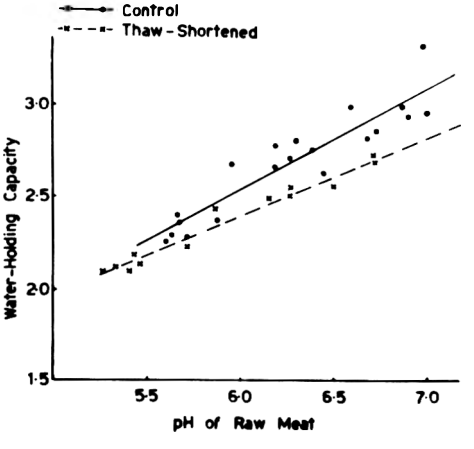

Fig. 9—Water-holding capacity (WHC), g water/g fat-free dried meat residue, as a function of ultimate pH obtained for raw control and thaw-rigor shortened deep pectoral muscles from 3-yr-old sheep.

state of the sarcomere and hence to the number of water binding sites available. These differences are less marked once the muscles have been cooked but this may be because stretched muscles contract more on cooking than do contracted muscles.

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Ms received 12/1/71; revised 3/3/72; accepted 3/4/72.

This work was supported in part by funds from the Australian Meat Research Committee.

The expert technical assistance of Miss Denise Lehmann and Mr. R.F. Dickinson and advice on statistical presentation of Dr. R.I. Baxter is gratefully acknowledged.

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THE EFFECTS OF ULTIMATE pH ON OVINE MUSCLE: MECHANICAL PROPERTIES

INTRODUCTION

MEAT TENDERNESS has been shown to depend on the contraction state of the myofibrillar structure (Locker, 1960; Locker and Hagyard, 1963; Herring et al., 1965a, b; 1967b; Marsh and Leet, 1966; Davey et al., 1967). The contribution from connective tissue has been shown to depend on both the content and on the animal age (Goll et al., 1964a, b; Hill, 1966; Herring et al., 1967a). A further factor known to influence tenderness (Hamm, 1960; Deatherage, 1963; Bouton et al., 1971) is the water-holding capacity (WHC) of the meat proteins. Changes in WHC have been shown to be closely related to the pH of the muscle or muscle homogenate (Hamm, 1958a, b; 1959). Much of the previous work relating pH to meat tenderness was reviewed by Bouton et al. (1971).

Miles and Lawrie (1970) investigated the relationship between pH and tenderness in cooked rabbit muscle. Studying a range of pH values from 5.4–7.2 obtained by cooking at different times pre-rigor or by pre-slaughter injections of adrenaline they found that tenderness, as assessed with a Volodkewich shear device, was pH-dependent.

Bouton et al. (1971) used pre-slaughter injections of adrenaline to produce a range of ultimate pH values in sheep muscles and found that tenderness was closely correlated with ultimate pH.

The present experiments extended the studies of Bouton et al. (1971). Several techniques have been used to produce variations in the structural components of muscle which affect tenderness. The contribution of connective tissue has been varied by using two groups of sheep of different ages. Further differences in tenderness have been produced by sampling muscles in different contraction states and by aging to produce changes in the myofibrillar tenderness component without concomitant connective tissue changes. Tensile measurements of fiber strength and of adhesion between fibers were carried out, in addition to compression/penetrometer and shear measurements, in order to assess the effects of pH on the structural components.

MATERIALS & METHODS

Animals, pre-slaughter and slaughter procedures

24 (1-yr-old) and 20 (3-yr-old) Merino sheep were used. Injections of adrenaline were used to produce meat with a high pH. The details of pre-slaughter feeding, adrenaline injections and slaughter procedures have been described in a previous paper (Bouton et al., 1972).

Measurement of pH

The pH values of both raw and cooked meats were measured directly on the meat using a Radiometer pH meter (Model 29b, Radiometer Inc., Copenhagen) with a Phillips probe-type combined electrode (C64/1). pH determinations were made on raw and cooked meat samples; 10 pH readings were made on each sample and the mean used as a measure of the pH. The pH values referred to in the text were either ultimate pH values taken at least 3 days

Table 1—Treatments carried out on selected muscles from lamb and sheep carcasses

Muscle[a]	Animal age yr	Time removed from carcass hr		Pre-rigor treatment		Time after death the muscle was used—days		Cooking temp °C			Mechanical measurement[b]			
		< ½	72	0–1°C	Frozen in liquid N_2	3	17	70	80	90	W-B[c]	Ch[d]	F[e]	A[f]
Biceps femoris	1	–	2	–	–	1	1	–	2	–	2	2	2	–
	3	–	2	–	–	1	1	–	2	–	2	2	2	–
Semitendinosus	1	1	1	1	–	2	–	2	–	–	2	–	–	–
	3	1	1	1	–	2	–	2	–	–	2	–	–	–
Semi-membranosus	1	–	2	–	–	2	–	–	–	2	1	1	1	1
	3	–	2	–	–	2	–	–	–	2	1	1	1	1
Longissimus dorsi	1	–	2	–	–	1	1	–	2	–	2	2	–	2
	3	–	2	–	–	1	1	–	2	–	2	2	–	2
Psoas major	1	1	1	1	–	2	–	2	–	–	–	2	–	–
	3	1	1	1	–	2	–	2	–	–	–	2	–	–
Deep pectoral	1	1	1	1	–	2	–	–	2	–	2	–	–	–
	3	1	1[g]	–	1	Frozen at −40°C		–	2	–	2	–	–	–

[a]Both muscles used for designated treatments
[b]One of the two muscles only used for designated treatments
[c]WB = Warner Bratzler shear force
[d]Ch = Chewiness
[e]F = Fiber tensile strength
[f]A = Adhesion
[g]Frozen in liquid N_2 after removal from carcass

postmortem or values taken immediately prior to cooking.

Measurement of sarcomere length

Samples from selected muscles were homogenized for 15 sec in cold (0–1°C) 0.08M KCl solution in a Ultra-Turrax Type TP18/2 Blender (Janke and Kunkel KG). The suspension of myofibrils was examined directly in a phase contrast miscroscope equipped with an ocular micrometer. Sarcomere length was determined as an average on 20–30 myofibrils containing a minimum of 6–8 sarcomeres.

Cooking methods

Samples were cooked for 1 hr in polyethylene bags totally immersed in water baths at the selected temperatures of 70, 80 or 90°C (± 0.5°C). Weights of samples taken from individual muscles were approximately equal so that variations in the time taken for different samples to reach bath temperature were negligible.

Mechanical measurements

The compression method and the version of the Warner-Bratzler (W-B) shear method used have been described previously (Bouton et al., 1971). The tensile testing methods have been described by Bouton and Harris (1972a, b, c).

Selection and treatment of muscles for mechanical measurements

Table 1 summarizes the muscles and treatments used. Sample size did not permit all four mechanical measurements to be made on all muscles. The carcasses were hung from the Achilles tendons for 3 days at 0–1°C before the selected muscles were removed. The biceps femoris (BF) and longissimus dorsi (LD) muscles, which were aged for 17 days, sealed in evacuated Cryovac bags and stored at 0–1°C. Cooking temperatures up to 80°C for the BF and LD muscles and 90°C for the semimembranosus (SM) muscles were selected to accentuate any collagen solubility differences in the connective tissue that could have been due to pH.

The deep pectoral (DP) muscles from the 3-yr-old sheep were removed within 30 min of death and frozen in liquid nitrogen, then stored at −40°C with its post-rigor frozen control. These frozen muscles were removed from −40°C storage when required and thawed at room temperature (about 22°C). Length changes during thawing were measured and only those pre-rigor frozen muscles which contracted by 50% or more when thawed were used.

Statistical methods

Animals of each group were selected at random. Regression analysis was carried out on the data of particular muscles from the animals in each group to show the dependence of selected mechanical measurements on pH, to estimate the regression coefficients and to establish the best fitting lines to the corresponding data. Regression coefficients were tested for homogeneity, and analysis of covariance used to test the significance between adjusted treatment effects and between adjusted age groups. All data represent the mean of 8–10 observations per muscle per animal.

RESULTS & DISCUSSION

Effect of pH on some mechanical properties of the SM muscles

The relationship between the compression and adhesion measurements and pH are shown in Figure 1 and for the W-B shear and fiber tensile strength measurements and pH in Figure 2. Significant ($P < 0.001$) linear regression lines are fitted to the compression, adhesion and fiber tensile strength measurements, but quadratic components significantly improved the regression of W-B shear measurements on pH ($P < 0.05$). When the results are adjusted to the mean pH of the noninjected animals from both age groups, only the W-B shear measurements show no significant animal age effect. This result agrees with those of Bouton and Harris (1972c) who found that shear measurements are more closely related with measurements of fiber strength than with measurements of adhesion between the fibers. The adhesion measurements show least change and fiber tensile strength measurements the greatest change with pH. Measurements of heat-induced length changes in the SM muscles

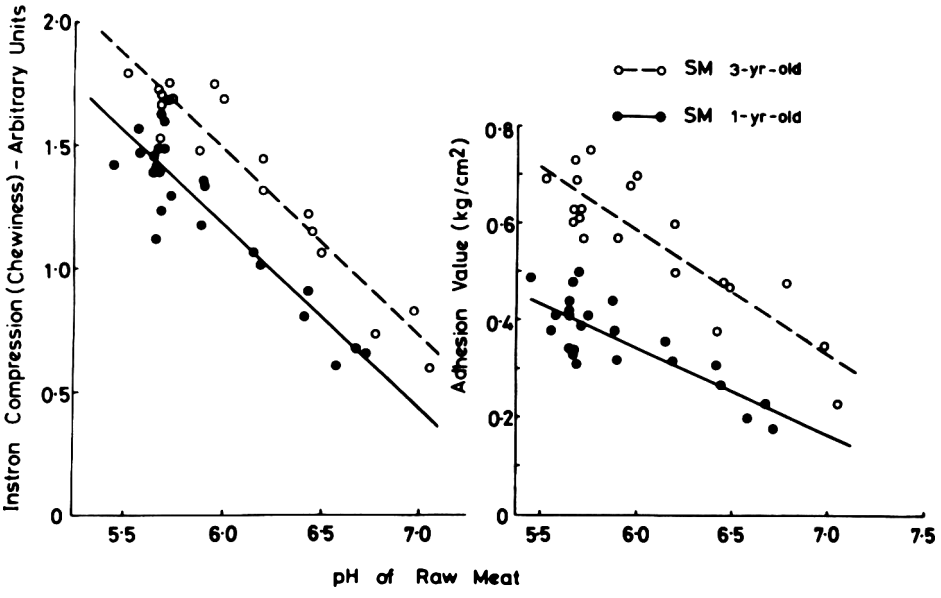

Fig. 1—Instron compression and adhesion measurements on sheep semimembranosus muscles cooked at 90° C for 1 hr as a function of the pH of the raw meat.

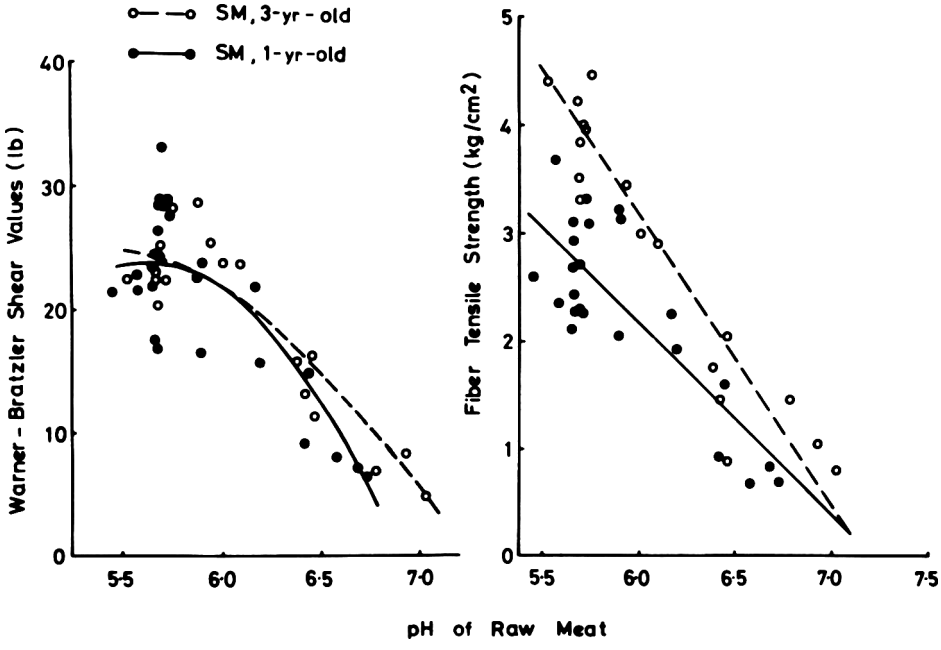

Fig. 2—W-B shear and fiber tensile strength measurements on sheep semimembranosus muscles cooked at 90° C for 1 hr as a function of the pH of the raw meat.

of the older group of sheep are negatively related to pH values and this regression is highly significant ($P < 0.001$).

Effects of aging on the pH dependence of the mechanical properties of BF and LD muscles

Table 2 lists the statistical results obtained for the changes in compression, shear and fiber tensile strength of the BF muscles. The results obtained from the 3-day-aged samples are similar to those obtained for the SM muscle in that the compression and fiber tensile strength are dependent on animal age and the shear measurements are not. The relationship between W-B shear values and pH are linear in the case of the young sheep's BF muscles, both 3- and 17-day-aged, and for the 17-day-aged BF muscles from the older sheep. However, for the 3-day-aged BF muscles from the older group of sheep this relationship between pH and shear value was curvilinear (Fig. 3 and Table 2). All measurements on the BF muscle, when adjusted for pH effects, are significantly ($P < 0.001$) affected by aging. While the effect of pH on the shear measurements of the aged meat samples is reduced (Fig. 3) the shear values are still significantly ($P < 0.001$) related to pH. Figure 4 shows that the mechanical strength of the aged fibers, as evidenced by the fiber tensile strength measurements, is no longer related to pH. The aging of meat has been shown by many authors, including Davey et al. (1967), Davey and Gilbert (1969), Davey and Dickson (1970) and Bouton and Harris (1972b) to reduce fiber tensile strength without significantly affecting adhesion between the fibers.

Fiber adhesion measurements on the LD muscles show that the values for the older animals are significantly ($P < 0.001$) greater than those obtained for the younger animals. No significant effect due to aging is found for the adhesion measurements on either the young or old animals. The adhesion measurements are highly negatively linearly related to pH: ($P < 0.01$) for the younger and ($P < 0.001$) for the older sheep. Similar adhesion measurements on raw adductor muscles also reveal ($P < 0.05$) significant relationships with pH.

Table 2—Analyses of variance for compression, W-B shear and fiber tensile strength measurements to show effects of pH, animal age and aging treatments on the BF muscle

Age of sheep	Source of variation		d.f.	Mean squares: Compression	W-B shear	Fiber tensile strength
1 yr	3 days P-M	Linear regression	1	2.5587***	3640.41***	3.7597***
		Residual error	22	0.0294	68.39	0.0339
	17 days P-M	Linear regression	1	1.0812***	672.78***	0.0048 N.S.
		Residual error	22	0.0304	30.53	0.0115
3 yr	3 days P-M	Linear regression	1	4.5598***	2084.70***	6.6504***
		Quadratic regression	1	–)	392.51**	–)
		Residual error	17	0.0431)	42.37	0.1322)
3 yr	17 days P-M	Linear regression	1	1.2628***	538.30***	0.0054 N.S.
		Residual error	18	0.0683	34.63	0.0208
		Animal age effect	1	5.0100***	216.30 N.S.	2.3363***
		Residual error	41	0.0563	75.08	0.0565
		Aging effect	1	2.3494***	4308.34***	7.2384***
		Residual error	42	0.0438	38.45	0.1222

**P < 0.01
***P < 0.001

Effect of pH upon the mechanical properties of noncontracted and contracted DP, ST and PM muscles

Miles and Lawrie (1970) have reported that severe shortening, such as can occur in thaw-rigor samples, resulted in samples with high shear values and these were not offset by the effect of high pH.

In the present work the amount of cold shortening obtained for the PM and ST muscles was pH-dependent. This pH-effect was less marked for the ST muscles: only two muscles contracted by less than about 40% of the sarcomere length of their controls. Substantial cold shortening in the PM muscles was largely confined to muscles which had an ultimate pH of less than about 6.2.

Table 3 lists the results of statistical analyses obtained for the compression measurements on control PM muscles (sarcomere lengths 3.0–3.6 μm) and cold-shortened PM muscles (sarcomere lengths approximately 1.3–1.5 μm). Sarcomere lengths of the order achieved with these cold-shortened muscles are probably equivalent to the 40% shortening of Marsh and Leet (1966). The compression values of both the control and cold-shortened muscles are linearly and significantly ($P < 0.001$) related to pH. The values for the older animals are greater ($P < 0.01$) than those obtained for the younger animals. The contracted muscles are considerably tougher ($P < 0.001$) than their nonshortened controls.

Table 3 lists the statistical results obtained for shear measurements on normal (sarcomere length about 2.2 μm) and cold-shortened (sarcomere length about 1.4 μm) ST muscles. The effects of both pH and contraction state on the shear measurements are very significant

Table 3—Analyses of variance for compression measurements on control and cold-shortened PM muscles and for W-B shear measurements on control and cold-shortened ST muscles to show the effects of pH, animal age and differences in contraction state

Age of sheep	Source		d.f.	Mean squares: PM compression measurement	ST shear measurement
1 yr	Control	Linear regression	1	0.9516***	636.30***
		Residual error	22	0.0181	7.32
1 yr	Cold-shortened	Linear regression	1	3.1080***	7856.60***
		Residual error	22	0.0240	42.20
3 yr	Control	Linear regression	1	1.0080***	441.73***
		Residual error	18	0.0249	1.87
3 yr	Cold-shortened	Linear regression	1	2.5805***	6988.30***
		Residual error	18	0.0313	34.83
		Animal age effect	1	0.2896**	33.78 N.S.
		Residual error	41	0.0318	25.84
		Contraction effect	1	0.6979***	4211.29***
		Residual error	42	0.0251	109.45

**P < 0.01
***P < 0.001

($P < 0.001$), but no significant effect attributable to animal age is found. Figure 5 illustrates that the pH effect on the shear values obtained for contracted muscle is larger that that obtained for the control or nonshortened muscles.

Shear values obtained for the DP muscles, which had been frozen pre-rigor and had contracted by 50–60% when thawed, and their post-rigor controls, are shown in Figure 6. The values obtained for the controls are not significantly different from the values obtained for the severely contracted samples. This result agreed with those obtained by Marsh and Leet, 1966. The shear values of both the control and severely contracted deep pectoral muscles are significantly ($P < 0.001$) and linearly related to pH.

The results obtained for the PM, ST and DP muscles show that pH is significantly and linearly related with mechanical measurements even when the muscle fibers have contracted by 50–60%.

The results presented in this paper show that the mechanical properties of ovine muscle at 3 days postmortem are closely correlated with pH. The measurements of changes in the adhesion between the fibers reveal clear differences attributable to both animal age and pH. However, effects of pH on adhesion values are small compared with its effects on other measurements, such as shear and fiber tensile strength. These latter measurements, which are most dependent on differences in the meat fiber structure, show very large pH effects. Aging, to produce degradative changes in the fibers, reduces fiber tensile strengths and the results are no longer pH dependent. The relationships between pH and shear values of aged meat are still significant ($P < 0.001$) but the shear measurements are less affected by changes in pH. The shear values of cold-shortened muscles are more dependent on pH than those obtained for their controls. Very severely contracted samples produced by thawing pre-rigor frozen muscle gave shear values and a pH dependence similar to their controls. All these results indicate that the measurements most affected by pH are those which indicate fiber structural strength.

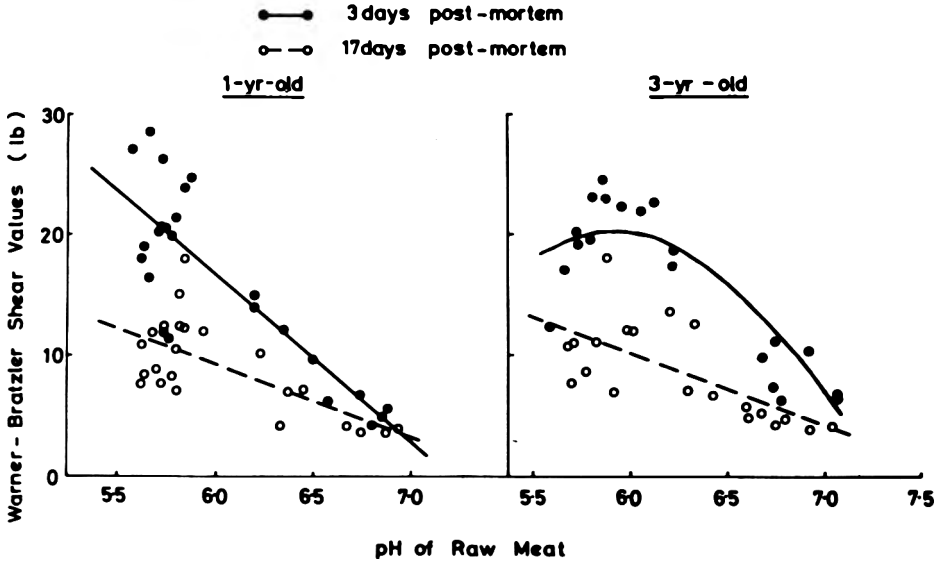

Fig. 3–W-B shear measurements on sheep biceps femoris muscle, aged at 0–1°C, for 3 and 17 days, then cooked at 80°C for 1 hr as a function of the raw meat pH.

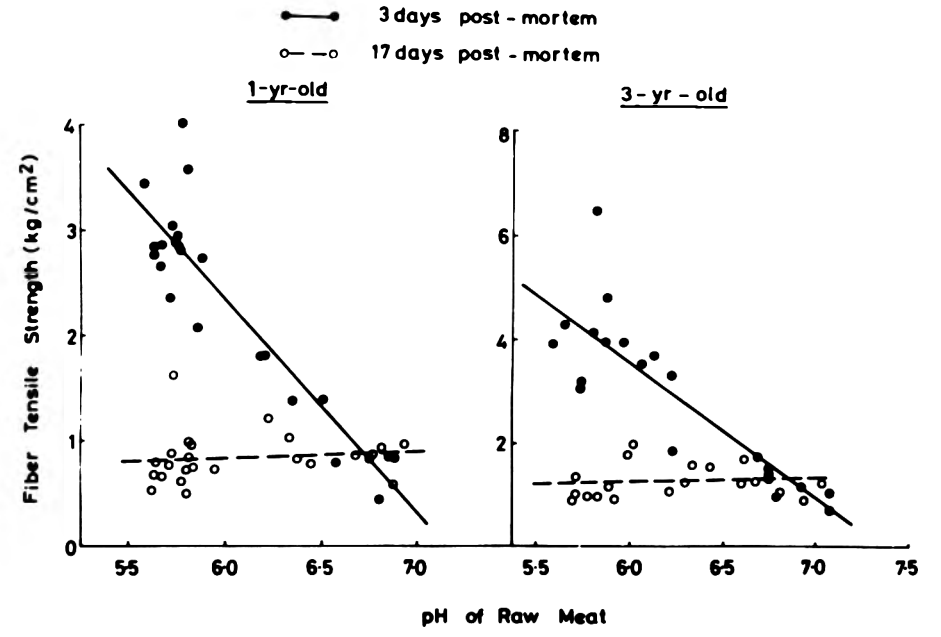

Fig. 4–Fiber tensile strength measurements on sheep biceps femoris muscle, aged at 0–1°C for 3 and 17 days then cooked at 80°C for 1 hr, as a function of the raw meat pH.

The shear force measurements on the SM muscles from both age groups and on the BF muscles from the older sheep show relationships between shear and pH values which are curvilinear. Curvilinear relationships between pH and toughness have been reported by Bouton et al., 1957, 1971. These muscles all have sarcomere lengths of less than 2.0 μm, which according to Davey et al. (1967) represents shortening of the order of 20–40% where quite small changes in contraction state give disproportionately large changes in shear values. The shear strength of the meat fibers is influenced by fiber contraction state (Davey et al., 1967; Marsh and Leet, 1966), and by WHC (Hamm, 1960; Bouton et al., 1971). It has been shown by Bouton et al. (1971, 1972) that changes in the WHC of meat over the pH range 5.4–7.2 are large and linear, for both the raw meat and meat cooked at temperatures of less than 80–90°C. For cooking temperatures of 80–90°C the relationship between WHC and pH is curvilinear (Bouton et al., 1971, 1972; Hamm and Deatherage, 1960) and there is little change in the WHC of the cooked meat when the raw meat pH is in the 'normal' pH range of 5.4–6.0.

It seems likely that fiber toughness in the 5.4–6.0 pH range is decided more by fiber contraction state than by the physical state of the muscle proteins, as represented by WHC. Differences in pre- and post-mortem glycolysis have been related to differences in toughness by Khan and Nakamura (1970) as has pre-slaughter stress, which Howard and Lawrie (1956) have shown increases ultimate pH. Sink et al. (1965) have shown that the rate of

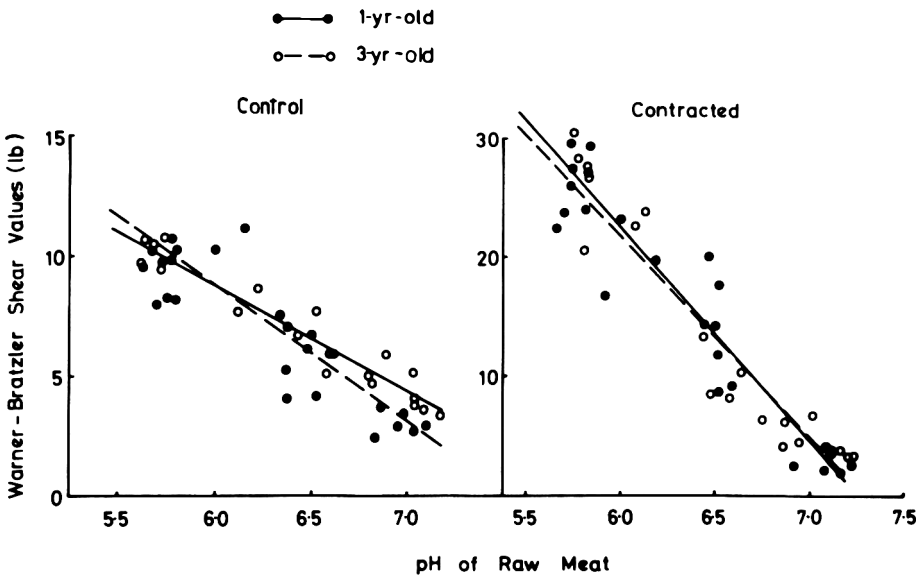

Fig. 5—W-B shear values on normal and cold shortened sheep semitendinosus muscles, cooked at 70°C for 1 hr, as a function of raw meat pH.

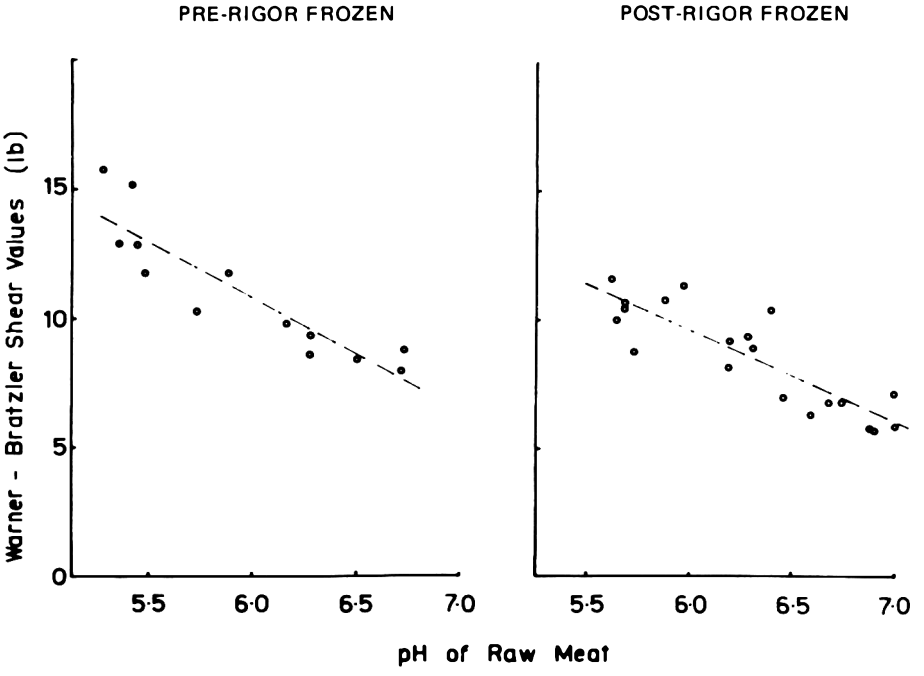

Fig. 6—W-B shear values on normal and thaw rigor shortened sheep deep pectoral muscles cooked at 80°C for 1 hr as a function of raw meat pH.

onset of rigor is directly related to the resulting sarcomere length. It seems possible that differences in the 'stress-state' of animals at slaughter could induce variations in fiber contraction and thus account for some of the variation in toughness not directly associated with pH.

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Ms received 12/1/71; revised 3/3/72; accepted 3/4/72.

This work was supported in part by funds from the Australian Meat Research Committee.

The expert technical assistance of Miss Denise Lehmann, Mr. R.H. Turner and Mr. R.F. Dickinson and advice on statistical presentation of Dr. R.I. Baxter is gratefully acknowledged.

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CHARACTERIZATION OF THE RED PIGMENTS PRODUCED FROM FERRIMYOGLOBIN BY IONIZING RADIATION

INTRODUCTION

IONIZING RADIATION affects the primary and higher order structures of proteins, especially in aqueous solutions. In the case of myoglobin, peptide bond cleavage has been the subject of recent studies (Ho, 1967). In the dose range contemplated for the "pasteurization" of fresh meats (50–150 Krad), the radiation effect on the muscle heme pigments, which is significant from the standpoint of color, involves the heme prosthetic chromophore. The oxidation state of the heme iron and the nature of its "sixth position" ligand influence the electron configuration of both the iron and the entire chromophore, and thus affect the magnetic and the light-absorbing properties of the pigment.

Uncertainty regarding the oxidation state and sixth position ligand of the iron of the red pigment generated by irradiation of myoglobin arose in the 1950's and continues to persist. Ginger et al. (1955) and Ginger and Schweigert (1956) observed that irradiation of meat extract containing a high proportion of metmyoglobin produced a bright red compound spectrally indicative of oxymyoglobin, and similar to oxymyoglobin in its reactions with hydrosulfite, ferricyanide and cyanide, and carbon monoxide. (In this paper the prefixes "ferri" and "met" are used interchangeably. Both denote the +3 oxidation state of the heme iron. "Ferri" is preferred over "met" and has replaced it in the official nomenclature.) When the extract to be irradiated contained a high proportion of oxymyoglobin the formation of metmyoglobin and/or a green oxidized porphyrin derivative was favored. Also based upon spectral evidence Tappel (1956) concluded that oxymyoglobin is formed by irradiation of metmyoglobin in meat and in purified solution. Bernofsky et al. (1959) reported that, upon irradiation, oxymyoglobin in partially purified solution is first converted to metmyoglobin which is in-turn converted by further irradiation to a red compound. This compound is stable to still higher doses of irradiation and appears spectrally similar to oxymyoglobin except for a lower A_{540}/A_{560} ratio. Irradiation of recrystallized metmyoglobin produced the same red pigment. They also observed that addition of irradiated water to nonirradiated metmyoglobin solution produced a red pigment having an absorption spectrum identical to that of "peroxymetmyoglobin" and distinct from that of both oxymyoglobin and the red compound produced by irradiation of metmyoglobin solutions. Brown and Akoyunoglou (1964) concluded that absorbance changes in the visible region suggested that irradiation of nitrogen-flushed pigment solutions caused partial oxidation of oxymyoglobins and converted metmyoglobins to substances with spectra similar to that of oxymyoglobins, differing by only a few nm in the positions of the absorbance maxima. Ho (1967) similarly removed practically all oxygen in myoglobin solutions by repeated nitrogen flushing and immediate sealing of the vials before irradiating to 500 Krad. The irradiated metmyoglobin was converted to a red pigment having an absorption spectrum very similar to that of oxymyoglobin. Clarke and Richards (1971) concluded from absorbance spectral peaks at 411, 542, 582 and 620 nm that the iron of metmyoglobin, myoglobin, or a mixture of oxy- and metmyoglobin irradiated in either oxygen, air, or nitrogen atmosphere was in the ferric (+3) oxidation state, but that the normal metmyoglobin structure was destroyed. They concluded that the 411 nm Soret peak ruled out oxymyoglobin and that the 620 nm band was "a result of the irradiation." Satterlee et al. (1971) reported that irradiation of metmyoglobin in meat and in several differing states of purity converted it to a red pigment that is similar to but not identical with oxymyoglobin because of its Soret peak at 412 nm. They speculated that the red pigment might be formed by the addition of a small molecule to the heme iron followed by reduction of the heme iron from the ferric to the ferrous state. They noted that the red pigment formation was greatest in a nitrogen atmosphere, was

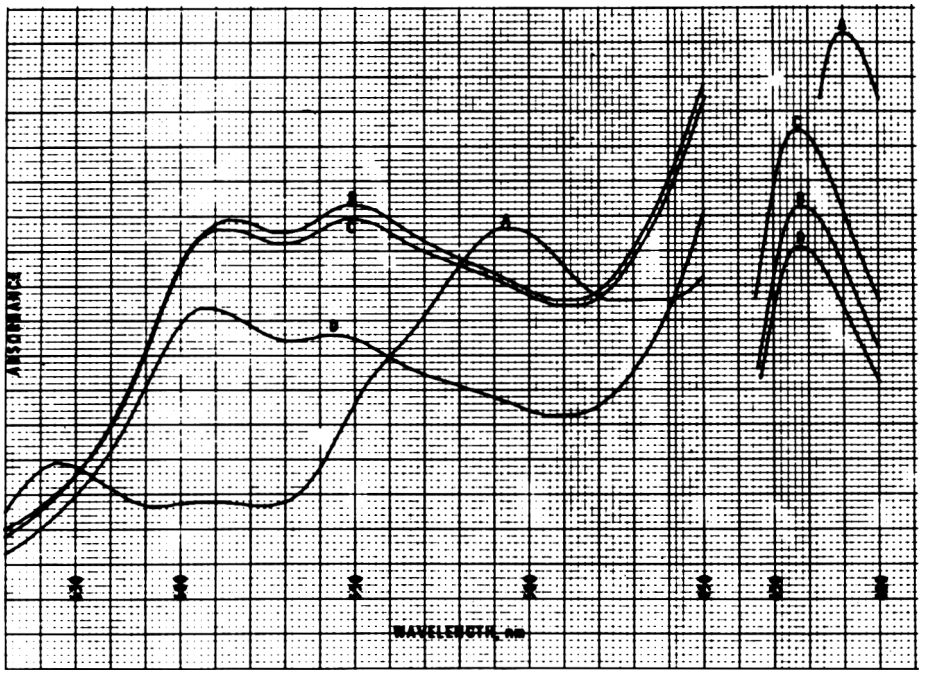

Fig. 1—Absorbance spectra of oxygen containing ferrimyoglobin solution which was: untreated, Curve A; treated with H_2O_2, Curve B; treated with 40 Krad, Curve C; treated with irradiated water, Curve D.

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slightly inhibited in air, and was greatly inhibited in an oxygen atmosphere, implying that differences due to atmosphere were quantitative only.

In a related development a number of investigators have been studying the interaction of hemoproteins with hydrogen peroxide. George (1952) revived a long-overlooked theory according to which the products of the interaction of ferricatalase, ferriperoxidase, ferrihemoglobin and ferrimyoglobin with H_2O_2 have a ferryl, (+4), oxidation state. The simple higher oxidation state theory was at variance with the then accepted hemoprotein-peroxide, enzyme-substrate complex model advanced by Chance (1951) and others. George and Irvine (1955, 1959) subsequently demonstrated the ferryl structure. King and Winfield (1963), using a combination of electron spin resonance, manometric and low temperature spectrokinetic techniques provided further evidence for the quadrivalent heme iron resulting from the metmyoglobin–H_2O_2 reaction. Additional confirmation of the ferryl structure was presented by Peisach et al. (1968), who described the ferrylmyoglobin iron as having four 3d valence electrons and an effective spin of 1, complexed to an oxygen atom, in agreement with the structure proposed by George and Irvine (1955). More recent support for the ferryl hemoprotein structure was presented by Mochan and Nicholls (1971), Coulson et al. (1971), and by Dolphin et al. (1971). Ferricytochrome c has also been shown to react with H_2O_2, thereby catalyzing the peroxidatic oxidation of ferrocytochrome c (Mochan and Degn, 1969; Davison and Hulett, 1971).

Hydrogen peroxide is one of the two known molecular products of water radiolysis (H_2 being the other) and its production is enhanced by the presence of oxygen in the irradiated aqueous medium, being minimal in the absence of oxygen. This suggests that irradiation of oxygen containing metmyoglobin solutions gives rise to ferrylmyoglobin via the reaction with radiation-generated H_2O_2.

Another development related to the irradiation of metmyoglobin is the demonstration of the reduction of heme proteins by solvated electrons resulting from water radiolysis. Three of the more recent reports on this subject are by Pecht and Faraggi (1971), Land and Swallow (1971) and Wilting et al. (1971). The absence of oxygen, an effective scavenger of hydrated electrons, favors reduction reactions and generally establishes a reducing environment in irradiated media. Therefore it is reasonable to expect metmyoglobin to be reduced to the ferrous state when oxygen is at low concentration or absent, and to be further oxidized to the ferryl state in the presence of substantial oxygen. The evidence presented in this paper demonstrates that this is, in fact, the case.

MATERIALS & METHODS

Myoglobin

Myoglobin was prepared from the semitendinosus muscle of USDA Commercial beef rounds. Partially purified oxymyoglobin (preparation A) was prepared by homogenizing x grams of ground lean beef with 3x ml of chilled 75% saturated $(NH_4)_2\ SO_4$ solution. After centrifuging, the red supernatant was exhaustively dialyzed against cold deionized water and finally against 0.01M phosphate buffer, pH 7.0. The oxymyoglobin solution was then vacuum-filtered. A highly purified metmyoglobin preparation (preparation B) was made according to the procedure of Hardman et al. (1966).

Reagents

Hydrogen peroxide was 30% Baker analyzed reagent grade, used either full strength or diluted to 1%.

Sodium hydrosulfite (dithionite) was Mallinckrodt reagent grade, added to solutions as the dry powder.

Potassium ferricyanide and ferrocyanide, Baker analyzed reagent grade, were added to solutions both as dry crystals and in aqueous solution form.

Gamma irradiation

A 30,000 curie Cobalt-60 research irradiator was employed. Samples prechilled at 0–3°C were positioned in the isotropic center of the source annulus during exposure at a dose rate of approximately 1 Mrad/hr. Anoxic solutions were prepared by alternately evacuating and backfilling with high purity nitrogen gas several times prior to irradiation under vacuum. Oxygenated solutions had oxygen bubbled into them for several minutes prior to irradiation. Otherwise solutions were in equilibrium with the atmosphere prior to irradiation.

Spectrophotometry

A Bausch & Lomb Spectronic 505 recording spectrophotometer was used to record spectra from 350–650 nm. Figures shown are original recorder tracings, photoreduced for publication. Matched 1 cm quartz cells and a distilled water blank were used. The Soret peaks (400–440 nm) were recorded after dilution of the samples used to obtain the 450–650 nm tracings.

RESULTS & DISCUSSION

FIGURE 1 compares visible and Soret spectra of (a) untreated, oxygen-containing metmyoglobin solution with that which had been treated with (b) H_2O_2, (c) 40 Krad of radiation and (d) irradiated water which stood at room temperature for 1 hr post-irradiation before adding 5 ml of it to 25 ml of metmyoglobin. The Soret peaks at 420–423 nm are indicative of ferrylmyoglobin as are the absorption bands in the 550 and 580–590 nm regions (curves B, C and D). H_2O_2, and possibly some molecular hydrogen, should be the only radiolysis products in the irradiated water at the time of addition to the metmyoglobin. The slightly different pattern of that spectrum no doubt reflects fine electronic detail. Clearly, all three treatments resulted in ferrylmyoglobin production via the metmyoglobin–H_2O_2 reaction, as further demonstrated by the following evidence.

Figure 2 shows the absorption spectra of (a) oxygenated metmyoglobin solution exposed to 40 Krad, (b) "a" after ferricyanide addition, (c) "a" after ferrocyanide addition and (d) "a" after hydrosulfite addition. The same results were obtained with metmyoglobin solu-

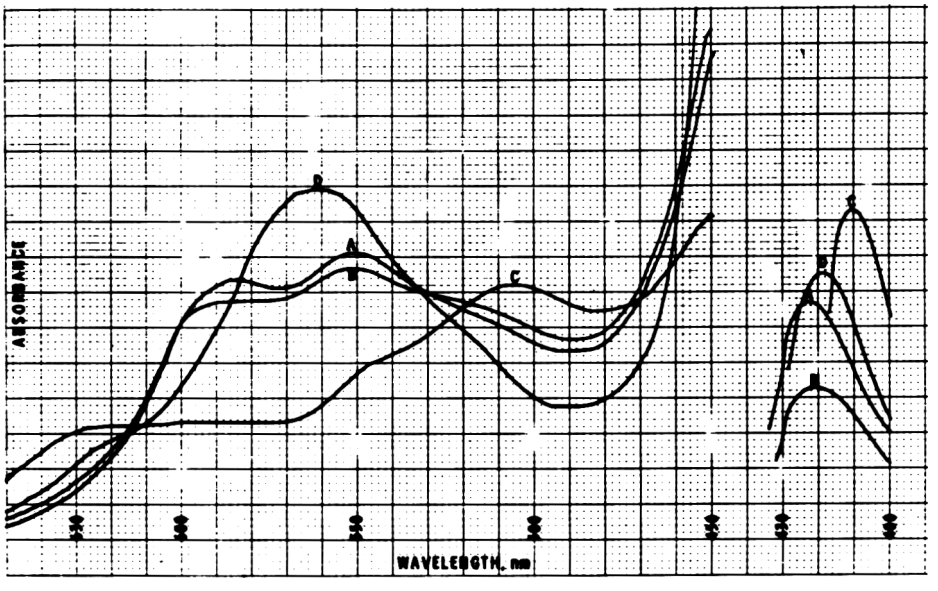


Fig. 2–Absorbance spectra of oxygenated, 40 Krad irradiated ferrimyoglobin solution which was: untreated, Curve A; treated with ferricyanide, Curve B; treated with ferrocyanide, Curve C; treated with hydrosulfite, Curve D.

tion that was merely equilibrated with the atmosphere. The ferrylmyoglobin spectrum due to irradiation of oxygenated metmyoglobin is virtually unchanged by post-irradiation addition of ferricyanide, whereas ferrocyanide addition caused an instantaneous reduction back to metmyoglobin. The ferrocyanide/ferricyanide redox couple is ideal for this type of study in that ferrocyanide instantaneously reduces quadrivalent myoglobin to the trivalent (met) state but not a lower one, and ferricyanide instantaneously oxidizes ferrous myoglobin to the trivalent (met) state but not a higher one, thus enabling one to distinguish the ferryl red pigment from oxymyoglobin and unequivocally establish the valency of the heme iron. The red myoglobin derivative from irradiation of oxygen-containing metmyoglobin has a quadrivalent iron. The powerful, general purpose reducing agent, hydrosulfite, reduces ferrylmyoglobin to purple deoxyferrous myoglobin via a two equivalent reduction of the heme iron.

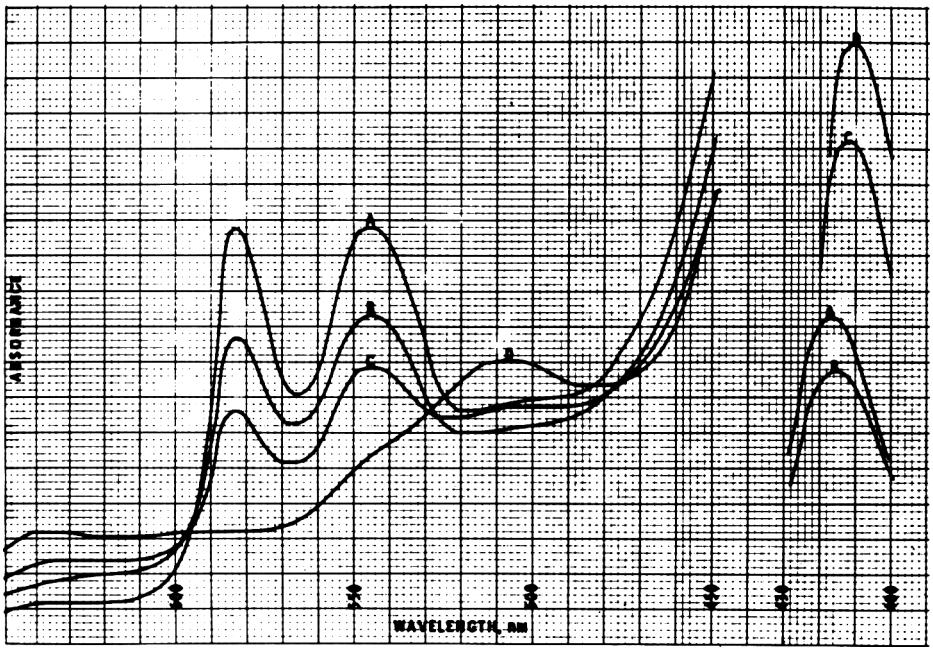


Fig. 3–Absorbance spectra of: untreated oxymyoglobin, Curve A; deoxygenated ferrimyoglobin exposed to 40 Krad, Curve B; "B" treated with ferrocyanide, Curve C; "B" treated with ferricyanide, Curve D.

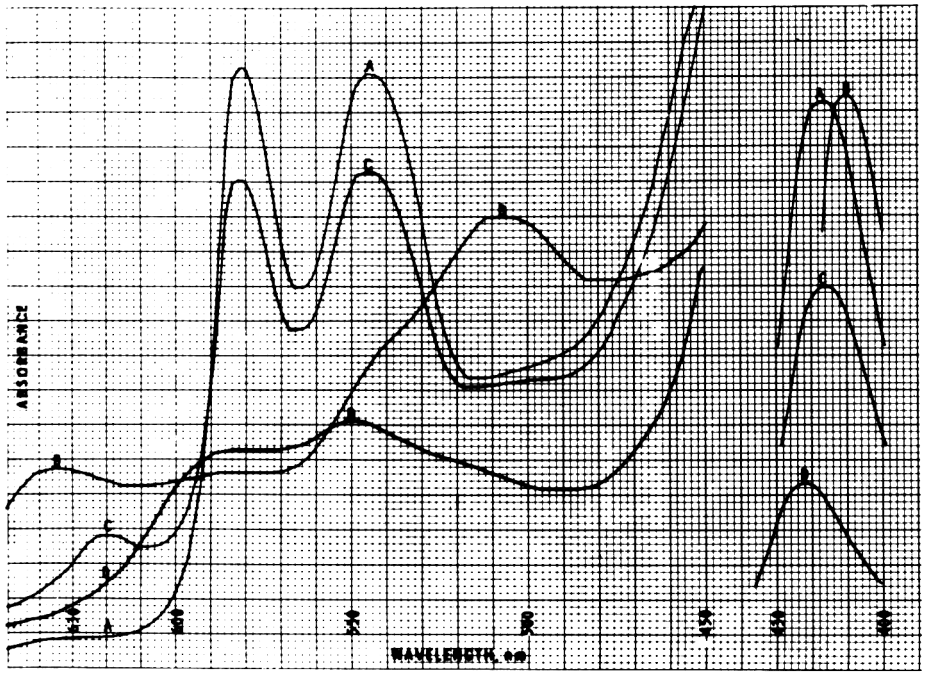


Fig. 4–Absorbance spectra of: untreated oxymyoglobin, Curve A; "A" exposed to 40 Krad, Curve B; "B" immediately exposed to an additional 40 Krad, Curve C; "B" exposed to an additional 40 Krad after dialyzing and oxygenating the solution, Curve D.

Figure 3 shows the absorption spectra of (a) oxymyoglobin, (b) deoxygenated metmyoglobin exposed to 40 Krad, (c) "b" treated with ferrocyanide and (d) "b" treated with ferricyanide. When metmyoglobin solution was depleted of oxygen and subsequently exposed to 40 Krad of gamma radiation the absorption spectrum of oxymyoglobin was obtained. Further evidence that the 6th position ligand of this radiation-generated pigment is oxygen was obtained by bubbling carbon monoxide gas into solutions of "natural" and radiation-generated oxymyoglobin. In both cases the spectra changed to that of MbCO (not shown). It is well known that CO readily replaces O_2 as 6th position ligand of oxyhemoproteins. In addition to the spectral evidence regarding the nature of the radiation-generated product the following two reactions confirm the ferrous state of the heme after irradiation: ferricyanide instantaneously oxidized the radiation product to metmyoglobin as shown by the absorption spectra (curves D), whereas ferrocyanide caused essentially no change in the spectrum of the radiation product (curves C).

There are at least two possible mechanisms for the formation of oxymyoglobin under the conditions used in this experiment. The more likely one is the reduction of the heme iron by a reducing product of water radiolysis, such as the hydrated electron. This reduction would be followed by oxygenation with either residual oxygen remaining in the solution prior to irradiation, or with oxygen generated during irradiation. Some reactions leading to the generation of oxygen by the radiation are:

$$\cdot OH + HO_2\cdot \longrightarrow O_2 + H_2O$$

$$HO_2\cdot + Fe^{+3} \longrightarrow H^+ + O_2 + Fe^{+2}$$

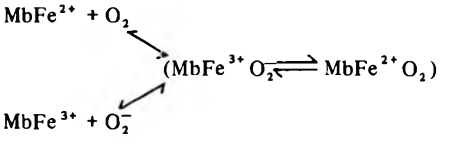
and $$2HO_2\cdot \longrightarrow H_2O_2 + O_2$$

Another possibility is the capture of hydrated electrons by oxygen generated as above to form the superoxide anion

$$\bar{e}_{aq} + O_2 \longrightarrow O_2^-$$

which may complex with the trivalent iron of ferrimyoglobin to form oxymyoglobin. This possibility is suggested by the

work of Wittenberg et al. (1970) and others confirming the theory first proposed by Weiss (1964) that when hemoproteins bind oxygen the heme iron becomes formally ferric by partial transfer of one 3d electron to the liganded oxygen which then becomes a bound superoxide anion. Thus if ferrimyoglobin is in fact able to bind superoxide anion, then the two suggested mechanisms of oxymyoglobin formation can be summarized as follows:



According to an energetics analysis by George and Stratmann (1954) both processes of oxymyoglobin formation would be about equally exothermic, and therefore equally favorable thermodynamically.

Figure 4 depicts the spectra of (a) oxymyoglobin (preparation A), (b) "a" exposed to 40 Krad of radiation, (c) "b" exposed immediately to another 40 Krad of radiation and (d) "b" exposed to another 40 Krad of radiation after first dialyzing the solution and then oxygenating it. The oxymyoglobin preparation was oxidized completely to metmyoglobin (curve B) by the initial 40 Krad and was immediately reduced back to oxymyoglobin by the additional 40 Krad (curve C). When the radiation-generated metmyoglobin solution was oxygenated prior to the second 40 Krad of radiation the ferrylmyoglobin spectrum (curve D) was obtained. Confirmation for the oxidation states of the radiation-generated oxymyoglobin and ferrylmyoglobin was provided by the ferrocyanide–ferricyanide reactions.

The regeneration of the oxymyoglobin after radiation-induced oxidation to metmyoglobin, also reported by Bernofsky et al. (1959), can be explained by the depletion of oxygen in the solution during the initial 40 Krad irradiation, thus favoring a reduction of the iron during the second phase of irradiation. The 620 nm absorption band of the regenerated oxymyoglobin indicates disruption of the normal porphyrin resonant double bond structure as a result of either oxidation of, or addition of a small molecule such as H_2S (Berzofsky et al., 1971) to a site on the ring. The dialysis step in the production of ferrylmyoglobin from radiation-generated metmyoglobin was found to be necessary to obtain complete oxidation to the ferryl state. Allowing the irradiated solution to stand for more than a day prior to oxygenation and further irradiation did not eliminate the need for dialysis. Also, pH was not found to be the factor inhibiting complete further oxidation. An interfering solute or solvent radiolysis product is therefore suggested.

In summary, it can now be unequivocally stated that there are in fact two red myoglobin derivatives produced by gamma irradiation of ferrimyoglobin solutions under the conditions usually employed in such work. When solutions are anoxic prior to irradiation a reductive oxygenation to oxymyoglobin takes place (as suggested by earlier work), and in oxygen-containing solutions ferrylmyoglobin is generated. This is consistent with our observations (unpublished) that when beefsteaks having an oxidized (brown) surface color are vacuum packaged and irradiated with a pasteurizing dose of ionizing radiation, a purple surface color develops indicative of a reduction of surface ferrimyoglobin. Upon exposure to the atmosphere this radiation-reduced surface pigment oxygenates, becoming bright red, whereas prior to anoxic irradiation the color was brown. Unlike oxygen-containing ferrimyoglobin solutions, however, we have not observed ferrylmyoglobin development on the surface of brown-appearing beefsteaks exposed to irradiation in the presence of oxygen. This may be because catalase activity in the meat tissue removes H_2O_2 as fast as it is produced.

The same results were obtained with human hemoglobin as are reported herein for bovine myoglobin.

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Ms received 10/25/71; revised 2/18/72; accepted 2/24/72.

Michigan Agricultural Experiment Station Journal Article No. 5703.

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PENTOSANS IN SORGHUM GRAIN

INTRODUCTION

SORGHUM GRAIN is the third largest U.S. cereal crop with more than 400,000,000 hundred weights produced annually. Over 98% of the grain is used domestically for livestock feed and export. Approximately 8–10,000 lines of sorghum have been collected throughout the world. The genetic diversity of this material offers an opportunity to improve sorghum through breeding programs. Improvement in quality of the grain is promising because many of these grains have been preferred for human food for centuries in Asia and Africa (Rooney et al., 1970).

The pentosan content of cereals has been thought to have an important role in the quality of some cereal grains (Pomeranz, 1961; Elder et al., 1953; Bechtel and Meisner, 1954). The only information on pentosan content of sorghum indicates a range of 2–3% for several varieties grown in the 1940's (Edwards and Curtis, 1943). The varieties were quite similar genetically. The purpose of the work reported in this paper was to obtain information on: (1) the pentosan content of sorghum grain with large differences in kernel size and endosperm characteristics; (2) distribution of pentosans in the kernel; (3) relation between pentosan content and processing properties of the grain; and (4) the influence of environment on pentosan content.

MATERIALS & METHODS

GRAIN from 31 varieties of sorghum (*Sorghum bicolor* L. Moench) used in this study was grown under comparable conditions at the South Plains Research and Extension Center, Texas Agricultural Experiment Station, Lubbock, Texas in 1967. The varieties represented yellow, waxy, sugary and normal endosperm types. To study the influence of environment on pentosan content of sorghum, grain samples were obtained from four varieties grown on the Texas A&M University Plantation, College Station, Texas in 1967 and 1968 and at Lubbock in 1967 and 1968. Grain from RS 608 was obtained from eight Texas locations in 1968. Laboratory tests were conducted on clean grain, free of glumes and broken kernels. Grain was ground to pass through an 0.010-in. slotted screen in a Weber Laboratory hammer mill. Replicate analyses were taken on the ground grain and should not be confused with field replicates.

The pentosan content of sorghum samples was determined by the method of Fraser et al. (1956).

Kernels were subjected to the dry milling technique of Rooney and Sullins (1969). By this technique, pericarp, germ, endosperm, a fine fraction and a germ-rich fraction, containing fragments of germ and endosperm, were obtained. Germ and endosperm fragments were separated by sodium nitrate flotation to provide relatively pure germ and endosperm fractions.

Grain from the sorghum variety, B 398, was used to determine the location of pentosans in the kernel according to the method of Johansen (1940) after kernels were prepared for histochemical studies by the procedure of Sass (1945).

Starch content was determined by the method of Norris and Rooney (1970a). Starch yield and peripheral endosperm content (PEC) were determined by a laboratory wet milling procedure (Norris and Rooney, 1970b). Starch recovery was defined as the amount of starch obtained by wet milling, divided by the amount of starch in the sample.

The dry milling fractions, i.e., coarse grits and fine grits were obtained by a laboratory method (Rooney and Sullins, 1969).

Kernel size index (KSI) and hardness determinations were conducted as described by Rooney and Sullins (1970).

A Beckman Model 930 air pycnometer was used to determine the density of grain from each variety.

Protein, lipid and moisture content were determined by AACC (1970) procedures.

Unless otherwise stated, all results are expressed on oven dry-weight basis.

RESULTS & DISCUSSION

Pentosan content of sorghum grain

The mean and range of pentosan content for grain from 31 sorghum lines and varieties are presented in Table 1. The

Table 1—Pentosan content of sorghum grain with different endosperm characteristics

Endosperm characteristics	Pentosan (% DB) mean	range	No. of varieties
Yellow	3.76	3.21–5.57	7
Waxy	2.77	2.51–3.03	2
Sugary	3.84	3.21–4.48	2
Corneous	3.51	3.21–4.01	6
Floury	3.56	2.87–4.87	7
Normal and Intermediate	3.40	2.8 –4.1	7

Table 2—Analysis of variance for pentosan content of grain from 31 sorghum varieties with different endosperm characteristics

Sources of variation	d.f.	M.S.
Varieties (treatments)	30	0.74**
Among endosperm groups	5	0.60**
Among varieties/yellow endosperm	6	1.50**
Among varieties/corneous	5	0.17**
Among varieties/floury	6	0.86**
Among varieties/sugary	1	1.61**
Among varieties/waxy	1	0.27**
Among varieties/normal	6	0.35**
Replication	1	0.03
Error	30	0.02
Independent and nonindependent comparisons among selected endosperm groups		
Corneous vs. floury	1	0.01
Waxy vs. sugary	1	2.31**
Yellow vs. normal	1	0.95**
Yellow vs. waxy	1	3.04**
Yellow vs. sugary	1	0.02
Normal vs. sugary	1	0.64**
Normal vs. waxy	1	1.20**
Normal vs. corneous	1	0.10*
Normal vs. floury	1	0.21**

*P < 0.05; **P < 0.01

Table 3–Distribution of pentosans within the sorghum kernel[a]

Parts of the kernel	% Pentosan[a]
Whole kernel	3.72[d]
Pericarp	21.54[a]
Endosperm	0.99[e]
Germ	6.17[c]
Fine fraction	11.16[b]
Recovery	87.73

[a]Expressed on dry weight basis
[b]If the small letters beside the mean are different, the means are significantly different at 0.01 level.

Table 4–Pentosan content of the endosperm fraction and whole grain from nine sorghum varieties[a]

Sorghum varieties	Pentosan %[b] Endosperm	Whole grain
SC 0283-6 (corneous)	0.87[a]	3.69[bc]
SA 216 (corneous)	1.06[a]	4.01[a]
SA 5875-6-1 (waxy)	0.96[a]	3.03[ef]
Tx 403 (normal)	0.82[a]	3.31[de]
7078 (normal)	0.90[a]	3.41[cd]
B 398 (normal)	0.97[a]	3.72[b]
B 3197 (normal)	1.20[a]	3.08[ef]
SA 394 (normal)	0.94[a]	4.10[a]
Tx 09 (normal)	0.88[a]	2.80[f]

[a]Expressed on dry weight basis
[b]If the small letters beside the mean are different, the means are significantly different at 0.05 level.

Table 5–Mean pentosan content of grain from four sorghum varieties grown under different conditions[a]

Variety	College Station 1967	College Station 1968	Lubbock 1967	Lubbock 1968
B 3197	3.42	3.39	3.07	3.22
TX 09	2.80	2.29	2.80	3.15
NSA 740	5.13	4.38	4.86	5.21
SCO 283	–	3.28	3.33	3.77

[a]Percent dry weight basis

Table 6–Analysis of variance for pentosan content of grain from three sorghum varieties grown under different conditions

Sources of variation	d.f.	M.S.
Location	1	0.07*
Year	1	0.09*
Variety	2	10.42**
Location × Year	1	0.98**
Location × Variety	2	0.31**
Year × Variety	2	0.05*
Location × Variety × Year	2	0.12**
Pooled within error	12	0.01

*P < 0.05; **P < 0.01

sorghums were divided into yellow, waxy, sugary, corneous, floury and normal groups based upon the endosperm characteristics. Yellow endosperm type means that the endosperm contains yellow carotenoid pigments. Waxy endosperm type means that the starch is composed of amylopectin rather than the 3:1 amylopectin to amylose ratio which is usually present in the starch of nonwaxy sorghums. Sugary type of sorghum is higher in soluble sugar content than other sorghums. Endosperm texture refers to the relative proportion of corneous to floury endosperm present in the kernel. Therefore, corneous sorghums are those with all corneous endosperm; while floury texture sorghums are those with all floury endosperm. Intermediate refers to those sorghums with varying proportions of corneous and floury endosperm within the kernel. The intermediate texture group of sorghums had normal endosperm type, i.e., they were not waxy, yellow or sugary.

An analysis of variance was conducted and several comparisons were made of pentosan content of the sorghums with various endosperm types and textures (Table 2). The pentosan content of floury texture sorghums was not different from that of corneous texture sorghums. The pentosan content was significantly different between the other groups; yet, there was significant variation among pentosan content of grain from varieties within each group.

Distribution of pentosans in the sorghum kernel

The major anatomical portions of grain from B 398 were separated and analyzed for pentosan content (Table 3). The high content of pentosan in the pericarp and germ compared to the low quantities in the endosperm indicates that a major part of the variability in pentosan content may be explained by variation in the proportion of pericarp and germ to endosperm in the kernel. The fines referred to in Table 3 contained mainly pericarp and germ which explains its high pentosan content. The fine fraction was included to provide recovery data. The inability to recover all the pentosans is partially explained by the 2–3% loss of fines during milling. Histochemical treatment of kernel sections showed clearly that the pentosans were in the cell walls which were most predominant in the pericarp and germ portions of the kernel. The pentosan content gradually decreases from the periphery to the center of the endosperm. Pentosans of the wheat kernel are similarly distributed (Elder et al., 1953); except that wheat endosperm (flour) contains 2.9–3.2% pentosan (Loska and Shellenberger, 1953).

The data on distribution of pentosans within the kernel strongly indicate that the proportion of pericarp and germ to endosperm significantly influences pentosan content. To substantiate this, grain from nine varieties of sorghum which differed in pentosan content and processing properties was milled into grits which were degermmed, debranned kernels and therefore, were representative of the endosperm of each variety. Pentosan

Table 7–Mean and range of physical and chemical properties of grain from 31 varieties of sorghum according to endosperm characteristics

Endosperm characteristics	No. of varieties	Kernel size index	1000 kernel wt (g)	Density (g/cc)	Test wt (lb/bu)	Hardness	Protein[a] %	Lipid[a] %	Starch[a] %
Yellow	7	50.3–67.7	26.6–59.2	1.32–1.36	57.3–61.1	16.4–29.6	11.4	2.7	69.7
							14.0	3.7	75.9
Corneous	6	68.5–74.9	19.0–28.5	1.31–1.40	57.4–63.5	17.9–63.8	13.8	2.8	65.1
							17.8	4.1	70.5
Floury	7	63.3–83.0	11.6–28.8	1.22–1.36	51.5–59.1	0.1–30.8	12.6	3.3	62.3
							14.6	4.2	67.5
Sugary	2	60.6–69.1	22.4–33.1	1.29–1.30	53.5–55.5	5.0–29.1	14.7	5.2	60.9
							17.3	5.4	64.1
Waxy	2	61.4–67.4	27.6–34.6	1.32–1.33	57.1–58.2	4.3–4.35	13.3	3.4	68.8
							15.7	3.7	72.6
Normal and intermediate	7	53.8–70.1	24.5–48.7	1.32–1.38	55.8–61.9	1.2–44.1	12.4	3.2	68.1
							15.6	4.2	71.4
Overall mean	31	66.2	29.1	1.33	58.2	19.8	14.0	3.7	68.8
Overall range	31	50.3–83.0	11.6–59.2	1.22–1.40	51.5–63.5	0.1–63.8	11.4	2.7	60.9
							17.8	5.4	75.9

[a]On an oven dry basis; Protein = N × 6.25

Table 8–Mean and range of dry milling and wet milling properties of grain from 31 varieties of sorghum according to endosperm characteristics[a]

Endosperm characteristics	No. of varieties	Coarse grits %	Fine grits %	Coarse & fine grits %	Starch yield %	Starch recovery %	Starch protein %	PEC %
Yellow	7	49.0–59.5	15.1–26.0	67.1–76.2	47.1–54.6	66.2–75.1	0.8–1.5	0.6–1.5
Corneous	6	43.6–79.3	4.1–33.5	74.7–83.2	41.5–49.3	59.9–70.0	1.7–2.6	1.8–2.9
Floury	7	0.1–55.8	17.3–58.5	31.0–73.0	46.9–53.6	71.9–83.9	1.4–2.1	0.3–1.4
Sugary	2	37.4–74.0	5.9–29.3	66.7–79.8	21.3–30.8	35.0–48.1	1.8–2.1	0.5–1.4
Waxy	2	35.1–38.5	32.5–39.9	71.0–75.0	43.8–49.7	63.7–68.5	1.1–1.5	1.3–2.4
Normal and intermediate	7	19.0–72.7	6.7–25.2	43.9–79.4	46.0–54.4	66.5–77.6	0.9–3.0	0.8–1.9
Overall mean	31	44.6	23.3	67.9	48.5	70.2	1.6	1.4
Overall range	31	0.1–79.3	4.1–58.5	31.0–83.2	21.3–54.6	35.0–83.9	0.8–3.0	0.3–2.9

[a]All values are expressed on oven dry basis.

content of whole grain and endosperm is compared in Table 4. The endosperm of the nine varieties did not differ significantly in pentosan content which again indicates that differences in the proportion of pericarp and germ in the kernels account for the difference in pentosan content of the whole kernel.

Influence of environment on pentosan content

Location of production significantly influences pentosan content. Mean pentosan content of grain from the hybrid, RS 608, grown at eight locations in Texas in 1968 was 3.74, 3.73, 3.52, 3.42, 3.31, 3.28, 3.08 and 3.05%. The grain was grown in replicated yield trials and the pentosan content was determined on a composite sample of grain representing the field replications at each location. This range although relatively small was statistically different and should be typical of that expected for grain from commercial sorghum hybrids grown under reasonably good yield conditions. A greater range would be expected if grain was grown under conditions where the proportion of endosperm in the kernel was reduced.

Another experiment to determine the influence of location, year of production and variety on pentosan content of sorghum grain consisted of analyses of grain from four varieties grown at two locations for each of two years. The data are presented in Table 5. The grain from SCO 283 produced at College Station in 1967 was lost, which caused problems in statistical analyses. Thus, a series of statistical analyses were performed to evaluate the data. The analysis of variance for three varieties analyzed over all locations and years is presented in Table 6. Pentosan content was significantly influenced by variety, location of production and year of production. The variety X location and year X variety interactions were significant, indicating that relative pentosan content of the grain from the different varieties changed among years and locations. The significant location X year interaction indicates that relative pentosan content was not consistent at any one location.

Table 9–Correlation between pentosan content and physical, chemical and processing properties of sorghum grain

Correlation between pentosan content vs.	Overall	Yellow endosperm	Corneous endosperm	Floury endosperm	Normal endosperm
Number of varieties studied	31	7	6	7	7
Kernel size index	–0.19	–0.64	–0.15	–0.22	0.85*
Hardness	0.17	–0.06	0.01	–0.33	0.88**
Starch content	–0.17	–0.34	–0.59	–0.07	–0.85*
Starch yield	–0.21	–0.59	–0.57	0.19	–0.57
Starch recovery	–0.18	–0.51	–0.37	0.09	–0.40
Total grits	–0.06	–0.88**	–0.37	–0.53	0.78*
Coarse grits	0.09	–0.33	0.42	–0.40	0.73
Fine grits	–0.20	–0.38	–0.56	0.06	–0.56
PEC	–0.11	0.23	0.67	–0.39	0.52
Protein of starch	0.22	0.19	0.68	0.31	0.71

*P < 0.05; **P < 0.01

These data indicate that environment (location of production) and variety influence pentosan content. This was expected because environment influences chemical, physical and processing properties of sorghum (Norris, 1971; Miller et al., 1964; Campbell and Pickett, 1968).

Relation of pentosan content to physical, processing and other chemical properties of sorghum grain

The grain in this study varied widely in chemical, physical (Table 7) and processing properties (Table 8). Therefore, it provided a good opportunity to relate pentosan content to some of the measurements taken on the grain (Table 9). None of the measurements was related to pentosan content when all 31 grain samples were considered. Therefore, the correlations were performed within the varieties representing each endosperm characteristic. Within the normal endosperm group, pentosan content was significantly positively correlated with kernel size index, hardness and total grit yield. The positive correlation of kernel size index with pentosan content indicates that varieties with the smaller kernels contained more pentosan than those with larger kernels. The smaller kernels have a higher proportion of pericarp than larger kernels. This might explain the higher pentosan content. However, the yellow endosperm varieties differ dramatically in kernel size but it was not significantly related to pentosan content.

Kernel hardness was positively related with pentosan content of grain within the normal endosperm group. Harder kernels are more resistant to breakage when dry milled, which is reflected in the significant positive correlation between pentosan content and total grits yield. The negative relation between starch and pentosan content is probably logical because high pentosan content in grain is probably related to a higher proportion of pericarp and germ in the kernel which would mean a decrease in starch content since starch is the major component of the endosperm.

CONCLUSION

PENTOSAN CONTENT is not a useful index for predicting processing properties

of sorghum. Pentosan content is influenced by environmental conditions as well as variety of sorghum grain. The major factor influencing pentosan content is the variation in proportion of pericarp and germ to the endosperm. Pentosan content of the endosperm is low and does not significantly vary among grain from different varieties.

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Ms received 7/26/71; revised 1/10/72; accepted 1/16/72.

Contribution from the Texas A&M University Cereal Quality Laboratory and is a portion of the senior author's dissertation submitted in partial fulfillment of the requirements for a Ph.D. in Food Technology.

We thank Dr. L.E. Clark for assistance with the statistics and Drs. D.T. Rosenow and J.W. Johnson for the grain samples.

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CHARACTERIZATION OF PENTOSANS IN SORGHUM GRAIN

INTRODUCTION

THE COMPOSITION and properties of pentosans have been studied in cereal grains including rice (Cartano and Juliano, 1970), wheat (Perlin, 1951a, b; Kuendig et al., 1961; Lin and Pomeranz, 1968; Cole, 1967), corn (Wolf et al., 1953) and barley (Preece and Mackenzie, 1952a, b). The pentosans of sorghum grain have not been characterized; although previous investigations (Karim and Rooney, 1972) at this laboratory compared pentosan content and its relation to physical, chemical and processing properties of sorghum grain.

The purpose of the work reported here was to characterize and compare the water-soluble and alkali-soluble pentosans from whole kernel, pericarp and endosperm of sorghum grain.

MATERIALS & METHODS

WATER-SOLUBLE and alkali-soluble pentosans were extracted from ground pericarp, endosperm and whole grain of the sorghum variety, Martin. The endosperm and pericarp fractions were obtained by the dry milling technique of Rooney and Sullins (1969). The fractions were ground to pass through an 0.010-in. mesh screen in a Weber Laboratory hammer mill and were defatted with petroleum ether. A 100-g sample was used for the extraction of water-soluble and alkali-soluble pentosans from whole grain and endosperm. For the pericarp, a 10-g sample was used.

Isolation of pentosans

Water-soluble pentosans were isolated by the procedure of Lin and Pomeranz (1968). Alkali-soluble pentosans were isolated by the procedure of Cartano and Juliano (1970). The schemes are outlined in Figure 1.

Purification of crude pentosan preparations

Soluble starches were removed from the various pentosan preparations by the procedure of Kuendig et al. (1961). 800 mg of crude pentosan were dissolved in 50 ml of 0.02M sodium phosphate buffer (pH 7.2). Then, 25 ml of 0.2% Wallerstein bacterial alpha-amylase (negative for pentosanase) in phosphate buffer was added and the mixture incubated for 20 hr at 70°C. By that time, the suspension gave a negative starch-iodine blue test. The suspension was adjusted with 5% trichloroacetic acid to a pH of 3 and centrifuged at 37,000g for 45 min. The supernatant was dialyzed against distilled water at 4°C for 48 hr and lyophilized.

Hydrolysis of pentosans

Approximately 40 mg of water-soluble and alkali-soluble pentosans from the whole grain, endosperm and pericarp fractions were hydrolyzed in sealed glass tubes with 10 ml of 0.1N H_2SO_4 for 4 hr at 100°C. The hydrolysates were neutralized with small amounts of Dowex-2 resin (HCO_3^- form) and stored at 4°C prior to chromatographic separation.

Identification and quantitative analysis of sugars in the pentosans

The hydrolysates of water-soluble and alkali-soluble pentosans were chromatographed on Whatman No. 1 paper with ethyl acetate:pyridine:water (8:2:1) according to the procedure of Kuendig et al. (1961). Xylose, arabinose, glucose and galactose were located by spraying the paper with an ammoniacal silver nitrate solution. The sugars appeared as dark brown spots against a light-brown background.

Quantitative determinations of the sugars in the chromatogram were made by the phenol-sulfuric acid method of Dubois et al. (1956). The quantity of sugar in the area containing glucose and galactose was determined as glucose because the two sugars were difficult to separate and galactose was present in small amounts. Glucose was also determined directly from the neutralized pentosan hydrolysates with a glucose-oxidase technique (Macrae and Armstrong, 1968) which permitted the calculation of galactose content by difference.

The total carbohydrate content of the neutralized pentosan hydrolysates was determined as glucose by the method of Dubois et al. (1956). The protein content (N × 6.25) was determined by the AACC (1970) procedure.

RESULTS & DISCUSSION

THE MAJOR pentosan fraction of the whole kernel and of the endosperm of sorghum grain was water-soluble. This made up 0.90% of the whole grain and 0.16% of the endosperm (Table 1). The alkali-soluble fraction comprised 0.42% and 0.09% of the whole grain and endosperm, respectively. In contrast, pentosans in the pericarp consisted almost entirely of the alkali-soluble fraction (Table 1). The distribution of water-soluble and alkali-soluble pentosans has been studied in other cereal grains. The water-soluble pentosans comprise about 0.50% of wheat flour (Montgomery and Smith, 1956; Lin and Pomeranz, 1968). One variety of milled rice contained 0.02% water-soluble pentosans and 0.10% alkali-soluble pentosans (Cartano and Juliano, 1970). Pentosans of wheat bran were reported to be less soluble in water than those from wheat endosperm and flour (Perlin, 1951a).

The carbohydrate content of the various pentosan preparations ranged from 68.72–85.67% (Table 1). Protein content of these preparations varied widely from 1.27% for the alkali-soluble pentosan of whole grain to 10.48% for the water-soluble pentosan fraction of whole grain. The composition of pentosan preparations from sorghum grain is similar to those obtained from rice and wheat. Cartano and Juliano (1970) isolated water-soluble and alkali-soluble pentosans which contained about 80% carbohydrate and 11–14% protein from milled rice. Cole

Table 1—Properties of water-soluble and alkali-soluble pentosans obtained from various anatomical portions of sorghum kernel[a]

Type of pentosan	Part of kernel	Yield %	Carbohydrate %	Protein[b] %
Water-soluble	Whole grain	0.90	76.25	10.48
	Endosperm	0.16	69.63	3.24
	Pericarp	0.62	68.72	6.83
Alkali-soluble	Whole grain	0.42	73.96	1.27
	Endosperm	0.09	85.67	3.74
	Pericarp	20.00	76.21	5.22

[a]Dry weight basis
[b]N × 6.25

Table 2—R_{g1} values of sugars constituting sorghum pentosans (ethyl acetate:pyridine:water, 8:2:1)

Sugar	R_{g1} values[a] Experimental	Literature value[b]
Galactose	0.82	0.85
Glucose	1.00	1.00
Arabinose	1.94	2.00
Xylose	2.58	2.60

[a]R_{g1} = ratio of distance traveled by the sugar to that traveled by glucose.
[b]From Hough (1962)

Table 3—Sugar composition of sorghum pentosans obtained from various parts of the kernel[a]

Type of pentosan	Part of kernel	Galactose	Glucose	Arabinose	Xylose	Ara/Xyl	Pentose/ Hexose
Water-soluble	Whole grain	9.36	68.73	16.68	5.22	3.19	0.28
	Endosperm	4.98	85.14	6.47	3.41	1.89	0.11
	Pericarp	13.67	30.19	37.75	18.38	2.05	1.28
Alkali-soluble	Whole grain	7.60	55.42	22.54	14.42	1.56	0.59
	Endosperm	3.85	73.46	14.53	8.16	1.78	0.29
	Pericarp	5.22	37.16	32.37	25.25	1.28	1.36

[a]Percent of total separated by paper chromatography

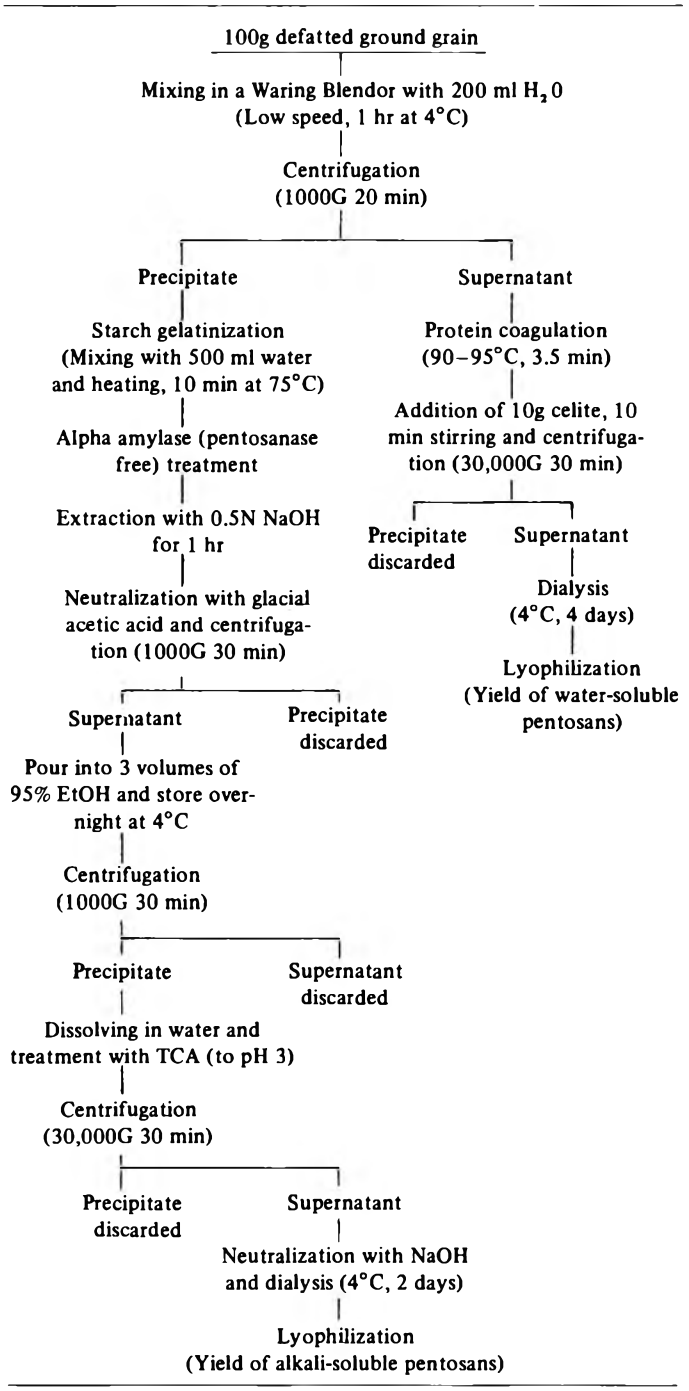

Fig. 1—Outline of preparation and purification of water-soluble and alkali-soluble pentosans from sorghum grain.

(1967) fractionated pentosans from wheat flour and reported 0–6% nitrogen in the various fractions.

Paper chromatography was used to identify galactose, glucose, arabinose and xylose in the hydrolysates of water-soluble and alkali-soluble pentosans obtained from the whole kernel, endosperm and pericarp fractions of sorghum grain (Table 2). The presence of these four sugars in the pentosans of cereal grains has been reported previously (Bechtel et al., 1954; Cartano and Juliano, 1970). The presence of ribose and mannose in barley pentosans has been reported (Luchsinger et al., 1958; Preece and Mackenzie, 1952a); but, these sugars were absent in our sorghum pentosans.

Glucose was the predominant sugar found in sorghum pentosans while galactose was present in smaller amounts (Table 3). This agrees with the results of Cartano and Juliano (1970) and Preece and Mackenzie (1952b) working with other grains. Pentosan preparations from endosperm contained more glucose than those from whole grain and pericarp. This was reflected in the pentose:hexose ratio of these preparations. Similar results were obtained by Preece and Mackenzie (1952b) working with barley and maize.

Glucose is absent in rice bran pentosans (Cartano and Juliano, 1970) and corn pericarp pentosans (Wolf et al., 1953). Unlike pentosans from rice bran and corn pericarp, both water-soluble and alkali-soluble pentosans from sorghum pericarp contained significant quantities of glucose.

Both the water-soluble and alkali-soluble pentosans from sorghum pericarp contained a higher proportion of xylose and arabinose than those obtained from whole grain and endosperm.

Both water-soluble and alkali-soluble pentosans from various parts of sorghum grain had the same constituent sugars. However, water-soluble pentosans contained more arabinose than xylose compared to the alkali-soluble preparation. Similar results were obtained by Cartano and Juliano (1970) working with milled rice.

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Ms received 8/20/71; revised 1/10/72; accepted 1/16/72.

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QUANTITATIVE DETERMINATION OF THE OLIGOSACCHARIDES IN DEFATTED SOYBEAN MEAL BY GAS-LIQUID CHROMATOGRAPHY

INTRODUCTION

THE OLIGOSACCHARIDE composition in soybean meal has been the subject of many investigations. Either paper chromatography, thin layer chromatography, column chromatography or gel filtration was employed previously to measure the relative percentages of the carbohydrates present (Kawamura, 1967a, b; Hardinge et al., 1965; De Stefanis and Ponte, 1968; Anon., undated). All of these methods are time-consuming and require some skill and experience to produce reliable results.

In the course of our work on soybean meal, it was necessary to analyze many samples as quickly as possible. A simple and rapid gas-liquid chromatographic method was developed to make it possible to analyze at least 20 samples/man/8-hr shift.

MATERIALS & METHODS

Sample preparation

10g defatted soybean meal was diluted to 50 ml by adding 46 ml of deionized water. The mixture was agitated for 15 min with a magnetic stirrer. About 3 ml of the suspension was poured into a test tube and centrifuged for 5 min in a clinical centrifuge. 1 ml of the supernatant was added slowly with agitation to 2 ml of a 1.8% barium hydroxide solution, followed by 2 ml of a 2% zinc sulfate solution. After standing for 5 min, the tube was centrifuged for 5 min. 1 ml of the supernatant was lyophilized to near dryness. 1 ml of Tri-Sil 'Z' (Pierce) was added to the tube and the silylation was completed in 1 hr at 65°C.

Analysis

The analysis was carried out using a F&M Model 500 gas chromatograph. The column consisted of 3 ft of stainless steel tubing, ¼ in. diam, packed with 3% OV-1 on Chromosorb W (HP) 80/100 (Pierce), conditioned with Silyl-8 (Pierce) at 200°C. The carrier gas was helium, flowing at 30 cc/min. The oven temperature was set at 150°C and raised after injection to 340°C. The column heater voltage was adjusted to 90v to bring the temperature to 340°C in 10 min. The injection temperature was 350°C and the thermo-conductivity detector temperature was 350°C. The injection volume was 10 μl.

RESULTS & DISCUSSION

Extraction procedure

Water extraction at 25°C was compared with hot water, 80% ethanol and with the method used by Kawamura (1967a, b): after defatting the sample, he refluxes it 1 hr in 10 parts of 80% EtOH, filters, centrifuges the filtrate and adds 10 parts of water to the solids. He stirs for 30 min, filters and washes until negative anthrone reaction of the washing. The extracts and washings are combined and concentrated below 40°C. The variation in the results was ± 5%. Since the cold water extract yielded slightly higher recoveries than the other three and because it is the quickest, this method of extraction was adopted. However, it should be emphasized that this study was concerned only with fully defatted soybean meal. Another extraction method may be required for partially defatted or whole soybeans.

Lyophilization to complete dryness was not pursued in order to gain time. The sample was simply dried to a highly viscous, syrupy consistency or to a wet powder in 100–120 min. The small quantity of water left did not affect the silylation results.

Silylation

The silylation of all the sugars in this study was considered to be complete since known concentrations of the pure species all fit the same standard curve (Fig. 1). In addition, the data obtained by this method corresponded very well to the values reported in the literature. No decomposition of the trimethylsilyl sugars was observed when working with pure sugars or mixtures of pure sugars (Fig. 2). This indicates that the peaks of galactose, melibiose and manninotriose are real and do not represent artifacts. Silylation was

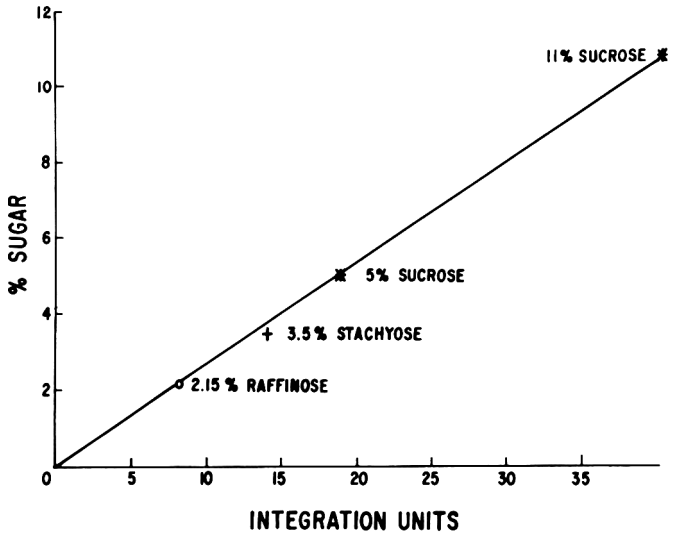

Fig. 1—Standard curve for peak integration.

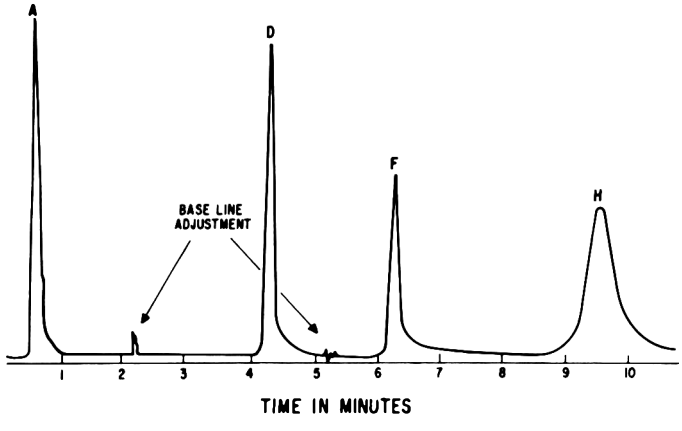

Fig. 2—Chromatogram of a known solution of pure sugars (see Table 1).

Table 1–Recovery of added stachyose from soybean meal

Stachyose added (30% H_2O)	Total stachyose	Yield (anhydrous stachyose)
0	4.6%	–
10 mg/g	5.2%	86%
20 mg/g	6.0%	100%
50 mg/g	8.2%	103%

Table 2–Quantitation of chromatograms in Figures 2, 3 and 4

		Fig. 2		Fig. 3		Fig. 4	
Peak	Compound	Atten	Conc %	Atten	Conc %	Atten	Conc %
A	Pyridine	1,024	–	1,024	–	–	–
B	Galactose	–	–	1	0.4		
						8	14
C_1C_2	Glucose	–	–	1	< 0.1	Monosaccharides	
D	Sucrose	8	11	8	10	–	–
E_1E_2	Melibiose	–	–	8	0.5	–	–
F	Raffinose	2	2.15	1	1	–	–
G_1G_2	Mannino-triose	–	–	1	< 0.1	–	–
H	Stachyose	2	3.5	1	4.6	–	–

found to be a very good method for volatilizing the soybean oligosaccharides since the main sugars do not form anomers.

Peak identification

Sucrose, raffinose and stachyose were identified by comparing retention times with those of pure sugar solutions. Galactose and glucose were identified by adding 0.1g of one of the sugars to 10g of soybean meal and observing peak coincidence and height increase. Melibiose and manninotriose were identified by observing an increase of the peaks when raffinose and stachyose in soybean meal were hydrolyzed with 10 units/g of yeast invertase for 1 hr at 55°C, pH 5.

Quantitation

The peaks were automatically integrated with a disk integrator and the areas compared with those of a standard solution (Fig. 1).

Although anhydrous sucrose was used for the standard curve, it was found that raffinose and stachyose could also be used (Fig. 1). When known amounts of stachyose were added to soybean meal, recoveries of essentially 100% were obtained (Table 1). The water in crystalline stachyose was determined by an anthrone test (Vomhof et al., 1966). The results of one analysis of a sample obtained from a local feed store are shown in Figure 3 and Table 2. A sample from Ralston-Purina was analyzed 11 times with the following results (mean percentage ± deviation): galactose 1.0 ± 0.1; sucrose 8.9 ± 0.4; raffinose 0.90 ± 0.1; and stachyose 4.2 ± 0.2. Trace amounts (about 0.1%) of glucose and manninotriose were found. Although verbascose has been reported previously (Kawamura, 1967a, b), no peak eluted from the column after the stachyose peak in the samples we analyzed.

Let us compare our results with those published previously in the literature. Kawamura (1967a, b) analyzed the defatted cotyledon, the hull and the defatted hypocotyl of six American varieties and three Japanese varieties. Our soybean meal samples could be compared to the defatted cotyledon, for which Kawamura gives the following average composition of six American varieties: sucrose 6.2%, raffinose 1.4% and stachyose 5.2%.

Our samples showed more sucrose and less α-galactosides. Unfortunately, we were not able to analyze reproducibly our samples by paper chromatography. This leaves open to question that the peaks derived from the extract represent only the sugar indicated. An indirect confirmation of identity of the peaks was found when the method was used to study the enzymatic conversion of soybean meal oligosaccharides: the sucrose peak disappears completely in 45 min when invertase hydrolyzes it; when a mixture of invertase and α-galactosidase is used, the oligosaccharides disappear completely and are quantitatively converted to monosaccharides. Table 3 shows the results obtained by hydrolyzing the soybean meal oligosaccharides with a crude yeast enzyme mixture at a concentration of 10 units of α-galactosidase and 100 units of invertase/g of soymeal. The temperature was 55°C and the pH 5.5. A chromatogram of soybean meal extract after 75 min is shown in Figure 4. It shows that the only peaks of any consequence come before 4 min and are monosaccharides: fructose, galactose and glucose, not completely separated. We found the method easy to use. Personnel could be trained to learn it in 2 days. One sample could be analyzed in 4 hr. This

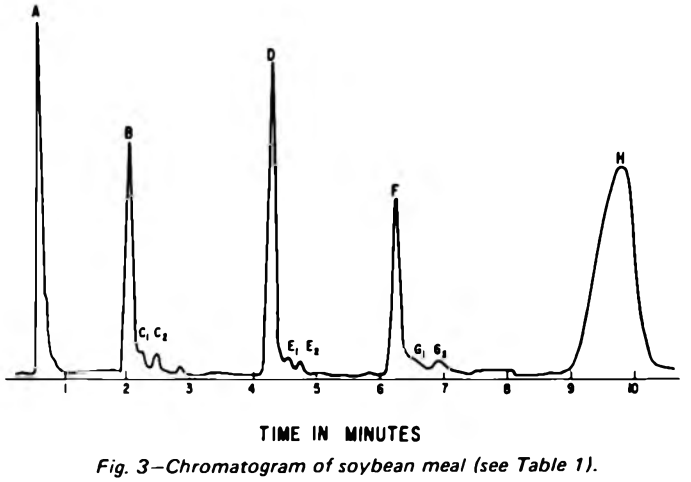

Fig. 3–Chromatogram of soybean meal (see Table 1).

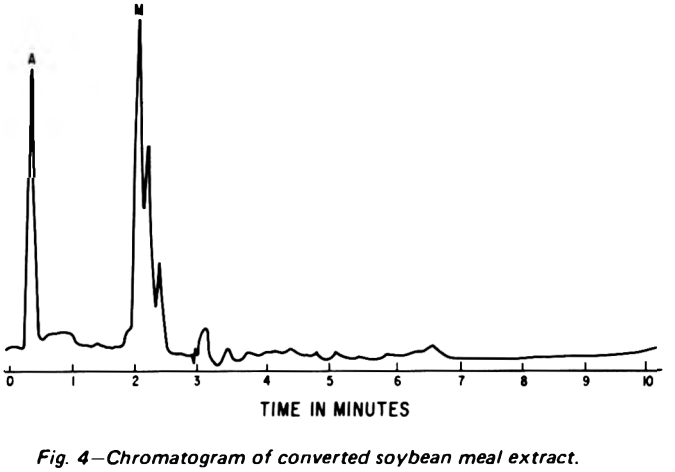

Fig. 4–Chromatogram of converted soybean meal extract.

Table 3—Hydrolysis of soymeal oligosaccharides with invertase and α-galactosidase mixture

% Sugar	Time 0 time	15 min	45 min	75 min
Monosaccharides	0.9	8.0	11	14
Sucrose	8.2	0.5	Together: 1.5	Trace
Melibiose	0.5	1.0		Trace
Raffinose	1.0	0.8	Trace	Trace
Manninotriose	Trace	1.0	0.8	Trace
Stachyose	4.7	3.1	0.5	Trace
Total	14.8	14.4	13.8	14.0

compares with about a week, as estimated from Kawamura (1967a, b), for paper chromatography. On a routine basis, we were able to analyze 20 samples per manshift. After 1 month of daily use, averaging 50 samples/wk, we found it necessary to repack the column, as the peaks were getting less sharp and the retention times shorter.

CONCLUSION

A RAPID METHOD has been developed to analyze oligosaccharides in soybean meal. One sample can be analyzed in 4 hr and during an 8-hr shift one man can analyze 20 samples. The method also can be used to follow the enzymatic conversion of soybean meal oligosaccharides. With a modification in the extraction procedure, it could probably be used for whole soybeans.

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Ms received 11/14/71; revised 1/18/72; accepted 1/9/72.

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AMINO ACID COMPOSITION OF SELECTED POTATO VARIETIES

INTRODUCTION

THE POTATO TUBER is commonly considered an "energy food" but its value as a protein source is not fully appreciated. Although the protein content of the tuber on a fresh-weight basis is only 2.1% (Watt and Merrill, 1963), the content on a dry-weight basis is 10.3% (this study), close to the 11.8% of wheat flour (Watt and Merrill, 1963). Furthermore, two acres of potatoes can supply as much protein as three to four acres of wheat (based on yields reported by Wittwer, 1969). The biological value of potato protein is high. Kofranyi and Jekat (1965) evaluated the protein of various foods by establishing the amount from each food needed to maintain nitrogen balance in three human subjects and found that the daily protein requirements in grams per kilogram body weight were: whole egg protein 0.50; potato protein 0.55; and milk protein 0.57. The same authors also demonstrated that mixtures of potatoes with eggs or milk or beef decreased the total protein requirements, e.g., one subject required 0.47g/kg of either egg or potato protein alone, but only 0.35g/kg of a mixture made of 65% potato protein and 35% egg protein.

Because the biological value and the amino acid composition of a protein are related, a number of analyses have been made on the concentration of a number of amino acids, free or bound as protein, in potatoes (Slack, 1948; Lyman and Kuiken, 1949; Hirsch et al., 1952; Schuphan, 1960; Bontscheff, 1965; Schwerdtfeger, 1969). In the present study a quantitative determination of 18 amino acids was attempted on one potato cultivar and five clonal selections of particular interest to the breeding program carried on in this Institution. The technique used provides a better estimate than earlier ones of the sulfur-containing amino acids, which limit the biological value of potato protein.

MATERIALS & METHODS

RANDOM SAMPLES, 15 lb each, of the established potato cultivar Russet Burbank (RB) and the clonal selections #58, #321-65, #322-6, #709 and #711-3 were obtained from the Crop and Soil Science Dept. of Michigan State University. All six varieties were grown in Montcalm sandy loam soil and fertilized with 128 lb N, 192 P_2O_5 and 192 lb K_2O per acre in 1969. The tubers harvested were stored at 4°C until March 1970. They were then thinly sliced, quickly frozen with dry ice and freeze-dried at a platen temperature of 38–80°C in a vacuum of less than 5μ.

The freeze-dried slices were broken up, passed through an 8 mesh sieve to remove peel and scar tissue, and stored at −18°C. Just before analysis, small samples were ground to pass an 80 mesh sieve and determinations were made as follows:

Total solids content

Total solids content was determined by drying in a vacuum oven at 70°C to constant weight.

Total nitrogen

Total nitrogen was determined by the AOAC (1970) micro-Kjeldahl method. The percent Kjeldahl N multiplied by 6.25 was indicated as "percent total protein."

Total amino acid analysis

Freeze-dried samples, 25 mg each, were weighed into 10 ml ampules, 5 ml of glass-redistilled 6N HCl was added and the mixture was frozen in a dry ice-ethanol bath. The ampules were then connected to a high vacuum line and their contents allowed to thaw slowly. On completion of the thawing the ampules were heat-sealed while still connected to the vacuum. The acid hydrolysis was allowed to proceed in an oil bath at 110°C for 22 hr in one analysis and 72 hr in another. After hydrolysis, 1 ml of 2.5M norleucine solution was added to each ampule as an internal standard indicating transfer losses. The hydrolysate and standard were evaporated to near-dryness under reduced pressure at 55°C. To remove the remaining HCl from the residue, a few ml water were added and the solution was evaporated. This procedure was repeated twice and the final residue was suspended in 5 ml 0.067M citrate-HCl buffer, pH 2.2. The suspension was centrifuged and 0.4 ml of the supernatant was analyzed for amino acid with a Beckman Model 120C amino acid analyzer. The chromatographic separation time was 55 min for the short column and 185 min for the long column. The resulting chromatograms were quantified by comparison with those obtained from a standard amino acid calibration mixture.

Sulfur-containing amino acids

Acid hydrolysis of proteins in the presence of large quantities of carbohydrates results in low analytical values for the sulfur-containing amino acids (Schram et al., 1953). Oxidation of cystine + cysteine to cysteic acid and of methionine to methionine sulfone by performic acid before hydrolysis prevents the loss of these amino acids (Schram et al., 1954; Lewis, 1966). Performic acid was prepared by mixing 1 vol of 30% hydrogen peroxide with 9 vol of 88% formic acid and letting the mixture stand 1 hr.

Table 1—Total amino acid composition of six potato varieties[a]

	Varieties							
Amino acids	RB[b]	#58	#321-65	#322-6	#709	#711-3	Avg	Std dev
Lysine	5.3	5.7	6.6	7.0	6.5	6.1	6.2	0.63
Histidine	2.3	2.4	2.1	2.1	2.3	2.4	2.3	0.14
Arginine	4.9	4.9	4.8	5.1	4.9	5.1	4.9	0.12
Aspartic acid	28.9	29.0	18.6	22.2	23.3	24.9	24.5	4.03
Threonine	3.6	3.4	4.9	4.2	3.5	3.4	3.8	0.60
Serine	4.3	4.0	5.7	5.0	3.7	3.5	4.4	0.84
Glutamic acid	15.9	17.0	13.2	13.3	16.9	16.6	15.5	1.77
Proline	3.0	3.0	4.7	3.8	3.3	4.8	3.8	0.82
Glycine	2.9	2.4	3.6	3.4	3.1	2.5	3.0	0.48
Alanine	2.3	2.8	3.6	3.4	3.3	3.1	3.1	0.47
Cystine + Cysteine	1.2	1.1	1.5	1.4	1.4	1.1	1.3	0.17
Valine	5.8	5.4	5.5	5.9	5.7	6.0	5.7	0.23
Methionine	1.6	1.8	1.4	1.4	1.7	1.6	1.6	0.16
Isoleucine	3.9	3.6	4.8	4.4	4.3	4.2	4.2	0.41
Leucine	4.8	5.0	7.9	6.8	6.1	5.6	6.0	1.17
Tyrosine	3.7	3.4	4.2	3.9	3.7	3.4	3.7	0.31
Phenylalanine	3.9	3.9	5.2	5.1	4.7	4.3	4.5	0.57
Tryptophan	1.7	1.3	1.7	1.6	1.6	1.4	1.5	0.16

[a]Grams of amino acid per 16 grams of total nitrogen
[b]Russet Burbank

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10 ml of performic acid, chilled to 0°C, and 600 mg of freeze-dried potatoes were mixed and left at 0°C for 16 hr. When oxidation was complete, ice-cold water, 20 ml, was added and the mixture was again freeze-dried. The oxidized samples were then hydrolyzed with 6N HCl at 110°C for 22 hr and subjected to amino acid analysis as described for the non-oxidized samples.

Tryptophan

Tryptophan, which is destroyed during acid hydrolysis, was determined colorimetrically after hydrolysis by pronase as described in "Procedure W" by Spies (1967).

RESULTS & DISCUSSION

THE PER CENT total nitrogen content on a dry weight basis and as an average of two determinations together with the percent total protein (in parenthesis) for each of the six samples is as follows: Russet Burbank 1.70 (10.6); #58 1.96 (12.3), #321-65 1.29 (8.1) #322-6 1.58 (9.9), #709 1.46 (9.1), #711-3 1.90 (11.9) and the average 1.65 (10.3). Results of the amino acid analysis are summarized in Table 1. The values for lysine, arginine, aspartic acid, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine and phenylalanine are averages obtained from the 22- and 72-hr hydrolysates. The data for histidine, threonine and serine are zero time extrapolation values calculated according to Hirs et al. (1954) from the 22- and 72-hr results. Table 1 also shows that for each amino acid no values are higher or lower than the average plus or minus twice the standard deviation.

The values for methionine and for cystine + cysteine are based solely on data obtained by the performic acid method. An average of 52.3% methionine and 62.2% of cystine + cysteine was destroyed during acid hydrolysis (Table 2). The destruction was as high as 70.6% for the former and 71.4% for the latter acids. The wide range and the average destruction of methionine correspond to those reported by Jennings and Lewis (1969) for leaf material.

When the nitrogen contained in all the amino acids, plus the nitrogen of the ammonia peak, was calculated for each variety and compared with the corresponding total nitrogen value, it became apparent that about 20% of the total nitrogen could not be accounted for. This undetermined nitrogen probably was in the form of compounds such as gamma-aminobutyric acid, pyrrolidone-2-carboxylic acid, choline, certain vitamins, amides, purines, pyrimidines and nitrates.

Protein scores

The essential amino acid (EAA) composition of the six potato samples, expressed in mg of each amino acid per g of total nitrogen, appears in Table 3, along with the EAA composition of whole egg, the protein of which is assumed to have the highest biological value among all foods. Tyrosine and cystine + cysteine were included because of their sparing action on phenylalanine and methionine, respectively. The protein scores of these varieties, calculated according to the method suggested by the Joint FAO/WHO Expert Group (FAO/WHO, 1965), are presented in Table 4. These protein scores verified Slack's (1948) suggestion that methionine is the limiting amino acid in potatoes. Tubers of clone #58, which had the highest score, 78, in sulfur-containing amino acids, might prove valuable in the breeding program related to this work. The average protein score for all varieties, based on the limiting amino acids, was 69, which is very near the value of 70 given in the FAO/WHO Report (1965). For comparison, we cite other protein scores from the same FAO/WHO Report: egg 100; cow's milk 60; beef muscle 80; fish 75; oats 70; rice 75; corn meal 45; wheat flour 50; soy flour 70; navy beans 42; peas 60; spinach 90; sweet potato 75; and cassava 40. A comparison of our results with those in other reports appears in Table 5.

Table 2—Loss of methionine and cystine + cysteine content in unoxidized samples vs. oxidized samples of six potato varieties[a]

	Methionine			Cystine + Cysteine		
Varieties	Unoxidized	Oxidized	Loss, %	Unoxidized	Oxidized	Loss, %
Russet Burbank	0.7	1.5	53.3	0.6	1.2	50.0
#58	0.8	1.8	55.6	0.4	1.1	63.6
#321-65	1.0	1.4	28.6	0.6	1.5	60.0
#322-6	0.8	1.4	42.9	0.5	1.4	64.3
#709	0.5	1.7	70.6	0.4	1.4	71.4
#711-3	0.6	1.6	62.5	0.4	1.1	63.6
Average loss, %			52.3			62.2

[a]Grams of amino acid per 16 grams of total nitrogen

Table 3—Essential amino acid composition of six potato varieties and whole egg

	Milligrams of amino acids per gram of total nitrogen							
	Varieties							Whole
Amino acids (AA)	RB[a]	#58	#321-65	#322-6	#709	#711-3	Avg	egg
Isoleucine	244	225	300	275	269	263	263	415
Leucine	300	313	494	425	381	350	377	553
Lysine	331	356	413	438	406	381	388	403
Total "aromatic" AA	475	456	588	563	525	481	515	627
Phenylalanine	(244)	(244)	(325)	(319)	(294)	(269)	(283)	(365)
Tyrosine[b]	(231)	(212)	(263)	(244)	(231)	(212)	(232)	(262)
Total S-cont. AA	175	182	176	176	194	169	179	346
Methionine	(100)	(113)	(88)	(88)	(106)	(100)	(92)	(149)
Cystine + Cysteine	(75)	(69)	(88)	(88)	(88)	(69)	(80)	(197)
Threonine	225	213	306	263	219	213	240	317
Tryptophan	106	81	106	100	100	88	97	100
Valine	363	338	344	369	356	375	358	454
Total essential AA	2219	2164	2727	2609	2450	2320	2417	3215

[a]Russet Burbank
[b]Tyrosine and cystine + cysteine are included in amounts not exceeding those provided by phenylalanine and methionine, respectively. Whole egg data are from FAO/WHO Report (1965).

Table 4—Protein scores of six potato varieties based on the essential amino acid pattern of whole egg

	Varieties						
Amino acid (AA)	RB[a]	#58	#321-65	#322-6	#709	#711-3	Avg
Sulfur cont. AA	73	78	60	62	73	68	69
Isoleucine	85	81	85	81	85	88	85
Leucine	79	84	>100	95	91	88	91
Threonine	>100	99	>100	>100	90	93	100
Valine	>100	>100	89	>100	>100	>100	>100
All others	>100	>100	>100	>100	>100	>100	>100

[a]Russet Burbank

Table 5—Comparison of the total amino acid composition of potatoes reported by various authors[a]

Amino acids	Slack (1948)	Lyman and Kuiken (1949)	Hirsch et al. (1952)	Schuphan (1960)	Bontscheff (1965)	Schwerdtfeger (1969)	This study
Lysine	5.0	5.2	5.5	6.2	5.3	5.4	6.2
Histidine	1.7	1.4	1.5	2.0	1.6	1.5	2.3
Arginine	4.4	5.3	5.0	5.2	5.5	4.0	4.9
Aspartic acid	–	–	–	–	10.3	24.8	24.5
Threonine	3.7	2.5	3.9	4.1	2.6	3.8	3.8
Serine	–	–	–	–	1.6	3.7	4.4
Glutamic acid	–	–	–	–	9.0	15.1	15.5
Proline	–	–	–	–	–	3.1	3.8
Glycine	–	–	–	–	–	3.8	3.0
Alanine	–	–	–	–	–	3.6	3.1
Cystine + cysteine	1.7	–	–	.8	1.9	.5	1.3
Valine	4.8	4.8	5.8	5.3	5.0	4.8	5.7
Methionine	1.6	1.5	1.5	1.6	1.8	1.5	1.6
Isoleucine	11.3	3.7	4.3	6.9	12.8	3.3	4.2
Leucine		4.6	4.8	6.7		6.1	6.0
Tyrosine	–	–	–	–	1.8	2.8	3.7
Phenylalanine	5.4	3.2	4.7	4.5	4.5	4.8	4.5
Tryptophan	.8	1.8	.1	1.6	.8	1.2	1.5

[a]Grams of amino acids per 16 grams of total nitrogen

It may be concluded on the basis of the protein scores we obtained that the protein of potatoes is of high nutritional quality. The human feeding studies of Kofranyi and Jekat (1965) show an even greater nutritional value than we obtained. This could be due to the difference between acid or enzyme hydrolysis in vitro and digestion and absorption in vivo. Because of its high lysine content, potato protein appears to be especially valuable as a supplement for cereal proteins.

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Ms received 12/29/71; revised 3/14/72; accepted 3/16/72.

J. Article No. 5763 by the Michigan Agriculture Experiment Station.

The authors deeply appreciate the cooperation of Professor N.R. Thompson of the Dept. of Crop & Soil Sciences and the technical assistance of Miss Ursula Koch in the amino acid analysis.

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EFFECT OF AMINO ACID SUPPLEMENTATION OF DEHYDRATED POTATO FLAKES ON PROTEIN NUTRITIVE VALUE FOR HUMAN ADULTS

INTRODUCTION

PLANT PRODUCTS are the primary source of dietary protein for most humans throughout the world even though plant proteins usually are of poorer nutritional value than animal proteins for the human (Jansen and Howe, 1964). Potato tubers, although usually of low protein content, may be an important source of dietary protein even among some groups within the United States because of high consumption (Clark, 1970). Current research on isolation of high protein strains of potatoes may make potatoes an even more important source of dietary protein (Jones, 1969). Examination of the amino acid proportionality pattern of potato tuber protein (Orr and Watt, 1957) with the Rose minimum animo acid requirement pattern for young men (Rose, 1957) suggest that the sulfur-containing amino acids are supplied in lowest amount in reference to need. Since it has been demonstrated that lysine is the first limiting amino acid in wheat, corn, triticale, rice, opaque-2 corn, milo and rye, substitution of a part of diets based on these cereals with potatoes might result in improved protein nutriture via the mutual supplementary effect (Kies, 1972).

In spite of the importance of potato tubers in the diets of many humans, controlled laboratory studies on the protein value of this food for the human have not been conducted. Animal feeding studies (rats) indicate the protein nutritive value of potato tubers to be superior to that of rice (Chang, 1969).

The objective of the current study was to determine the first limiting amino acid in dehydrated potato flakes for maintenance of nitrogen equilibrium (adequate protein nutriture) in human adults.

PROCEDURE

Experimental plan

The 33-day study consisted of an introductory 3-day nitrogen depletion, a 5-day nitrogen adjustment period and 5 experimental periods of 5 days each. The experimental periods were arranged at random for each subject to eliminate the variables of time and order of presentation. Thus, order of presentation of dietary periods differed for each subject. The experimental plan is given in Table 1.

During the preliminary nitrogen depletion period, nitrogen intake per subject per day totaled 0.68g as provided by the basal diet composed of wheatstarch, fat, instant coffee and tea, nonprotein bouillon, and a few low protein fruits and vegetables. Purposes of this period included the introduction of subjects to their duties and responsibilities, determination of individual caloric requirements for weight maintenance and utilization of a very low nitrogen diet to speed the adjustment of subjects to the later experimental diets.

During all experimental and adjustment periods, nitrogen intake was maintained at 5.0g N/subject/day–4.0g N from dehydrated potato flakes plus 0.68g N from the basal diet (see Table 1), and systematically variable amounts of nitrogen from the amino acid supplements. Urea was used to maintain diets isonitrogenous at the 5.0g N intake level. This level of total test nitrogen intake has been selected in this laboratory as a standard in studies designed to determine the first limiting amino acids in food proteins for human adults. If subjects are not in negative balance while receiving nonamino acid supplemented diets, the effects of amino acid additions are difficult to demonstrate. No amino acid supplements were used during the nitrogen adjustment period. The purpose of this period was to allow the subjects time to adjust to both the level and source of dietary protein used in each of the studies. During the 5 randomly arranged experimental periods, the following purified crystalline L-amino acids were used individually as supplements: methionine (1.568g/day), leucine (0.956g/day), or phenylalanine (1.096g/day). During one experimental period no amino acid supplements were given (negative control) and during one period, a combination of all three amino acids was given (positive control). Determination of level and kind of amino acid supplements used was made by comparison of the amino acids provided by 4.0g potato tuber nitrogen with human daily need as listed by the Rose Provisional Recommended Intake Pattern of Essential Amino Acids for Young Men (Rose, 1957) as shown in Table 2.

The three amino acids selected for biological testing as first limiting amino acid were those predicted as being first, second and third most limiting on the basis of chemical composition. It would be highly improbable that chemical and biological evaluation would differ so drastically that an amino acid other than one of the first three on the basis of chemical evaluation would be first limiting according to biological assay. Under such unlikely circumstances, no positive response would be achieved by the amino acids tested, thus eliminating the possibility of false results.

Diets

Caloric intake for each subject was kept constant during the experimental periods of each study at the level required for weight maintenance by varying the intake of sucrose,

Table 1–Experimental plan

Period[a]	No. of days	N intake-potato[b] (g N/day)	Amino acid supplement[c]	Total N intake[d] (g N/day)
Depletion	3	0	None	0.8
Adjustment	5	4.0	None	5.0
Expt. 1	5	4.0	None	5.0
Expt. 2	5	4.0	L-leucine	5.0
Expt. 3	5	4.0	L-phenylalanine	5.0
Expt. 4	5	4.0	L-methionine	5.0
Expt. 5	5	4.0	Leu. + Phe. + Met.	5.0

[a]Expt. periods 1–5 randomly arranged for each subject.
[b]Dehydrated "instant" mashed potato flakes
[c]In amounts needed to supplement cereal so that the total intake would meet Rose Recommendations of amino acid intake for young men
[d]Includes N provided by basal diet. Urea used to maintain diets isonitrogenous. The basal diet (per subject per day) consisted of 100g applesauce, 100g green beans, 100g tomato juice, 100g peaches, 100g pears, 3.5g dry bouillon flakes, 10g dry instant decaffeinated coffee and varying amounts of butteroil, carbonated beverages and hard candy. A vitamin supplement provided 5000 IU of Vitamin A (synthetic), 900 IU of Vitamin D and (in milligrams) thiamine, 2; riboflavin, 2.5; ascorbic acid, 50; pyridoxine, 1; niacin, 20; Ca pantothenate, 1; and B_{12}, 1 μg. Mineral supplements provided the following per subject per day: (in grams) Ca, 1.00; P, 1.00; Mg, 0.199; Fe, 0.015; Cu, 0.002; K, 0.00005; I, 0.00015; Mn, 0.002; and Zn, 0.0009. NaCl was allowed ad libitum.

Table 2–Comparison of the essential amino acid composition of dehydrated potato flakes and the amino acid requirements of young men

Essential amino acid	Amino acids in potato flakes[a] (g AA/4g N)	Recommended intake of amino acids[b] (g AA/day)	Percent of minimum requirement supplied by potato flakes (%)
Tryptophan	0.27	0.50	54.0
Threonine	1.08	1.00	108.0
Isoleucine	0.90	1.40	64.3
Leucine	0.98	2.20	44.5[c]
Lysine	1.45	1.60	90.6
Methionine	0.28	2.20	12.7[c]
(Total-s-)	(0.60)	(2.02)	(29.7)
Phenylalanine	1.00	2.20	45.4[c]
Valine	1.50	1.60	93.7

[a]Essential amino acid composition determined by auto-analyzer techniques except for tryptophan. Tryptophan value based on handbook value (Orr and Watt, 1957) for potato tubers.

[b]Rose Provisional Recommended Intake pattern of essential amino acids for young men (Rose, 1957). This represents a doubling of minimum requirement figures.

[c]Selected for supplementation in this study

soft drinks, jelly and butteroil among the subjects. A few low protein fruits and vegetables were part of the daily basal diets. Vitamin and mineral supplements were also included. The dehydrated potato flakes were weighed for each subject for each of the three daily meals. These were reconstituted with boiling water immediately before being served. The purified amino acid and urea mixtures were administered in water solutions at the evening and noon meals. Methods used in the preparation and administration of the other food items were basically the same as described in an earlier paper (Kies et al., 1967).

Laboratory methods

Evaluation of protein nutriture of the subjects was primarily by the nitrogen balance technique. Nitrogen content of the dehydrated potato flakes, other dietary items and excreta were determined according to the boric acid modification of the Kjeldahl method (Scales and Harrison, 1920). Creatinine in urine was analyzed by the method of Folin (1914) in order to check the accuracy of collections. Urinary nitrogen and creatinine excretions were determined daily on each 24-hr collection, and fecal nitrogen data were obtained from 5-day composite collections for each individual. Details are given in an earlier paper (Linkswiler et al., 1958). Amino acid composition (except tryptophan) was determined via analysis with a Technicon amino acid auto-analyzer. Handbook values were used for estimations of tryptophan content (Orr and Watt, 1957).

Statistical analysis of data including analysis of variance and Duncan's Multiple Range Test (nitrogen balance data) were done by the Statistical Laboratory of the Nebraska Agricultural Research Station, University of Nebraska.

Experimental subjects

The seven young men who volunteered to be subjects for these studies were all college students of the University of Nebraska or Nebraska Wesleyan University. Subject description data are given in Table 3. All maintained their usual daily activities in regard to work or study but reported to the human metabolism unit for meals. Health records of all were reviewed by a physician of the Student Health Div. of the University of Nebraska to ascertain the desirability and/or safety of their participation in studies of this type.

Sensory evaluation

A seven member sensory evaluation team was used to evaluate reconstituted dehydrated mashed potatoes to which L-methionine had been added at the 0.0, 0.5, 1.0, 2.0 and 3.0% level (dry weight basis). A 10-point rating scale was used to evaluate product flavor in order to establish the practical upper limit of methionine supplementation from a product palatability standpoint.

RESULTS

MEAN NITROGEN balances of each individual subject as well as collective means for each experimental period are shown in Table 4.

Subjects showed increases in nitrogen retention when methionine was used as a dietary supplement either singly or in combination with leucine or phenylalanine. Neither phenylalanine or leucine supplementation resulted in an improvement in nitrogen balance. Mean nitrogen balances of subjects while receiving the potato diet alone or plus methionine, phenylalanine, leucine or a combination of methionine, phenylalanine and leucine were −1.18, −0.27, −0.91, −0.83 and −0.30g nitrogen per day, respectively.

Analysis of variance indicated significant difference among supplements at the 1% level. Table of least significant differences indicates that only methionine is not significantly different from combined amino acid supplement at the 5% level.

Mean crude protein digestibility of potato protein was 78%. No significant effect of amino acid supplementation on digestibility was demonstrated.

The results of the current project suggest that the protein value of dehydrated potato flakes in human diets could be improved by increasing the methionine content (or total sulfur-containing amino acid content). To achieve this result, various theoretical approaches may be taken. These include genetic selection of potato tubers having a higher methionine content from which to manufacture dehydrated potato flakes, improved processing techniques resulting in less destruction of methionine, addition of purified methionine in the industrial processing of dehydrated potato flakes, or education of

Table 3–Vital statistics of subjects[a]

Subject No.	Age (Yr)	Height (cm)	Weight (kg)
316	18	180	74.5
317	34	168	66.7
318	20	179	68.6
320	18	179	63.6
321	20	188	84.0
322	19	183	80.4
323	20	185	83.5

[a]All subjects were white Americans, male sex

Table 4–Effect of amino acid supplementation on protein value of potato diets for humans

Subject No.	N balance (g N/day) while receiving potato diet plus: No amino acids	Combined amino acids	Methionine	Leucine	Phenyl-alanine
316	−1.01	−0.12	−0.42	−0.73	−1.87
317	−0.48	−0.27	−0.13	−1.34	−0.45
318	−1.18	−0.75	−1.16	−1.26	−1.33
320	−1.44	+0.58	+0.61	−0.13	−0.05
321	−1.50	−0.19	+0.27	−0.38	−0.49
322	−1.87	−1.16	−1.05	−1.42	−1.36
323	−0.79	−0.22	0.00	−0.55	−0.79
Mean	−1.18	−0.30	−0.27	−0.83	−0.91

consumers in useage of desirable food combinations. The discovery of the opaque-2 corn mutant in improvement of the protein value of that cereal for humans has created interest in genetic control as a vehicle for improvement of the nutritional value of other food products (Mertz and Nelson, 1966). Whether or not this is workable in the case of potato tubers is questionable and at best is time consuming. Comparison of handbook values of potato tuber methionine content (Orr and Watt, 1957) with determined values for dehydrated potato flakes are quite similar suggesting that current processing techniques are not particularly destructive to methionine in this further processed food. Consumer education in nutrition is slow and difficult.

Further processed foods such as dehydrated potato flakes are frequently subjected to criticism on the ground that processing results in nutrient destruction. By the very fact that they are undergoing processing in which controlled additions of ingredients can be made, they offer ideal medias for nutrient supplementation, fortification and enrichment. Thus, further processing of foods can be looked at as a nutritional enhancer under special circumstances.

In this project purified L-methionine was fed at a level equivalent to a 0.37% supplementation level (dehydrated potato). Results of sensory panel evaluations indicated that methionine supplementation would be feasible up to 1.0% level (dehydrated potato) without adversely affecting palatability.

Potato tubers and further processed foods derived from them such as dehydrated potato flakes, like many other cereal/plant products, have a double disadvantage as protein resources for the human. In addition to frequently exhibiting limitations in essential amino acid proportionality patterns in comparison to human need, plant products tend to simply have a low protein content. Thus, it is difficult to overcome low content of one essential amino acid by feeding excesses of total protein. If high protein lines of potatoes are developed, it would be feasible perhaps to meet human methionine requirement by feeding large amounts of potato protein. This alternative is not open with a low protein product because of practical limitations on human consumption. Therefore, another method for improving the protein value of potato tubers and products produced from them such as dehydrated potato flakes could be achieved by simply increasing the total protein content even though methionine content per gram potato protein remained unchanged or was even slightly lowered.

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Ms received 12/19/71; revised 3/10/72; accepted 3/16/72.

Paper no. 3302, Journal Series, Nebraska Agricultural Experiment Station. The work reported was conducted under Nebraska Agricultural Experiment station project no. 91-7.

The authors thank and acknowledge the cooperation and support of the seven young men who served as subjects for this research project. The technical assistance of the following individuals is also acknowledged: Mrs. Elsie Bishop, Mrs. Marge Kunzman, Mrs. Mary Rhodes, Mrs. Deanna Sivers, Mrs. Jean Malzer and Mrs. Evelyn Coufal.

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PEROXIDASE LOCALIZATION AND LIGNIN FORMATION IN DEVELOPING PEAR FRUIT

INTRODUCTION

THE PRESENCE of sclereids, "grit cells" or "stone cells" in the pulp of pear fruit imparts a characteristic texture. Deposition of large numbers and sizes of these cell aggregates has long been known to lower the quality of fruit (Crist and Batjer, 1931). Sclereids may proliferate as a species characteristic or as a consequence of adverse growing conditions (Ryugo, 1969). The intractable nature of sclereids led Crist and Batjer to suggest they were chemically similar to the lignin which occurs in wood pulp. Recently, sclereid tissue has been definitively identified as lignocellulosic in nature (Ranadive and Haard, 1972).

Peroxidase is thought to be one of the principal enzymes functioning in the deposition of lignin on vascular tissue (Brown, 1969; Siegel, 1956). While other enzymes participate in lignin biosynthesis, evidence points to the peroxidase catalyzed reaction as an important site of control (Freundenburg, 1966; Siegel, 1957). Studies of lignin formation in vascular regions of *coleus* indicated that peroxidase was localized in the secondary and primary cell wall regions where lignin deposition occurs (Helper et al., 1970). Lipetz and Garro (1965), working with crown gall tissue cultures, observed that the presence of Ca^{++} caused release of wall bound peroxidase and a consequent decrease in lignin deposition.

Kieffer pears, which contain a large number of sclereids, were shown to contain more peroxidase than Bartlett fruit (Crist and Batjer, 1931). More recently, Ryugo (1969) noted that catalase and peroxidase of *Pyrus serotina* were concentrated in tissue regions where sclereids developed.

The above findings have led us to examine the peroxidase activity and localization in pear varieties containing wide differences in stone cell development.

MATERIALS & METHODS

Source of fruit

Two pear varieties, Bartlett (*Pyrus* Communis, L. Bartlett) and an interspecific hybrid between *Pyrus serotina* and *P. communis* (Rutgers University, Experimental Variety 500371746) were used in these studies; the latter exhibited symptoms of "Yuzuhada disorder" as manifested by excessive sclereid development and contained relatively low levels of free phenolic substances (Ranadive and Haard, 1971). This variety will be referred to as "Yuzuhada fruit." Fruit were collected at weekly intervals for 7 wk prior to full maturity during the 1970 growing season. Mature fruit were harvested at a Ballauf pressure test of 15 lb. Fruit were ripened in a ventilated chamber maintained at 20°C and 80–90% relative humidity after 4–8 wk storage at 5°C.

Peroxidase isolation

A 20g aliquot of pulp was sampled from four peeled and cored fruit. The pulp was sliced to 1 mm thickness and vacuum infiltrated with 50 ml isolation medium utilizing a water aspirator. The isolation medium contained insolubilized polyvinylpyrrolidone (Polyclar-At, GAF Corp.), 0.75g; sodium maleate, 5 mmoles; and as variables, $CaCl_2$, 0–15 mmoles; and Na_3PO_4, 0–15 mmoles in 50 ml and was adjusted to pH 6.0. The infiltrated tissue and isolation medium were macerated for 1 min at high speed in a Serval Omnimix blender. The homogenate was passed through "Rapid Flo" filter discs (Johnson & Johnson, Chicago), and the filtrate was centrifuged for 10 min at 28,000 × G. All operations were performed at 4°C. The clear supernatant was made to 100 ml volume with 0.1M sodium maleate, pH 6.0 and assayed.

Peroxidase assay

Peroxidase (EC 1.11.1.7) was assayed by a method utilizing o-dianisidine as H-donor (Anonymous, 1968). Absorbancy change at 460 nm was recorded with a DB-G spectrophotometer. The initial slope (ΔOD/min) was calculated from the linear portion of the curve. One unit of peroxidase activity was that amount of enzyme decomposing 1 μmole of H_2O_2 per minute at 25°C. Peroxidase activity was expressed as units per milligram N in the sample assayed. Total nitrogen was determined by a micro Kjeldahl (Umbreit et al., 1964).

Histochemical assay of peroxidase

The localization of peroxidase in cross sectional slices of pulp was judged histochemically by incubating tissue in the o-dianisidine assay medium or a water saturated solution of benzidine containing 0.03% H_2O_2. Development of color was followed with a binocular microscope.

Polyacrylamide gel electrophoresis

Peroxidase isozymes were resolved on polyacrylamide gels with Tris-glycine buffer (pH 8.8) according to Ornstein and Davis (1962). Electrophoresis was at 4 ma/tube for 45 min. Soluble peroxidase was prepared from pulp samples as described in the materials and methods section. The sedimented residues of homogenates were re-extracted with isolation media containing 0.1M $CaCl_2$ to obtain the bound enzyme in soluble form. Cell-free extracts containing peroxidase were dialyzed against 100 volumes of distilled water for 12 hr at 4°C. Dialyzates were made 40% in sucrose

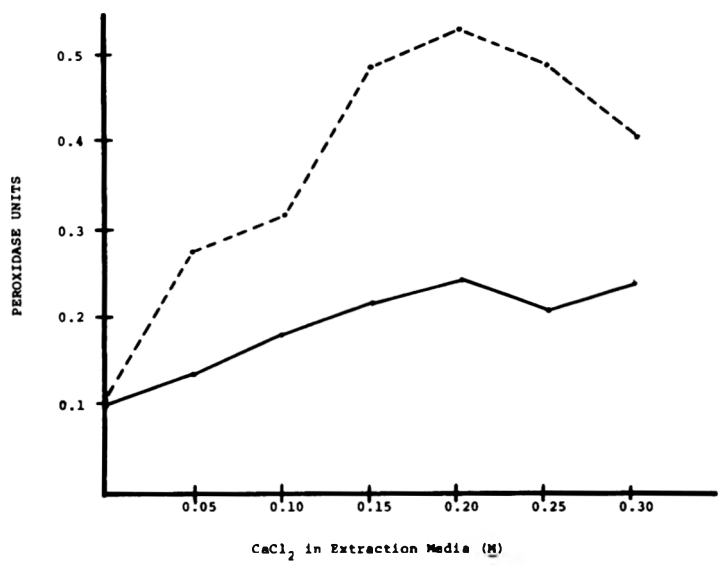

Fig. 1—Effect of calcium chloride on peroxidase release from pulp tissue of Bartlett (——) and Yuzuhada (----) pear fruit. Results are average of duplicate experiments.

Table 1–Soluble peroxidase[a] activity of developing fruit

Days before harvest	Peroxidase activity[b]	
	Bartlett	Yuzuhada
21	0.65	0.39
14	0.81	0.29
7	1.08	0.37
0	1.18	0.59

[a]Peroxidase extracted with 0.025M sodium maleate and 1.5% w/w Polyclar-At. Results are an average of duplicate experiments and are representative of experimental data obtained during a second growing season.
[b]ΔOD @ 460 nm/min/mg N at 25°C

Table 2–Total peroxidase[a] of developing fruit

Days before harvest	Peroxidase activity[b]	
	Bartlett	Yuzuhada
35	1.19	0.62
28	1.28	1.18
21	0.84	0.82
14	0.89	0.52
7	1.10	0.63
0	1.18	0.82

[a]Peroxidase extracted with .025M sodium maleate, 1.5% Polyclar-At and 0.1M $CaCl_2$. Results are an average of duplicate experiments and are representative of experimental data obtained during a second growing season.
[b]ΔOD @ 460 nm/min/mg N at 25°C

Table 3–Bound peroxidase[a] of developing fruit

Days before harvest	Peroxidase activity[b]	
	Bartlett	Yuzuhada
21	0.19	0.43
14	0.09	0.23
7	0.02	0.26
0	0.00	0.23

[a]Peroxidase extracted with 0.025M sodium maleate, 1.5% Polyclar-At and less peroxidase recovered with medium not containing $CaCl_2$. Results are an average of duplicate experiments and are representative of data obtained during a second growing season.
[b]ΔOD @ 460 nm/min/mg N at 25°C

and a 0.1-ml sample (0.1–0.2 mg protein) was applied to the gel. Isozymes were indicated by incubating gels in o-dianisidine assay medium.

RESULTS & DISCUSSION

Extraction of peroxidase

The extraction medium containing sodium maleate (0.1M, pH 6.0) and Polyclar-At (0.75%) gave consistent levels of peroxidase in cell-free extracts prepared from pear fruit. Pulp extracts from ripe Bartlett and Yuzuhada fruit contained similar activity, at 1.02 and 0.88 units per mg N, respectively. Polyclar-At, a scavenger of free phenolic substances, prevented extensive browning of extracts.

Histochemical analysis

Sections of pulp tissue underwent extensive darkening when incubated in the o-dianisidine-H_2O_2 assay solution or in benzidine-H_2O_2. Peroxidase activity appeared to be concentrated in the parenchyma cells surrounding grit cells and on cell walls throughout the pulp. Reaction was most evident in the core and cortical areas where grit cells were most abundant. In addition, pellet residues obtained after extraction and washing with maleate buffer, contained significant levels of bound peroxidase when judged by histochemical assay.

Effect of calcium on peroxidase recovery in cell-free extracts

Increasing the concentration of $CaCl_2$ present during tissue maceration and centrifugation facilitated greater release of peroxidase in the resulting supernatant fraction. In both pear varieties, enzyme activity increased with increased Ca^{++} to a concentration approaching 0.2M and declined at higher levels up to 0.3M (Fig. 1). Preliminary studies showed that other salts, such as $MgCl_2$, were also effective in this capacity, while increasing the concentration of Na_3PO_4 to 0.2M had little efficacy in releasing the enzyme from the particulate fraction. Addition of equimolar $CaCl_2$ to supernatants after separation from the particulate fraction had no appreciable influence on the activity of the preparation. The salt was facilitating release of bound peroxidase and was not acting by activating the soluble enzyme. Recently, Whitmore (1971) observed that $Ca(NO_3)_2$ elicited release of bound peroxidase from wheat coleoptiles.

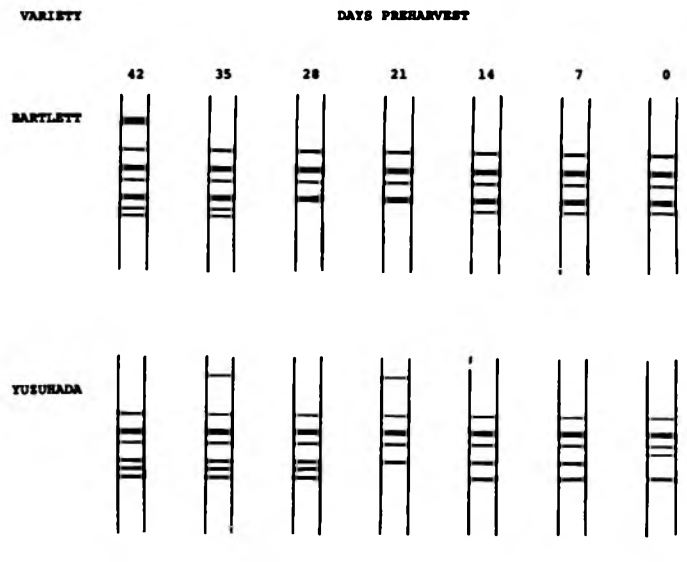

Fig. 2–Diagrammatic representation of electrophoretic profiles of isoperoxidases extracted from Bartlett and Yuzuhada fruit. Similar patterns observed for "soluble" and "bound" peroxidase fractions.

Changes in soluble and wall-bound peroxidase with maturation

The soluble peroxidase activity obtained from developing Bartlett fruit extracted with maleate buffer (exogenous Ca^{++} absent) was consistently higher than for that from Yuzuhada fruit during the final 4 wk of maturation (Table 1). Both varieties showed an overall increase in activity at maturation. When pulp was extracted with a medium containing 0.1M $CaCl_2$, to obtain a measure of total extractable peroxidase, the results shown in Table 2 were obtained. Total peroxidase activity recovered in extracts was consistently higher in those obtained from Bartlett fruit. Estimation of wall bound-peroxidase was done by subtracting soluble peroxidase from total peroxidase (Table 3). The data shows that a portion of the peroxidase remained bound to the particulate debris of homogenates in Yuzuhada throughout maturation. Although total peroxidase was consistently higher in Bartlett fruit, the wall-bound peroxidase was most pronounced in Yuzuhada fruit. Histochemical analysis of peroxidase was also consistent with the conclusion that wall-bound peroxidase was higher in Yuzuhada fruit. Tissue residues which were extracted with media containing 0.2M $CaCl_2$ exhibited negligible peroxidase activity by histochemical analysis.

Table 4—Calcium content of maturing pears

Days before harvest	Calcium (ppm)[a]	
	Bartlett	Yuzuhada
41	48	16
34	56	30
27	68	32
21	56	40
14	54	36
7	48	36
0	52	52

[a]Calcium was determined by atomic absorption spectrophotometer on pulp; results are the average of duplicate experiments.

Gel electrophoresis

The electrophoretic profiles of peroxidase isozymes obtained from Bartlett and Yuzuhada varieties are shown in Figure 2. Isozyme patterns did not change significantly during maturation of fruit and were identical for bound and soluble preparations of peroxidase. The data indicate that peroxidase localization rather than total content or isozyme distribution may affect lignin deposition.

Calcium content of pulp

The calcium content of pulp obtained from Bartlett and Yuzuhada varieties is shown in Table 4. The lower calcium levels in Yuzuhada fruits throughout early maturation were consistent with the greater extent of wall-bound peroxidase.

The data presented are consistent with the thesis that mineral nutrition (Ca^{++} and perhaps other ions) delimits peroxidase localization and accordingly, the control of peroxidase catalyzed lignin deposition in pear fruit.

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Ms received 12/3/71; revised 3/10/72; accepted 3/16/72.

Presented at the 31st Annual Meeting of the Institute of Food Technologists in New York.

Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University—The State University of New Jersey, New Brunswick, NJ 08903.

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THE APPLICATION OF AMINO ACID COMPOSITION TO THE CHARACTERIZATION OF CITRUS JUICE

INTRODUCTION

THE PROBLEM of determining juice content or authenticity of citrus-based products has been of major concern to citrus processors and governmental agencies for a long time. Of the numerous systems proposed and tried over the years, many have involved the amino acids in one form or another. They have several advantages over other constituents such as sugar, acid, or minerals in that they cannot be cheaply or easily added to compensate for a diluted product.

Underwood and Rockland (1953) did much of the early work on the qualitative identification and estimation (Rockland and Underwood, 1956) of the citrus amino acids. Ting and Deszyck (1960) studied the proportions of proline and the ninhydrin blue amino acids of Florida orange juice. Clements and Leland (1962) made a preliminary survey of the amino acid composition of six citrus varieties. Alberola and Primo (1969) made a study of the gas chromatographic separation of amino acids in orange juice, fermentation citric acid and sugar to detect adulterations by changes in the amino acid pattern. In that paper they also presented an excellent literature review of citrus amino acid work. From the same laboratory Aranda et al. (1969) studied the amino acid composition of Spanish varieties of orange juice by thin-layer chromatography. Recently Elahi and Khan (1971) studied the free amino acids of Pakistani citrus fruits and made some quantitative estimations by paper chromatography. Lifshitz and Stepak (1971) investigated the individual amino acids of fresh Israeli lemon juice. Early work in this laboratory (Vandercook et al., 1963) indicated that the total amino acids of lemon juice were useful in characterizing the authentic products. The purpose of this work was to study the composition of the individual amino acids of commercial citrus juices with the aim of determining their usefulness in detecting adulteration and in estimating juice content.

Table 1—Amino acid composition of orange and lemon juices

	Orange Juice (27 samples)		Lemon Juice (35 samples)	
	Mean[a]	S.D.	Mean[a]	S.D.
Aspartic acid	6.5	2.0	16.6	4.6
Asparagine	6.9	1.7	9.8	2.5
Serine	4.9	0.7	9.8	1.4
Glutamic acid	2.1	0.3	5.3	0.7
Glutamine	0.5	0.1	0.3	0.2
Proline	44	6	30	5
Alanine	3.2	0.6	6.5	1.2
Cystine	–	–	0.3	0.2
Valine	0.50	0.12	0.7	0.2
Methionine	0.12	0.08	0.2	0.1
Isoleucine	0.18	0.07	0.3	0.1
Leucine	0.24	0.08	0.4	0.1
Tyrosine	0.33	0.08	0.2	0.2
Phenylalanine	0.71	0.22	0.8	0.2
γ-Aminobutyric acid	17	4	7.2	1.6
Lysine	1.2	0.4	0.4	0.2
Histidine	0.22	0.09	0.2	0.1
Ammonia	1.5	0.9	8.5	3.5
Arginine	10.4	3.1	1.0	0.8
°Brix	12.3	0.5	–	–
Total amino acids (formol)[b]	2.54	0.59	1.91	0.30
Citric acid[b]	13.5	2.1	87.5	11.5
Total phenolics[c]	0.694	0.086	0.625	0.172

[a]Amino acids reported in mol percentages of the total amino acids
[b]Meq/100 ml
[c]Absorbance at 330 mμ of 1:20 dilution with ethanol

EXPERIMENTAL

Equipment

The buffer pumping system (Hare, 1966) consisted basically of a ¼ in. × 100-ft coil of plastic tubing which held the eluting buffers. The buffers were forced through the column with an air pressure of about 55–60 psi. The buffers passed through a Chromatronix sample injection valve (SV-8031) with a 0.25 ml sample loop onto a Chromatronix column (LC-9M-29). For the neutral and acidic amino acids a 0.9 × 55 cm column of Aminex Q-150S (BioRad, Berkeley, Calif.) was used. A 0.9 × 15 cm column of the same resin was used for the basic amino acids. Both columns were water jacketed and heated to 50°C. The flow rate for both columns was 55–58 ml/hr. The column effluent was split, and a portion was sampled by a Technicon Pump II. The sample, 0.67% buffered ninhydrin solution, and 0.002M hydrazine sulfate (Technicon, 1968) were mixed in the pump manifold and passed through a 40-ft coil in the heating bath at 93°C. After an air cooling coil, the solution went through a colorimeter with a 570 mμ filter. The signal was recorded on a Technicon single pen recorder. The areas of each peak were estimated by triangulation and were proportional to concentrations in the ranges measured.

Buffer system

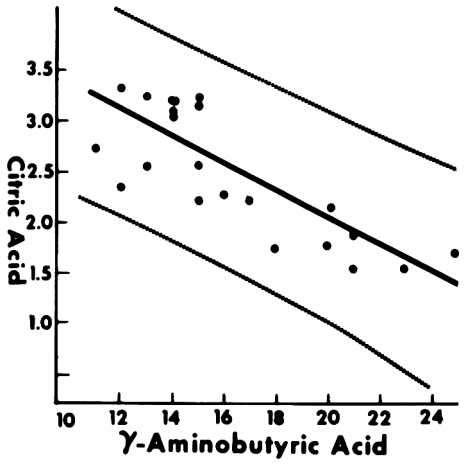
The buffers used in the neutral and acidic column were adapted from the lithium citrate system reported by Benson et al. (1967). The concentrations were as reported (0.3N Li); however, the pH of some had to be altered to give satisfactory separations with citrus juices. All buffers were boiled to remove air and stored under toluene. The buffer coil was loaded in reverse order to the elution starting with 75 ml pH 6.0, 35 ml pH 4.15, 10 ml pH 3.0, and a linear gradient of 85 ml each of pH 2.8 and pH 2.6 buffers. The column was regenerated after each run with 50 ml 0.3N LiOH, 75 ml pH 6.0 buffer, and 75 ml of the pH 2.6 starting buffer.

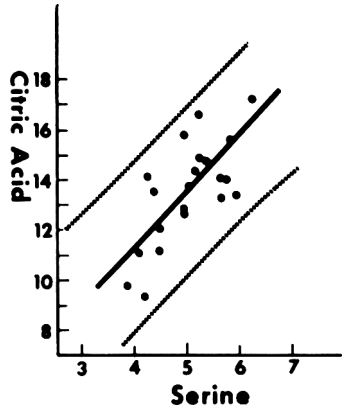
The buffer system for the basic amino acids (Moore et al., 1958) consisted of 0.35N sodium citrate pH 5.28. The column was regenerated with 0.35N NaOH and equilibrated with starting buffer after each run.

Juice samples

13 California orange samples with both pure Valencia and navel concentrates and 14 Florida orange samples were chosen to cover an entire season's production. The lemon juice concentrates covered several seasons and the major

processors; they consisted of 13 samples from California, 15 samples from Arizona and 7 samples from Florida. Orange and lemon juice concentrates were diluted with water according to the manufacturers recommendations to single strength composition. The juices were filtered with Celite to give a clear serum. The serum was diluted with equal parts of the internal standard in pH 2.2 buffer. (Some orange juice samples with high amino acid concentrations had to be diluted further to keep the peaks on scale.) For internal standards, taurine was used with the neutral and acidic amino acids, and δ-guanidinopropionic acid was used with the basic amino acids.





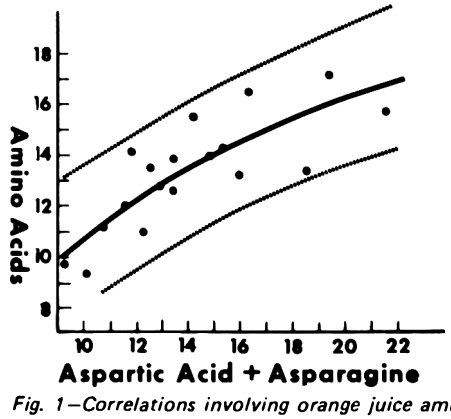


Fig. 1—Correlations involving orange juice amino acids (in mol percentages of the total amino acids).

Supplementary analyses

The soluble solids (°Brix) were measured by refractive index. The acidity was measured by titration to pH 8.4 and expressed in meq/100 ml. The total amino acids were estimated by formol titration (Vandercook et al., 1963). The total phenolics were determined by UV absorption (Vandercook and Rolle, 1963).

RESULTS & DISCUSSION

BY MEANS OF the experimental conditions described above, all of the amino acids in citrus juices were well resolved. The peaks were identified by using pure standards of the amino acids reported in citrus juices both alone and mixed with the juice. A set of constants was obtained for our experimental system relating the peak areas to amino acid concentration for the internal standard and all of the major amino acids. The standard deviations of replicates were 6–7% of the mean for the α-amino acids and 10% for proline.

Table 1 presents the average amino acid mol percentages and standard deviations for orange and lemon juices. The averages are expressed in terms of mol percentages of the total amino acids for easier comparisons since the total amino acid concentrations varied with the sample. The data are comparable to those reported by other authors except for the Israeli lemon juice (Lifshitz and Stepak, 1971). The buffer system they used did not resolve asparagine or γ-aminobutyric acid which were reported as threonine and phenylalanine, respectively. The data for the soluble solids (°Brix), citric acid, total amino acids by formol titration, and total phenolics are included for comparison.

The varietal effects of lemons on the amino acids were studied. Three lots of four lemons each were picked in Nov. 1969 and worked up separately from two Lisbon and two Eureka lemon trees grown in Indio, Calif. The samples showed no significant differences in any of the amino acids. In Meyer lemon concentrate, serine and arginine were different ($P < 0.05$). The average mol percentages for serine were 6.7 and 10.2 and for arginine were 2.6 and 0.7 for Meyer and regular lemons, respectively.

Since lemons are frequently stored for several months before processing and compositional changes have been reported (Sinclair and Eny, 1945; Eaks, 1961; Vandercook et al., 1966), it was decided to investigate the individual amino acids during storage. Stored California coastal lemons, from another study, were analyzed for amino acids at 0, 45 and 102 days. There were no significant ($P < 0.05$) changes in any of the amino acids over the storage period.

Several of the amino acids of California oranges changed significantly over the course of the season. The mol percentages of serine, asparagine and aspartic acid decreased for navel orange concentrate from December through May, and alanine increased over the same period. Valencia concentrate showed an increase in the mol percentage of aspartic acid and asparagine over the period of May through October.

Table 2 shows the effect of growing areas on the composition of lemon and orange juices. The mol percentage of aspartic acid was lower in lemons grown in the coastal areas of California than in desert or Florida fruit. The proline was highest in California coastal lemons, lower in desert lemons and lowest in Florida lemons. Florida lemons were higher in ammonia than either California-coastal or desert fruit. For orange juice the total amino acids were lower for Florida than California. Of the individual amino acids, the mol percentages of asparagine and serine were lower and γ-aminobutyric acid was higher for Flor-

Table 2—Effect of growing area on the composition of orange and lemon juices

	California	Arizona	Florida
Orange juice (No. of samples)	13		14
Asparagine	7.9**		5.8**
Serine	5.3**		4.4**
γ-Aminobutyric acid	14**		20**
Methionine	0.17**		0.08**
Histidine	0.26*		0.18*
Total amino acids (formol)	3.05**		2.06**
Total phenolics	0.761**		0.620**
Lemon juice (No. of samples)	13	15	7
Aspartic acid	12.5*	19.4	18.3
Proline	35*	29*	24*
Ammonia	8.0	8.0	12.2*

*$P < 0.05$; **$P < 0.01$

ida than for California orange concentrate.

The compositional differences with both season and growing area suggested possible correlations between constituents. The relationships between total amino acids (AA) and γ-aminobutyric acid (γ-AB), citric acid (CA) and serine (SER), and CA and aspartic acid plus asparagine (ASP-ASN) in orange juice are quite interesting. Figure 1 shows the regression lines and 95% confidence intervals for some of these parameters. (Equations for the regression line have been omitted since the data are intended to show potential applications rather than a finished juice content equation. Additional data are currently being gathered to develop equations for estimating juice content.) The mol percentage of γ-AB would be independent of added sugar, water, or acid. Thus, a dilution factor could be estimated by comparing the measured AA value with that predicted from γ-AB. Likewise, the mol percentages of SER and ASP-ASN are unaffected by sugar, water, or acid and might be used to estimate a dilution factor.

It should be pointed out that although there were significant differences in some of the constituents between California and Florida orange juice the data from all samples tested fell on the same regression lines. The same was true for the seasonal trends noted above. This is fortunate in regards to estimating juice content since most of the time the source or history of a sample is unknown and concentrates are frequently blended. The regressions could possibly provide an adjustment for the natural sample variations. Thus, a parameter might be compared with its corresponding regression value rather than with its mean value.

The quantitative determination of the amino acid composition could be quite useful in detecting adulterations with amino acids. For example, the total amount of the minor amino acids (valine, methionine, isoleucine, leucine, cystine, tyrosine, phenylalanine, lysine and histidine in lemon and orange juice is about 3 mol percent with a range from 2–6%. The addition of amino acids from a protein hydrolysate would be readily detected since the minor amino acids of citrus comprise a much larger percentage of commonly available proteins (soy, 30%; casein, 43%; cottonseed, 35%). Paper or thin-layer chromatographic patterns or spot intensities might indicate this addition, but quantitative data offer more reproducible and convincing proof of adulteration than subjective evaluations of chromatograms. The addition of amino acid mixtures which approximated that of the juice does not appear to be practical, since the cost of the amino acids alone would exceed the price of authentic juice.

Work is currently underway to develop a simpler, more rapid method to separate and analyze for just the amino acids which have the highest correlations with juice content.

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Ms received 12/16/71; revised 2/18/72; accepted 2/24/72.

This work was supported in part by the Lemon Products Technical Committee, Los Angeles, Calif.

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FATTY ACID DISTRIBUTION IN ORANGE JUICE PHOSPHOLIPIDS

INTRODUCTION

FLAVOR DETERIORATION in lemon and other citrus juices has been associated with the presence of PUFA, such as linolenic acid, present in the juice and seeds (Gorbach and Hekal, 1968). Other studies have listed the major phospholipids in citrus juices and the effects of storage on the fatty acids and phospholipids of commercial orange juice (Vandercook et al., 1970; Nagy and Nordby, 1970). These recent studies have outlined the complexities of citrus juice lipids and raised questions regarding the nature of reactions and interactions of these compounds with other constituents in the juice. The present paper describes in detail the positional distribution of the major fatty acids in two orange juice phospholipids, and includes new information on the effect of commercial processing on the phospholipids in the juice.

EXPERIMENTAL

Orange juice samples

Juice from freshly harvested 'Valencia' oranges was extracted with a commercial extractor (FMC Corp.) on nine different dates during March–May, 1971. Duplicate 100 ml samples of juice were analyzed immediately after extraction for fresh juice investigations and after heat stabilization (90.5°C) and frozen concentrate manufacture for processed juice investigations. Quantitative analyses and comparisons were all based on 12° Brix readings for either single-strength juice or reconstituted frozen concentrate.

Lipid analyses

Lipids were extracted and purified from fresh juice samples (100 ml) using a published procedure (Nordby and Nagy, 1969). Phosphatidyls-ethanolamine (PC), -choline (PC), -serine (PS), -inositol (PI) and phosphatidic acid (PA) were isolated from the phospholipid fraction of the juice lipids by two-dimensional TLC on silica gel G. Plates were developed in the following solvent systems: lst dimension, $CHCl_3$:MeOH:H_2O (65:25:4); 2nd dimension, $CHCl_3$:MeOH:NH_4OH (70:20:4). For preparative quantities, the same solvent systems were used with consecutive development of PE and PC bands scraped from the 1st plate, eluted with $CHCl_3$:MeOH (50:50) and chromatographed in the 2nd solvent system. An antioxidant (0.2% BHT) was added to all solvent systems to prevent sample oxidation (Nordby and Nagy, 1971).

Fatty acid methyl esters were prepared and purified from PE, PC and lyso-derivatives using BF_3-methanol and following a procedure described for citrus juice lipids (Nagy and Nordby, 1970). Gas chromatographic analyses of fatty acid methyl esters were performed using a Micro Tek Model 2000 gas chromatograph equipped with flame ionization detectors. Operation was isothermal at 190°C using a 6 ft × 1/8 in. stainless steel column packed with 10% EGSS-X on 100/120 mesh Gas Chrom Q (Applied Sciences Labs., Inc.).

Phospholipids were detected on TLC plates through the use of ninhydrin and a molybdate spray reagent (Dittmer and Lester, 1964). Quantitative analysis involved scraping the phospholipid from the plate into micro-Kjeldahl flasks for digestion with perchloric acid (Harris and Popat, 1954). Following digestion, the following reagents were added in the order listed: 5 ml distilled H_2O, 1 ml 2.5% ammonium molybdate, 1 ml 10% ascorbic acid and 2 ml distilled water to rinse the flask. The samples were then placed in a boiling water bath for 5 min, cooled, centrifuged at 3000 × G and the absorbance at 820 nm was compared to a standard curve prepared from potassium dihydrogen phosphate.

Enzymatic hydrolysis

An enzymatic hydrolysis using crude porcine pancreatic lipase (pancreatin, Sigma) was adopted to determine the positional distribution of the major fatty acids on PE and PC from orange juice. Pure samples (20 mg P) of PE and PC were converted by phospholipase D hydrolysis to phosphatidic acid using the procedure of Possmayer et al., 1969. Phospholipase D was prepared from cabbage and partially purified by preparation of an acetone powder (Davidson and Long, 1958).

The purified phosphatidic acids from PE and PC were converted to their dimethylphosphatidates using 2 ml 0.2M diazomethane in ether plus 0.1 ml methanol and incubating at 20°C for 30 min (Possmayer et al., 1969; Renkonen, 1968). The dimethylphosphatidates were incubated at 30°C for 1–3 hr in a pH 8.0 borate buffer system containing pancreatic lipase (Slotboom et al., 1970). Several drops of ether were also added to the reaction mixture to increase the hydrolysis efficiency. Following incubation, the mixture was extracted with $CHCl_3$ and the lysodimethyl-phosphatidates were purified by TLC on silica gel G using petroleum ether:ether:acetic acid (20:80:1.0) as a developing solvent. Bands were scraped from TLC plates for direct preparation of methyl esters as described above.

RESULTS & DISCUSSION

Positional distribution of fatty acids

Slotboom et al., 1970 have shown that crude pancreatic lipase can be used to hydrolyze specifically the l-acyl ester bond of phosphotriesters without the interference of contaminating phospholipase A. For this reason, crude lipase was used to hydrolyze orange juice PE and PC dimethylphosphatidates to their respective 2-acyl lyso-derivatives. Table 1 gives the results of the fatty acid analysis of both the intact phospholipids and the 2-acyl derivatives of PE and PC from fresh 'Valencia' orange juice. From this data, it is clear that palmitic (16:0), palmitolecic (16:1) and stearic (18:0) acids are located primarily at the 1-position, while oleic (18:1), linoleic (18:2) and linolenic (18:3) are most concentrated at the 2-position of the 2 phospholipids studied. This distribution follows closely the pattern of palmitic and stearic acids being preferentially esterified at the 1- and 3-positions and linoleic acid at the 2-position of plant seed oil glycerides (Mattson and Volpenheim, 1961, 1963; Evans et al., 1969).

One of the more marked characteristics of the fatty acid composition found in the two major orange juice phospholipids was the considerable amount of palmitic acid. This high amount of pal-

Table 1—Fatty acid composition of 'Valencia' orange juice phospholipids and of fatty acids in the 2-position

	% GLC peak area[a]					
	16:0	16:1	18:0	18:1	18:2	18:3[b]
PE						
Phospholipid	24	4	2	19	42	9
2-position	3	0	0	23	62	12
PC						
Phospholipid	22	5	2	26	34	9
2-position	2	0	0	31	57	10

[a]Average of nine different juice samples
[b]Notation signifying carbon chain length:number of double bonds.

Table 2–Concentration of phospholipids in fresh, heat stabilized and frozen concentrated 'Valencia' orange juice

	% of total lipid phosphorus					
	PE	PC	PI	PS	PA	Lipid P[c]
Fresh[a]	39	29	11	7	3	1.0
Stabilized[a]	40	32	9	7	5	1.3
Concentrate[b]	34	29	3	9	9	1.3

[a]Average of nine different juice samples
[b]Average of six different juice samples
[c]Mg lipid phosphorus per 100 ml juice

mitic acid persists in the seed lipids as well as in the juice (Nordby and Nagy, 1969) and has been documented previously, when the similarities between citrus seed oils and cottonseed oil were recorded (Dunn et al., 1948).

Effects of commercial processing

Table 2 presents data showing the concentration of several phospholipids in fresh and processed 'Valencia' orange juice. These data were taken from samples of fresh and freshly processed juice, intending to show the effects of processing on the phospholipid fraction of the juice. Experiments were performed on juice processed using commercial-sized industrial equipment. No storage studies were conducted, since a previous paper thoroughly described the effects of storage time and temperature on phospholipid stability in aseptically filled glass containers of single strength orange juice (Nagy and Nordby, 1970).

Several important aspects ot the data in Table 2 should be pointed out. More lipid phosphorus was extracted from the processed juice than from the fresh juice, a result which could be explained by considering that heat treatment and evaporation during processing caused physical disruption of phospholipid-containing membrane and tissue in the juice, hence allowing greater extractability. Additional extractions of fresh and processed juices did not significantly increase the yield beyond the figures reported. The percentages of total lipid phosphorus of the PE and PI fractions were considerably decreased and the PA fraction increased as a result of processing fresh juice into frozen concentrate. Lesser changes were apparent for juice which had been heat stabilized to inactivate pectic enzymes. No explanation could be given for the negligible change in the PC and PS fractions, except for the speculation that the ionic nature of these molecules may in some way affect their stability in the juice.

The percentages of the various phospholipids listed in Table 2 are similar to values reported elsewhere (Vandercook et al., 1970). However, the values reported in this table for total lipid phosphorus, PI and PS for fresh and processed 'Valencia' juice are not in complete agreement with the values published by the above authors. These differences may be due to seasonal, varietal or experimental differences which are in accordance with biological variations between samples. Some unidentified phospholipids or phosphorus-containing reaction products were also present, partially accounting for the reason that the values in Table 2 do not add up to 100%. The amount of PE was greater than the amount of PC in the average values reported in Table 2. This difference was most pronounced in fruit harvested in March, while fully mature fruit harvested in May contained slightly more PC than PE. However, these changes were observed for only one growing season and more studies are needed to determine the effect of maturity on phospholipid concentrations in the fruit.

In summary, these studies have shown that the specific fatty acid distribution in the two major phospholipids of orange juice is in keeping with the general pattern found for most plant lipids; i.e., esterification of palmitic and stearic acids is predominantly at the 1-position and the polyunsaturated fatty acids at the 2-position of the phospholipids. With regard to the effect of commercial processing on juice phospholipids, no serious damage was apparent to the phospholipids studied, even though the concentrations of PE and PI were reduced following processing.

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Ms received 12/26/71; revised 3/6/72; accepted 3/16/72.

Florida Agricultural Experiment Station Journal Series No. 4261.

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COLORIMETRY OF FOODS: ORANGE JUICE

INTRODUCTION

THAT COLOR PLAYS an important role in the consumption of a food is amply demonstrated by the allotment of 40 out of 100 points in the USDA grading system for orange juice. Orange juices vary from pale yellow in the early season to red-orange late in the season and a desirable color is achieved by blending. Color has been evaluated visually by comparing juice samples to USDA plastic standards.

Two instruments used to measure the color of orange juice are the Hunterlab Color Difference Meter and the Hunter Citrus Colorimeter. The latter is a simple, rugged instrument designed specifically for use with citrus juices (Hunter, 1967). Its development was based on data obtained with the Hunterlab Color Difference Meter and visual scores using USDA color standards. Correlations of 0.855–0.939 were obtained for panel scores with citrus colorimeter values (Wenzel and Huggart, 1969).

A method of extracting orange peel carotenoids to be used as a colorant for orange juice was proposed by Ting and Hendrickson (1969). Under the Florida Citrus code no foreign matter, including colorants, is permitted in pure orange juice and frozen orange concentrates. It may be that orange peel carotenoids would not be considered foreign matter. Ting (1961) found that total carotenoid content of oranges harvested late in the season was higher than for those harvested earlier. A highly significant correlation coefficient (0.903) was obtained for total carotenoids against the Hunter "aRd" value. The addition of orange peel carotenoids to orange juice resulted in large increases in Hunter "aRd" values and only small changes in "Rd" and "bRd" (Ting and Hendrickson, 1969).

The Kubelka-Munk colorant layer concept as applied to translucent foods has been discussed by Clydesdale and Francis (1971), Huang et al. (1970a, b), Joslyn and Little (1967), Mackinney et al. (1966) and Little (1964). A translucent sample has some of the light from below the surface reflected as a result of internal transmission of light. Therefore, traditional methods of color measurement may not accurately reflect the color of the sample as seen by the human eye. Kubelka and Munk developed equations for calculating the reflectance of a layer of infinite depth (Judd and Wyszecki, 1963). This method uses the measurement of reflectance of a thin layer of the sample presented against a white and a black background. In some cases, presentation alone, of a thin layer against a white or black background was enough to increase numerical separation of samples and thus increase accuracy of the instrument in ranking the samples (Little, 1964; Mackinney et al., 1966; and Joslyn and Little, 1967). Also the thin layer presentation with small aperture and small area of illumination, was at least as good, for the products tested, as the large aperture with small area of illumination presentation generally recommended for translucent samples.

Tristimulus values X, Y and Z were not equally efficient in detecting color differences in the samples. The same was true for calculated T_i, K and S values (Little, 1964; Mackinney et al., 1966; Joslyn and Little, 1967).

Huang et al. (1970a, b) applied the Kubelka-Munk concept for color measurement to samples of pureed squash and carrots containing very small color differences. They found that a panel could rank visually, samples differing in approximately 0.2 delta E units (Delta $E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$). Huang et al. also observed that a single color parameter was not sufficient for ranking samples instrumentally, the best correlations being obtained when all three parameters either X, Y and Z or Hunter "L," "a" and "b" were included. Huang et al. found the Hunterlab D25 colorimeter measured small color differences more precisely than the GE spectrophotometer for the foods studied.

Rummens (1970) designed an illuminating hollow sphere, with the sample placed in the center, to measure both transmitted and reflected light from the sample. He found that the color coefficients of a 5 cm cylindrical cell filled with orangeade, measured with this all-side illumination technique, were in substantive agreement with those of a colored card selected visually to match the color of the orangeade sample.

Orange juice is a good example of a translucent food. This study was undertaken to determine whether the application of the Kubelka-Munk colorant layer concept to orange juice provided a better means of evaluating or measuring the color than the methods now used.

EXPERIMENTAL

FROZEN ORANGE concentrate was obtained from the Citrus Experiment Station, Lake Alfred, Fla. (courtesy of F.W. Wenzel). The concentrate had a Brix of 58° and was made from juice with the characteristics: total acid, as citric, 0.76%; 11.6° Brix; Brix/acid ratio 15.3. Neither cutback juice nor orange oil was added to the concentrate. The juice was given a rating between 35 and 36 as judged against the USDA standard plastic OJ tubes and had a CR value of 27.2 as measured by the Hunterlab Citrus Colorimeter Model D45.

Orange peel carotenoids used as colorant for the orange juice were also obtained from the Lake Alfred Citrus Experiment station. The colorant solution was prepared by blending 5g concentrated orange carotenoids with 500 ml of light colored orange juice in a Waring Blendor.

Stock solutions of orange juice were made by diluting the concentrated juice to obtain a reconstituted juice with a Brix of 13.0° and by adding 29 ppm concentrated orange carotenoids so that the color approached the lower limits for a Grade A juice. A more highly colored juice was obtained by adding colorant in the amount of 56 ppm concentrated orange carotenoids to the stock juice. The two juices were blended to give 10 mixtures containing varying amounts of the more highly colored juice (0.0–56 ppm carotenoid colorant added to stock juice). Ten cell thickness (2, 3, 4, 5, 6, 7, 8, 16, 24 and 32 mm) and 1-in. diam test-tubes for the Citrus Colorimeter were tested. Each series of 10 samples for each of the various cell thickness were tested three times. Three cell thickness were tested at one time and the order was randomized so that different thicknesses were tested together.

Color was measured on the GE Spectrophotometer (General Electric Co., West Lynn, Mass.) with tristimulus integrator (Davidson & Hemmendinger, Inc., Easton, Pa.) operated with specular reflection included and pressed barium sulphate standards. Huang et al. (1970a) found that inclusion of the specular component did not alter the results for samples of carrot. Samples were presented against white and black backgrounds (Munsell Color Reference Guide Tobey Papers N9/, N 2.25/ respectively). In addition to the spectrophotometric measurements, color was measured on the Hunterlab D25 Color Difference Meter (Hunter Associates Lab., Fairfax, Va.). Measurements were made using an aperture 4.7 cm diam and mode of illumination which illuminated the area of the aperture. A standard tile of specification

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L = 83.0; a = −3.6; b = 25.3 was used to standardize the instrument.

The equations used for calculating the K/S ratios were as follows:

$$a = 1/2(R + \frac{Ro - R + Rg}{RoRg})$$

$$b = (a^2 - 1)^{1/2}$$

$$R\infty = a - b$$

$$K/S = \frac{(1 - R\infty)^2}{2\,R\infty}$$

where

R = reflectance of sample with white background
Ro = reflectance of sample with black background
Rg = reflectance of white background
R∞ = reflectance of infinitely thick sample
K = coefficient of absorption
S = coefficient of scattering

Color differences were calculated from Hunterlab data by the equation:

$$\Delta E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$$

The Hunter Citrus Colorimeter (Hunter Associates Lab., Fairfax, Va.) was also used to measure the color of the orange juice samples. Samples were presented in 1-in. test tubes as recommended by the manufacturer. Duplicate readings were made in two positions for each tube and the four readings averaged to obtain a CR value for the sample. The instrument was standardized using USDA plastic OJ4 tube #107 with a CR value 31.3. (The present USDA standard for the Citrus Colorimeter is the OJ5.) All measurements were made on juice samples in which the pulp had not been allowed to settle.

Samples were evaluated visually by a panel of six. The samples were examined in a MacBeth Lablite Booth (MacBeth Daylight Corp., Newburgh, N.Y.) under north sky light (7400°K) and were supported at a 45° angle for viewing. The panelists were asked to rank the 10 samples for increased redness. Ranking was done against the same backgrounds as used for instrumental measurements.

Linear correlation (r) and multiple correlation coefficients (R) were calculated to show the relationship between the theoretical rankings achieved by adding colorant and the color indices obtained by instrumental measurements and visual examination.

A second experiment was conducted in which the color differences were decreased to about one-half of those used in the first experiment. Juices were prepared by the same procedure except that concentrated carotenoid colorant added to the more highly colored juice was reduced to 25 ppm. Only the 2-, 3- and 8-mm cells and the test tubes for the Citrus Colorimeter were measured. In this part of the study, background Munsell papers were replaced with paint. (101-AO white and 101-C10 black, Reflective Products Div., 3M Co., St. Paul, Minn.)

A third part of the study included the effect of pulp on the color measurement of orange juice. For this, commercial frozen orange concentrate containing 13% pulp by volume was used. The pulp was separated from the juice by successive dilutions and centrifugations of 10-min periods at 2000 rpm in an IEC centrifuge Model PR6 (International Equipment Co., Needham Hts., Mass.). Both the pulp and supernatant liquid were collected. The amount of water required for four centrifugation processes was recorded and the pooled supernatant liquid further diluted to the 1:3 ratio recommended by the processor. This was used as the stock juice.

The pulp was freeze dried in a pilot size freeze drier (Repp Industries Inc., Gardiner, N.Y.) operated with shelf fluid at −50°F and condenser fluid at −60°F. Shelves were heated to +50°F for drying. The drying operation took 23 hr and the vacuum was broken by air. The dried pulp particles were ground in a Micro-Mill (Chemical Rubber Co., Cleveland, Ohio) and separated into two sizes by sieving. Large particles were those between 250 and 500μ and small particles were less than 250μ. Considerable carotenoid color was retained by the pulp particles and this was removed by successive washings with petroleum ether and 95% ethyl alcohol until no more color could be extracted. This resulted in almost white pulp particles which were then resieved to ensure desired particle size.

The dried pulp was added to the stock juice so that a juice containing 9.5% pulp by volume was obtained. This was blended with stock juice containing no pulp to achieve 10 samples varying in amount of pulp from 0.0–9.5%. Cell thicknesses and instrumental measurements were the same as described previously for the second part of the study. There was considerable difficulty in maintaining a suspension of the larger pulp particles for the time required for visual ranking so visual ranking of samples containing the larger pulp particles was omitted.

All statistical analyses and color indices were programmed on a CDC 3600 computer. Only a small sampling is presented here and the remainder may be found in the original thesis (Gullett, 1970).

RESULTS & DISCUSSION

Effect of pigment

The sensitivity of the human eye in its ability to discriminate between very small color differences (< 0.15 ΔE units) when a reference sample is available, such as the ranking of a series of samples for redness, is demonstrated by this study (Table 1). However, examination of correlations for the individual judges showed that a considerable range existed between judges for the same cell thickness. In most cases, correlations for average scores were higher than those for the individual scores. In contrast to the findings of Huang et al. (1970a, b) background effect on ability to rank samples was not significant as determined by a paired t-test for Z values calculated from r values. Reducing the amount of colorant added, lowered the correlations obtained for visual ratings with theoretical ranking only slightly, with the greatest reduction being obtained when samples were presented in 1-in. test tubes (Table 1).

Table 1—Visual ratings correlated with theoretical rankings for orange juice samples

	Cell thickness (mm)							
	2	4	6	8	16	24	32	1-in tubes
Correlation	Correlation coefficient (r × 1000)[a]							
Th[b] vs. V_W[c]								
56 ppm colorant	910	968	981	959	966	936	967	946
25 ppm colorant	870	–	–	931	–	–	–	850
Th vs. V_B								
56 ppm colorant	962	970	974	991	956	975	972	960
25 ppm colorant	922	–	–	936	–	–	–	802

[a]All correlation coefficients are the average of r from three experiments.
[b]Theoretical ranking
[c]Visual ranking for samples with a white (V_W) and a black (V_B) background

Table 2—Relationship between pigment concentration and ΔE for different cell thicknesses

Cell thickness (mm)	Amount of colorant (ppm)				
	7	19	32	44	56
	ΔE[a]				
White background					
2	0.74	0.98	1.25	1.50	1.75
4	1.17	1.19	1.21	1.22	1.24
6	0.31	0.66	1.04	1.40	1.75
8	0.25	0.55	0.88	1.19	1.49
16	0.34	0.61	0.89	1.16	1.42
24	0.47	0.68	0.90	1.10	1.30
32	0.27	0.56	0.87	1.16	1.45
Black background					
2	0.75	0.86	0.97	1.08	1.19
4	0.35	0.55	0.77	0.97	1.17
6	0.25	0.51	0.80	1.07	1.34
8	0.29	0.49	0.72	0.93	1.14
16	0.25	0.51	0.79	1.05	1.31
24	0.50	0.73	0.99	1.23	1.47
32	0.31	0.60	0.91	1.20	1.48

[a]ΔE obtained with a Hunterlab D25 colorimeter and calculated as $\Delta E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$. ΔE is the difference between readings for samples with added pigment and the controls.

ΔE values deduced from a regression equation of 10 different pigment concentrations are shown in Table 2. The background used for sample presentation affected the values obtained. White background presentation and increasing cell thickness resulted in a reduction in ΔE values obtained, whereas black background presentation increased ΔE values for thicker cells. Background effects on ΔE values were not reflected in the panelists' ability to rank samples visually (Table 1). A decrease in ΔE values for thicker cells and white background presentation was also observed by Huang et al. (1970b) for samples of squash puree. In addition to the effect of cell thickness, an expansion in color space, expressed as a larger ΔE value, was observed for 2 mm cells. This same phenomenon was noted for samples of Tang (Gullett et al., 1972) and by Huang et al. (1970a, b) for samples of squash and carrot purees. In all instances, the expansion in ΔE was not accompanied by a corresponding improvement in visual judgments. The ability of the panel to rank the samples appeared to be affected by the relative change in the components of the equation used to calculate ΔE.

Correlations of GERS data with theoretical ranking for 56 ppm added carotenoid colorant are shown in Table 3. As was observed by Huang et al. (1970a, b) the use of more than one color parameter improved the correlations obtained. Higher correlations were noted when K and S were both included than when the single ratio K/S was considered. The best correlations were obtained for the parameter Y. This is not to suggest that Y is the best indicator of redness but that the increase in redness was accompanied by a greater decrease in luminosity of the sample. Correlations for the tristimulus value Z were shown to be low and not included in most of the calculations (Gullett, 1970).

Correlations for theoretical ranking of orange juice samples containing increments of pigment with Hunter parameters and hue, calculated as $\tan^{-1}$ a/b, are shown in Table 4. The importance of the tridimensionality of color is demonstrated by the higher correlations obtained when two or more parameters were included. However, very good simple correlations were obtained for the Hunter parameter "a" and for hue, regardless of background. These correlations were as good as those for visual rating versus theoretical ranking. Wenzel and Huggart (1962, 1969) and Hunter (1967) all reported that tristimulus value "a" was the best single indicator of redness in orange juice. Gullett et al. (1972) observed that this was also true for the synthetic orange drink Tang.

Absorption and scattering coefficients for the tristimulus value Y are presented in Figures 1 and 2. Hunter data were converted to CIE tristimulus values for calculation of absorption and scattering coefficients. Tristimulus values obtained for the samples presented against white and black backgrounds were substituted for R values in Kubelka-Munk equations for calculation of K and S. Both absorption and scattering coefficients decreased with increasing cell thickness. This was true for solutions of Tang (Gullett, 1970; Gullet et al., 1972) and purees of squash and carrots (Huang et al., 1970a, b). Much less difference between coefficients of absorption and scattering existed for orange juice than for the other foods tested. This was accompanied by lower correlations for the ratio K/S. Coefficients for the tristimulus value X exhibited the same trends as Y.

Absorption and scattering coefficients were calculated for the Hunter parameter "L." The use of this parameter reversed the relation of K and S from those calculated for the other parameters. This was a result of a considerable increase in S and a reduction in K. This reversal was noted for other foods tested (Gullett, 1970; Gullett et al., 1972; Huang et al. 1970a, b). Obviously, the parameter "L" is more sensitive to scattering effects. Orange juice solutions exhibited a large ΔE accompanied by a large ΔL and a small K/S ratio for 2 mm cells (Fig. 3). The use of the white background has increased the effect of scattering for these

Table 3—Correlations of theoretical ranking with data for GE spectrophotometer (56 ppm carotenoid colorant)

	Cell thickness (mm)						
	2	4	6	8	16	24	32
Correlation[a]	Correlation coefficient (r or R × 1000)						
Tristimulus values							
Th vs. X_W	−717	−320	−478	−462	−186	−528	−040
Th vs. Y_W	−732	−389	−736	−701	−420	−610	−233
Th vs. $X_W \cdot Y_W$	791	745	979	907	925	977	942
Th vs. X_B	−018	−225	440	018	−151	−469	030
Th vs. Y_B	055	−270	339	−298	−294	−565	−125
Th vs. $X_B \cdot Y_B$	769	693	965	942	948	979	936
Kubelka-Munk treatment							
Th vs. $K_X \cdot S_X$	803	475	732	615	417	769	315
Th vs. $K_Y \cdot S_Y$	782	572	836	763	479	823	514
Th vs. $K_{660} \cdot S_{660}$	687	481	711	647	491	685	493
Th vs. $K_X \cdot S_X \cdot K_Y \cdot S_Y$	848	823	888	808	901	867	703
Th vs. K/S_X	732	341	−266	243	170	499	−020
Th vs. K/S_Y	729	443	−040	503	318	512	057
Th vs. K/S_{660}	544	085	−497	−261	013	356	−106

[a]X and Y are tristimulus values obtained from GERS. The subscripts W and B refer to white and black background, respectively. Th refers to theoretical ranking, K to coefficient of absorption and S to coefficient of scattering, 660 refers to reflection at 660 nm.

Table 4—Correlations of theoretical ranking of orange juice samples (56 ppm carotenoid colorant) with data from Hunterlab D25 colorimeter

	Cell thickness (mm)						
	2	4	6	8	16	24	32
Correlation[a]	Correlation coefficient (r or R × 1000)						
Th vs. L_W	−155	−552	−764	−749	−777	−521	−743
Th vs. a_W	717	905	972	976	978	983	954
Th vs. b_W	812	704	790	694	554	695	381
Th vs. $(\tan^{-1}$ a/b$)_W$	841	905	971	977	978	982	955
Th vs. $L_W \cdot a_W \cdot b_W$	906	970	995	984	991	986	992
Th vs. L_B	500	−173	486	−192	−513	−328	−732
Th vs. a_B	−079	841	959	958	968	977	938
Th vs. b_B	838	836	922	916	976	713	400
Th vs. $(\tan^{-1}$ a/b$)_B$	866	881	962	957	965	976	941
Th vs. $L_B \cdot a_B \cdot b_B$	985	990	990	988	988	988	976

[a]The subscripts W and B refer to samples with white and black background, respectively. The letters L, a, b refer to tristimulus values from Hunterlab D25 colorimeter. Th refers to theoretical ranking and $(\tan^{-1}$ a/b) refers to hue.

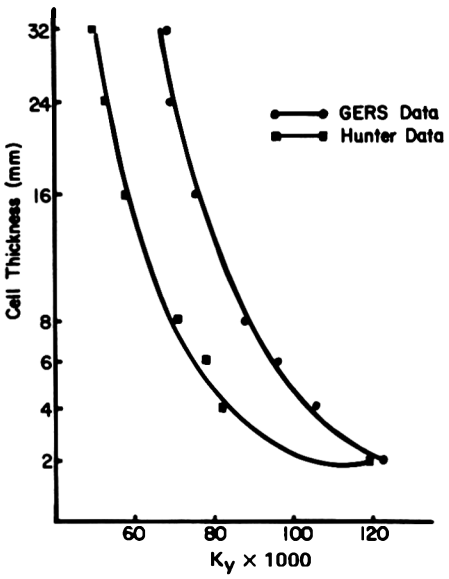

Fig. 1—Coefficients of absorption (K) calculated for GERS and Hunterlab data converted to CIE Y for samples of orange juice containing 56 ppm carotenoid colorant.

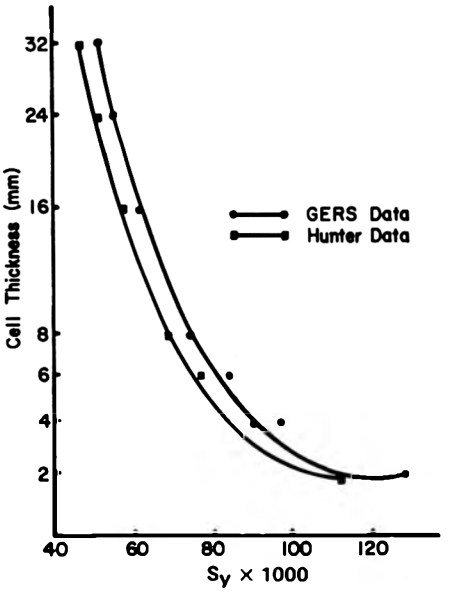

Fig. 2—Coefficients of scattering (S) calculated for GERS and Hunterlab data converted to CIE Y for samples of orange juice containing 56 ppm carotenoid colorant.

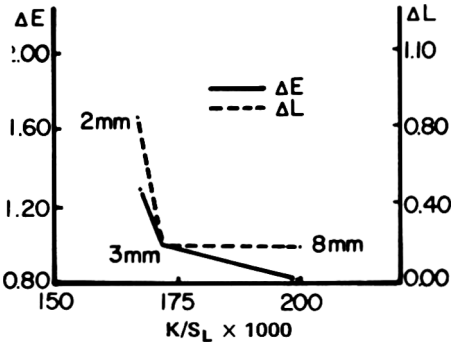

Fig. 3—ΔE values, $\Delta E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$ and ΔL values, $\Delta L = L_1 - L_2$ plotted against K/S calculated from Hunter "L" values for samples of orange juice.

thin cells. K/S values were found to increase with cell thickness but ΔE values decreased and ΔL values remained constant. This is in contrast to the findings for Tang (Gullett, 1970; Gullett et al., 1972) where an increase in K/S ratios was noted for 8 mm cells. Thus K could be considered to play a less important role in orange juice than in the Tang which has a higher chroma.

As cell thickness increases, more reflected light would be lost through peripheral scatter which would not be collected by the photocell in the colorimeter. This would account for the greater decrease obtained for S values than for K values. However, it does not account for the smaller increase in K/S observed for thicker cells for orange juice and for squash and carrot purees (Huang et al., 1970a, b) than for Tang (Gullett, 1970; Gullett et al., 1972). One would expect that greater trapping would occur for the foods containing more particles. Therefore, the phenomenon of light trapping probably plays a minor role in the effect of cell thickness on K/S ratios.

It follows that ΔE values are related to the absorption and scattering coefficients and in particular to the ratio of the two. The expansion in color space observed for thin cells is associated with a disproportionately large scattering coefficient as a result of the light reflecting ability of the white background. As cell thickness is increased the effect of the background is diminished and scattering is decreased more than absorption. Thus it should be possible to characterize optically a food by determining the coefficients of absorption and scattering for a few cells of appropriate thickness.

Correlations for theoretical ranking and visual rating with CR values from the Hunter Citrus Colorimeter for orange juice containing 56 ppm colorant fell within the range of the best correlations for a single parameter for the other instrumental measurements (0.973). Decreasing the amount of carotenoid colorant added to the samples by about one-half resulted in a lower correlation (0.935) for theoretical ranking with CR values but still above those obtained for the panel on the same juice (Table 1). Correlations for CR values were higher than for those obtained for the GERS data. Only two multiple correlations for the Hunterlab D25 colorimeter data were consistently as good as CR values. However, as cell thickness increased coefficients fell within the range of those for CR values, especially those for multiple correlations. When the amount of colorant added was decreased, fewer of the D25 correlations were as high as those for CR values.

Effect of pulp

When the pulp content was increased and pigment content remained constant, low correlations were obtained for pulp content with visual ranking on the basis of increased redness. The calculation of hue as $\tan^{-1} a/b$ showed that a very small shift in hue towards redness occurred with increased pulp content (0.9 for white background; 2.2 for black background and 2 mm cells). Wenzel and Huggart (1969) also noted a shift in hue towards redness when pulp content was increased. Particle size as tested here did not affect this shift. The shift in hue towards redness could not have resulted from carotenoids contained in the pulp since these were removed in the preparation of the pulp. The larger shift in hue for samples presented against a black

Table 5—Correlations of theoretical rankings for orange juice samples containing small differences in pulp content, small particle size, GERS data

	Cell thickness (mm)		
	2	3	8
Correlation[a]	Correlation coefficient (r or R × 1000)		
Tristimulus values			
Th vs. X_W	−731	−681	429
Th vs. Y_W	−703	−642	391
Th vs. $X_W \cdot Y_W$	806	778	591
Th vs. X_B	854	888	939
Th vs. Y_B	720	885	930
Th vs. $X_B \cdot Y_B$	858	905	961
Kubelka-Munk treatment			
Th vs. $K_X \cdot S_X$	903	899	950
Th vs. $K_Y \cdot S_Y$	902	894	944
Th vs. $K_{660} \cdot S_{660}$	887	884	952
Th vs. K/S_X	−430	−809	−917
Th vs. K/S_Y	−422	−770	−903
Th vs. K/S_{660}	007	−630	912

[a]Symbols same as in Table 3

background was attributed to the fact that the particles would increase the path length within the cell and thus increase the amount of blue absorption by the solution and at the same time lessen the absorption effect of the background. Butler and Norris (1960) found that the addition of light-scattering material to solutions intensified absorption bands as a result of an increase in path length. Huang et al. (1970a) also observed higher absorption coefficients for the highly light scattering purees of squash and carrots.

The white background presentation resulted in a smaller shift in hue, because of the light scattering effect of the background and the absorption is relatively less important. This is supported by the Hunter "L" values obtained for 2-, 3- and 8-mm cells and white background presentation. An increase in particle content resulted in a decrease in "L" values for 2- and 3-mm cells. However for 8-mm cells "L" values increased indicating that the light scattering properties of the particles in the solution had a greater effect than the background. Correlations for the ratio K/S with theoretical ranking were better than those obtained for orange juice containing pigment increments except for 2-mm cells (Table 5).

The addition of decolorized pulp to pulp-free orange juice resulted in a reduction in Citrus Colorimeter CR values and negative correlations for theoretical ranking with CR values (−0.594). This would seem to be in contradiction to the shift in hue towards redness indicated from the Hunterlab D25 data. The calculation of hue as $\tan^{-1} a/b$ does not take into account lightness of the sample represented in the "L" parameter. CR calculated as $CR = 200\ [(A/Y) - 1)]$ would take into account increasing lightness resulting from the presence of pulp particles, in the Y factor, and thus a decrease in CR values with increase in pulp content would be obtained.

Correlations for theoretical rankings with instrument parameters were generally lower for large particle pulp than small particle pulp but similar trends were exhibited. Wenzel and Huggart (1969) reported that fine pulp, presented in an infinitely thick sample, increased lightness, but had only a slight effect on the chromaticity for a corresponding amount of coarse pulp. In this study, no clear cut patterns emerged for the effect of particle size on Hunter parameters. This may have been a result of the thinner samples used or the differences in particle size may not have been as great.

CONCLUSIONS

WHILE the Kubelka-Munk treatment, particularly the K/S ratio, does not seem to be advantageous in the color measurement of orange juice, useful coefficients of absorption and scattering can be obtained. These may be useful in classifying carotenoid-containing translucent foods by both pigment content and particle size in order to predict the success of a color measurement technique.

The results of this investigation indicate that for orange juice, the Hunterlab D25 colorimeter, the single parameter "a," a cell 8-mm thick and white background presentation, is a suitable method of color measurement. If very exacting measurement is required then the more complicated regression calculation, including all three tristimulus parameters and Kubelka-Munk treatment would be beneficial. However it should be remembered that Kubelka-Munk equations were developed for reflectance at a single wave length and so the substitution of tristimulus values introduces considerable empiricism into the calculation of K/S ratios. These findings also indicate that the Citrus Colorimeter is at least as good as the D25 colorimeter and is especially sensitive to changes in pigment concentration. It has the added advantage of being simpler to operate in a practical situation.

The expansion in color space noted for thin cells and white background presentation is a result of expansion of "L" values, which in turn reflect the greater degree of light scattering for these cells as indicated by the coefficients of scattering (S_L) obtained.

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Ms received 6/13/71; revised 1/17/72; accepted 1/24/72.

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ODOR THRESHOLDS AND RELATIVE INTENSITIES OF VOLATILE AROMA COMPONENTS IN AN ARTIFICIAL BEVERAGE IMITATING WHISKY

INTRODUCTION

THE COMPOSITION of the volatile aroma portion of alcoholic beverages has been the subject of a number of investigations in recent years. Studies on the flavor of whisky have shown that it is mainly composed of numerous carbonyl compounds, fusel alcohols, esters and fatty acids (Nykänen et al., 1968; Ronkainen et al., 1968; Ronkainen and Suomalainen, 1969; Suomalainen and Nykänen, 1970). Attention should be paid to the sensory importance of these aroma constituents in order to gain knowledge of their influence on the nuances and strength of the aroma.

The determination of the flavor threshold of an individual component is one way of estimating its contribution to the total aroma intensity of a beverage. Several flavor threshold determinations have been made on beer (Meilgaard et al., 1970; Harrison, 1970). Palamand and Hardwick (1969) studied the actual effect of some beer constituents on the overall beer flavor at, below and above their threshold levels in order to relate the outstanding flavor notes to the gas chromatographic profiles. Sega et al. (1967) determined detection, identification and difference thresholds for beer components in order to find flavor interactions and to estimate the effects of these interactions on the overall flavor of beer. They found that an increase in the concentration of a beer constituent will not significantly change the flavor of the beer if kept below its detection threshold level, e.g., the normal concentration of ethyl acetate or β-phenylethyl alcohol can be doubled or tripled with no effect on beer flavor. Guadagni et al. (1966a) attempted to determine the relative contribution of the individual components to the overall odor intensity of hop oil, having already shown earlier that the mixture of compounds from different chemical classes gave an additive odor effect (Guadagni et al., 1963). Kendall and Neilson (1966) showed opposite effects with mixtures of four pure odorants. Baker (1963) studied aqueous mixtures of organic chemicals with a theoretical model and found strong proof of odor synergism—the enhancement of odor intensity—with a mixture of eight chemicals. Keith and Powers (1968) made an artificial peach beverage base and found that sub-threshold and additive sub-threshold effects resulted from only a few flavor combinations. Further they showed that a change in concentration of one compound in a mixture of six compounds was not readily detectable organoleptically.

In an earlier study (Salo, 1970a) a method was developed and standardized for the determination of sensory odor thresholds of volatile aroma compounds in alcoholic beverages. The aim of the present investigation was to study the importance of the volatile aroma portion of whisky from the standpoint of sensory evaluation. An endeavor has been made to use odor threshold values and quantitative data obtained by gas chromatography of volatile aroma compounds to determine the relative odor intensity of individual aroma compounds, the aroma fractions of alcohols, acids, esters and carbonyl compounds and some mixtures of these components. A further aim was to discover odor interactions between constituents and to examine the influence of these interactions on whisky aroma.

EXPERIMENTAL

IN ORDER that the concentrations of aroma compounds might be accurately known throughout the investigation, a synthetic whisky imitation was used instead of original whisky. The beverage model, imitating blended, light-flavored Scotch whisky, was made according to quantitative data obtained from gas chromatographic investigations (Nykänen et al., 1968; Ronkainen et al., 1968; Ronkainen and Suomalainen, 1969; Suomalainen and Nykänen, 1970). 576 mg alcohols, 90 mg acids, 129 mg esters and 17.4 mg carbonyl compounds (813 mg in all) were used per liter blended beverage. The chemical compounds used were of purest obtainable commercial grade, at least 98%. These were added to highly rectified grain spirit diluted with water purified with ion exchange columns and active charcoal to the required alcohol content 34% (w/w). The concentrations of the individual compounds can be found in Tables 1–4. Caramel coloring was used to give the artificial beverage the color of the original blended whisky. For odor determinations, the artificial beverage was diluted with water in the ratio 1:2 to decrease the pungency of ethyl alcohol.

The odor thresholds of the pure compounds were determined by the method published

Table 1—Alcohol fraction of artificial whisky model: relationships of concentrations, threshold levels and relative contributions to total odor

Alcohols	Concentration mg/l	Concentration %	Odor threshold mg/l	Odor unit	% of total odor units
n-Propyl	72		> 720	< 0.1	
n-Butyl	0.50		> 5.0	< 0.1	
sec.Butyl	1.00		> 10	< 0.1	
n-Hexyl	0.50		5.2	0.1	
n-Octyl	0.26		1.1	0.2	
n-Decyl	0.10		0.21	0.5	
n-Tetradecyl	0.50		> 5.0	< 0.1	
n-Hexadecyl	0.11		> 1.1	< 0.1	
n-Dodecyl	0.10		1.0	0.1	
Alcohol mixture I					
Total	75.10	13.0	35.0	2.1	4.7
Isobutyl	174	30.0	75.0	2.3	
opt.act.Amyl	91	16.0	32.0	2.8	
Alcohol mixture II					
Total	265	46.0	53.0	5.0	11.0
Isoamyl	232	40.0	6.5	36.0	79.0
β-Phenylethyl	4.3	0.7	7.5	0.6	1.3
Alcohols Total	576	100	12.7	45.0	

earlier (Salo, 1970a). The panel was selected from the laboratory staff and had gained some experience in the earlier threshold determinations. The triangular test was applied and a solution of grain spirit in purified water 9.4% (w/w) was used as solvent and for blank samples. If the threshold concentration proved to be more than tenfold, the concentration at which a compound was present in the beverage, precise determinations were not made. The caramel coloring was added to all the samples used in determining the thresholds of the alcohol, acid, ester or carbonyl compound mixtures and aroma fractions. The synthetic whisky imitation was compared with several types of original blended whiskies in order to assess the success of the imitation, taking into consideration that the beverage contained neither phenolic nor nonvolatile compounds. Furthermore, the beverage was mixed with a Scotch blended whisky bottled in Finland [40% (v/v) malt whisky]. The panel was required to decide at which mixing rate a difference was found between the aroma of the mixture and the Scotch whisky.

In the threshold trials of the compound mixtures and aroma fractions, samples were first presented to the panel in which the concentrations of the compounds were the same as in the artificial beverage. If the mixture was detectable at this level it was tested in diluted solutions until the threshold level was reached. Likewise, the amounts of the compounds were increased if the threshold level was above the beverage concentration. Furthermore, trials were made to determine whether the panel could detect a difference between the odor of a beverage model from which some compounds or a whole aroma fraction had been left out and the beverage composed of all the components. The purpose was to find which components could be omitted from the beverage without any perceptible alteration in the whisky-like aroma.

As individuals vary in their response to the odor of pure compounds and especially to olfactory mixtures (Baker, 1963; Sega et al., 1967; Keith and Powers, 1968), the odor detection threshold values were determined using the percentage-above-chance scores as a function of logarithmic concentration and assuming the distribution of the scores to follow the normal probability function. When thresholds are determined according to this function at the level of 50% positive responses, using a selected amateur panel, the values are mainly dependent upon the substances themselves (Salo, 1970b). The relative contribution of each component or mixture of components to the odor intensity of the aroma fraction or of the whole whisky imitation was estimated by dividing the concentration of each component or mixture of components present in the beverage by its threshold value (Guadagni et al., 1966a; Keith and Powers, 1968). The quotient was called the "odor unit" and indicated the relative importance of components in contributing to final odor characteristics. Furthermore, odor unit percentages were calculated for the alcohol, acid, ester and carbonyl mixtures and for the aroma fractions by the expression 100 times the number of odor units in the particular mixture or fraction divided by the total number of odor units in the total fraction or in the whole artificial beverage.

Table 2–Acid fraction of artificial whisky model: relationships of concentrations, threshold levels and relative contributions to total odor

Acids	Concentration mg/l	%	Odor threshold mg/l	Odor unit	% of total odor units
Propionic	0.81		20	< 0.1	
Valeric	0.05		> 0.5	< 0.1	
Enanthic	0.05		> 0.5	< 0.1	
Pelargonic	0.11		> 1.1	< 0.1	
Undecanoic	0.05		> 0.5	< 0.1	
Tridecanoic	0.05		> 0.5	< 0.1	
Myristic	1.20		>12	< 0.1	
Pentadecanoic	0.05		> 0.5	< 0.1	
Palmitic	0.92		>10	< 0.1	
Palmitoleic	1.10		>11	< 0.1	
Stearic	0.16		> 1.6	< 0.1	
Oleic	0.22		> 2.2	< 0.1	
Linoleic	0.12		> 1.2	< 0.1	
Acid mixture I					
Total	4.90	5.5	6.40	0.8	1.9
Isobutyric	2.60		8.2	0.3	
Butyric	0.81		3.4	0.2	
Caproic	2.30		8.6	0.3	
Acid mixture II					
Total	5.70	6.4	1.70	3.3	8.4
Acetic	36.0	40.0	26.0	1.4	
Isovaleric	3.2	3.6	0.75	4.3	
Caprylic	14.0	16.0	15.0	1.0	
Capric	17.0	19.0	9.4	1.8	
Lauric	8.8	9.8	7.2	1.2	
Acid mixture III					
Total	79.0	88.0	1.40	57.0	144.0
Acids Total	90.0	100	2.30	39.1	

RESULTS

WHEN THE whisky model was compared with original blended whiskies, it was considered to differ from the model in that the smoky aroma typical of Scotch malt whisky was less intense. Some kind of sweet nuance foreign to the aroma of original whiskies was noticed, and obviously indicates slightly too large amounts of esters or higher alcohols. The panel was able to differentiate between the artificial beverage and the Scotch blended whisky bottled in Finland in 93% of trials above chance ($p < 0.001$). However, when the artificial beverage and the whisky were mixed in the ratio 1:1 and the mixture compared with the original whisky, only 6% correct judgments above chance were obtained. The difference was significant when the proportion of the artificial beverage was 60% ($p < 0.05$) and highly significant when it was 70% ($p < 0.001$). These results were obtained with the laboratory panel; some trained experts could find a difference in mixtures with a proportion of 45% of artificial beverage.

Alcohols

The threshold values and quantity percentages of the alcohols and mixtures of alcohols, and their odor unit values are shown in Table 1. Only three alcohols (isoamyl, opt.act.amyl and isobutyl) have thresholds which are beneath their beverage concentrations. Isoamyl alcohol has the highest individual odor unit value of all the studied compounds. Isobutyl and opt.act.amyl alcohols have both about the same odor unit value as the whole mixture of minor alcohols (alcohol mixture I) 96% of which consists of propyl alcohol. However, some alcohols present in very small quantity (decyl, dodecyl and octyl alcohols) have the lowest threshold levels found among alcohols. The threshold value of β-phenylethyl alcohol proved to be so much lower than the level of isobutyl and opt.act.amyl alcohols that it could not be added to the mixture of these compounds. But its relative contribution to odor intensity is so great that it could not be added to the mixture of the minor alcohols, since its smell masked that of the others and the threshold level of the mixture decreased. The threshold level of the whole alcohol fraction is about twice that of isoamyl alcohol, the contribution of which comprises the bulk of the odor intensity in the alcohol fraction. The combined contribution of isobutyl and opt.act.amyl

alcohols is only about 1/7 of that of isoamyl alcohol. However, the contribution of the mixture of minor alcohols is greater than that shown by β-phenylethyl alcohol alone.

When all the alcohols were left out of the synthetic whisky the panel gave only 5–14% correct judgments above chance, and did not notice any difference between it and the whole beverage. The results did not change when six esters with important olfactory contribution were also omitted. Consequently, the relative contributions of the individual alcohols could not be studied.

Acids

In the acid fraction of the aroma (Table 2) five acids (acetic, isovaleric, caprylic, capric and lauric acids) have threshold levels lower than the respective beverage concentrations. Isovaleric acid contributes most to odor intensity in spite of its small concentration. Acetic acid with a tenfold concentration has an odor unit value only 1/4 that of isovaleric acid. Altogether 13 acids (acid mixture I) have insignificant odor unit values. The mixture of five acids (acid mixture III) has an odor unit value higher than the sum of the individual units. The relative contribution of these five acids proved to be greater than that of the whole acid fraction.

When all the acids were omitted from the synthetic whisky the panel gave only 10% correct judgments above chance. But when the ester mixture III was left out in addition to the acids, they gave 30% correct judgments above chance which represented significant differentiation ($p < 0.05$) between the incomplete and the whole beverages. When acetic acid and one or two of the other four acids (especially isovaleric and capric acids) were substituted for the acid fraction, the panel no longer noticed the difference. But acetic acid alone or together with the minor acids (acid mixture I or II) was not sufficient as a substitute.

Esters

The ester fraction of the aroma (Table 3) contains six esters (ethyl acetate, ethyl caproate, ethyl caprylate, ethyl caprate, ethyl laurate and isoamyl acetate), which have threshold levels below the concentration at which they are present in the synthetic whisky. All these esters proved to have nearly the same odor unit value–about ten units. The sum of the individual odor unit values of these six esters is also lower than the value of the whole mixture (ester mixture III). Nevertheless, the threshold value of this mixture is the highest of the ester mixtures. β-Phenylethyl acetate and β-methyl-γ-octalactone have odor unit values higher than one unit but the relative contribution of their mixture to the total odor intensity of the ester fraction is smaller than that of ester mixture I where the threshold levels of compounds are much higher than their concentrations in the beverage. In general the odor unit percentages of the ester mixtures seem to follow their concentration percentages. The threshold level of the whole ester fraction proved to approach the level of the mentioned six esters with the exception of ethyl acetate, the threshold of which deviated from the others.

When all the esters were omitted from the synthetic whisky, the judgments were 55% correct above chance, representing highly significant differentiation ($p < 0.001$). The results were not changed by the addition of ethyl acetate alone or the ester mixture I or β-phenylethyl acetate and β-methyl-γ-octalactone. If five esters of the ester mixture III or ethyl acetate and three of these esters were added, the proportion of correct judgments increased to 25–30% ($0.01 < p < 0.05$) indicating a possible sensory difference. When all six esters were added, the percentage of correct judgments was only equal to that of chance.

Table 3–Ester fraction of artificial whisky model: relationships of concentrations, threshold levels and relative contributions to total odor

Esters	Concentration mg/l	Concentration %	Odor threshold mg/l	Odor unit	% of total odor units
Ethyl propionate	0.36		> 4.0	< 0.1	
Ethyl-n-butyrate	0.10		0.15	0.7	
Ethyl lactate	1.80		14.0	0.1	
Ethyl myristate	0.57		> 5.7	< 0.1	
Ethyl palmitate	1.40		> 14	< 0.1	
Ethyl palmioleate	1.90		10.0	0.2	
Ethyl stearate	0.05		> 0.5	< 0.1	
Ethyl oleate	0.10		0.87	0.1	
Ethyl linoleate	0.10		0.45	0.2	
Isobutyl acetate	0.21		3.40	0.1	
Isoamyl isobutyrate	0.10		0.25	0.4	
Isoamyl isovalerate	0.10		> 1.0	< 0.1	
Isoamyl-n-valerate	0.001		> 0.1	< 0.1	
Isoamyl caproate	0.20		1.40	0.1	
Isoamyl caprylate	0.17		0.60	0.3	
Isoamyl caprate	0.50		> 5.0	< 0.1	
Ester mixture I					
Total	7.70	6.0	0.63	12.0	4.1
β-Phenylethyl acetate	1.20		0.70	1.7	
β-Methyl-γ-octalactone	0.22		0.051	4.3	
Ester mixture II					
Total	1.40	1.1	0.16	8.9	3.0
Ethyl acetate	100	77.0	17.0	5.9	
Ethyl caproate	0.85	0.7	0.076	11.0	
Ethyl caprylate	2.80	2.2	0.24	12.0	
Ethyl caprate	7.70	6.1	1.10	7.0	
Ethyl laurate	5.50	4.5	0.64	8.6	
Isoamyl acetate	2.60	2.0	0.23	12.0	
Ester mixture III					
Total	120	93.0	0.87	137.0	46.0
Esters Total	129	100	0.44	296.0	

Carbonyl compounds

The carbonyl compounds (Table 4) are divided into two clearly separate groups, one comprising acetaldehyde, propionaldehyde, furfural and 2,3-pentanedione with rather low odor unit values, and the other comprising butyr-, isobutyr-, valer- and isovaleraldehyde and diacetyl, which all have very high odor unit values. The threshold values of butyraldehyde and diacetyl are the lowest found among the compounds studied. Both the threshold level of the carbonyl compound mixture II and that of the whole carbonyl fraction are near these very low threshold levels. Isobutyraldehyde has the lowest odor unit value in the carbonyl compound mixture II in spite of having the highest concentration. Furthermore the sum of the odor unit values of the five individual carbonyl compounds is lower than the value of the whole mixture. The odor unit value of the carbonyl compound mixture I is about the same as the

value of butyr-, or isovaleraldehyde alone. The odor unit percentage of the carbonyl compound mixture I is only one 30th of the percentage of the main carbonyl compound mixture II.

When the whole carbonyl compound fraction was omitted from the synthetic whisky, 58% correct judgments above chance were received, which indicated a highly significant difference ($p < 0.001$). The re-addition of the carbonyl compound mixture I did not change the results significantly. When the three carbonyl compounds largest in quantity (isobutyr-, isovaler- and valeraldehyde) were re-added to the beverage, the difference was no longer significant. Furthermore, the carbonyl compounds in smaller quantities—although larger in number—could not substitute for the carbonyl fraction of the aroma.

Artificial whisky model

Data on all the mixtures and aroma fractions of the whisky imitation have been collected in Table 5 to provide a survey of the contribution of these mixtures and fractions to the total odor intensity. The threshold value of the whole artificial beverage is nearest to that of the ester fraction, and is about halfway between the thresholds of the carbonyl compounds and the alcohols, being a little closer to the level of the more important carbonyl compounds with low threshold values.

Both the odor unit values and the odor unit percentages of the alcohols and acids are about equal although the concentration percentage of the alcohols is 70% and that of the acids only 11%. Both these fractions make a very small contribution to the total odor intensity, their united contribution being only about 7.5% of the total odor intensity. The concentration percentage of the ester fraction is only a little greater than that of the acids but the odor contribution of the esters is about eight times as high as that of the acids and constitutes more than a quarter of the total odor intensity of the artificial beverage. The carbonyl compounds, which constitute only 2.2% of aroma components of the beverage, contribute more than half of the total odor intensity. The contributions of butyr-, isobutyr-, valer- and isovaleraldehyde and diacetyl are especially high, amounting to nearly half of the total intensity.

The next most important components are the main esters (ester mixture III), the contribution of which amounts to about one-fifth of the total odor intensity. Further, the contribution of the acid mixture III amounts to 5%, but the odor unit percentage of the whole acid fraction is smaller than the percentage of these five acids. In the alcohol fraction, isoamyl alcohol contributes nearly the whole proportion of the odor intensity of the fraction. The odor unit value of diacetyl is quite near that of isoamyl alcohol in spite of its low concentration. The mixtures of the minor acids and alcohols show only 0.1–0.2% contribution to the total odor intensity but the ester mixture I and the carbonyl compound mixture I contribute about ten times as much.

DISCUSSION

THE VOLATILE aroma fraction of whisky consists of numerous components. Nevertheless, it is becoming obvious that not all these components can be equally important in respect to sensory evaluation. Investigations have further shown (Guadagni et al., 1966b) that knowledge

Table 4—Carbonyl compound fraction of artificial whisky model: relationships of concentrations, threshold levels and relative contributions to total odor

Carbonyl compounds	Concentration mg/l	Concentration %	Odor threshold mg/l	Odor unit	% of total odor units
Acetaldehyde	2.80	16.0	1.20	2.3	
Propionaldehyde	0.48	2.8	2.00	0.2	
Furfural	1.10	6.4	5.80	0.2	
2,3-Pentandione	0.12	0.7	0.078	1.6	
Carbonyl mixture I					
Total	4.50	26.0	0.25	18.0	2.7
Isobutyraldehyde	8.00	46.0	1.30	6.2	
n-Butyraldehyde	0.56	3.2	0.028	20.0	
Isovaleraldehyde	2.50	14.0	0.12	21.0	
n-Valeraldehyde	1.20	7.0	0.11	11.0	
Diacetyl	0.60	3.5	0.020	30.0	
Carbonyl mixture II					
Total	12.90	74.0	0.024	540	82.0
Carbonyl compounds					
Total	17.40	100	0.027	660	

Table 5—Synopsis of data on aroma constituents of artificial beverage imitating whisky

Constituent	Concentration mg/l	Concentration %	Odor threshold mg/l	Odor unit	% of total odor units
Alcohol mixture I	75	9.2	35.0	2.1	0.2
Alcohol mixture II	265	32.6	53.0	5.0	0.4
Isoamyl alcohol	232	28.5	6.5	36.0	3.2
β-Phenylethyl alcohol	4.3	0.5	7.5	0.8	0.1
Total alcohols	576	70.8	12.7	45.0	4.0
Acid mixture I	4.9	0.6	6.4	0.8	0.1
Acid mixture II	5.7	0.7	1.7	3.3	0.3
Acid mixture III	79.0	9.8	1.4	57.0	5.1
Total acids	90.0	11.1	2.3	40.0	3.5
Ester mixture I	7.7	1.0	0.63	12.0	1.1
Ester mixture II	1.4	0.2	0.16	8.9	0.8
Ester mixture III	120.0	14.7	0.87	137.0	12.1
Total esters	129.0	15.9	0.44	296.0	26.0
Carbonyl mixture I	4.5	0.6	0.250	18.0	1.6
Carbonyl mixture II	12.9	1.6	0.024	540.0	48.0
Carbonyl compounds	17.4	2.2	0.027	660.0	58.0
Total beverage	813	100	0.72	1130	

of the amount of a volatile component is not sufficient to tell how much a particular component contributes to the total aroma of a product. The determination of the odor threshold levels of individual components and some mixtures made it possible to estimate the contribution of the components and mixtures to the total odor intensity.

The threshold determinations of individual components of a synthetic whisky imitation indicate that many acids and alcohols but also some esters and two carbonyl compounds make a low contribution to the total odor intensity. Moreover, there are components with rather high concentrations but also rather high threshold levels, e.g., propyl and isobutyl alcohols and acetic acid, whose contribution to the total odor intensity is also slight. A third group consists of components with rather low threshold levels, whose concentrations are also so low that their contribution is not particularly important. Examples are decyl and dodecyl alcohol, ethyl butyrate, isoamyl isobutyrate and β-methyl-γ-octalactone. On the other hand, the threshold determinations revealed components, especially the carbonyl compounds, which make a very important contribution to the total odor intensity despite their small concentrations. These findings regarding different types of components are in agreement with the flavor threshold values of Harrison (1970), taking into consideration the differences in the composition of beer and whisky.

The threshold values merely represent the situation when a compound is alone in a medium, here a solution of rectified grain spirit and water. However, the aroma of an alcoholic beverage is typically a mixture of many components, and moreover, the quantities and the relations of these components can vary within certain limits without any considerable change in the total aroma (Sega et al., 1967; Suomalainen and Nykänen, 1970). The results indicate that the prepared whisky imitation can be mixed with a Scotch blended whisky without any significant change in the whisky aroma. In view of these findings it can be assumed that the relative contributions of, and the interactions among the aroma components and their mixtures revealed by this synthetic whisky model, reflect the composition of the aroma intensity in original whiskies.

An examination of the relative contributions of the aroma constituents to the total odor intensity indicates that the esters and carbonyl compounds contribute the greatest part. Especially some carbonyl compounds with the quantity proportion of 1.6% (butyr-, isobutyr-, valer- and isovaleraldehyde and diacetyl) and a few esters with the combined quantity proportion of 15% (ethyl acetate, ethyl caproate, ethyl caprylate, ethyl caprate, ethyl laurate, and isoamyl acetate) may be considered to constitute the core of the total odor intensity in a beverage like whisky. This does not mean that these components alone can create the harmonious, mellow aroma of whisky, but their contribution to aroma intensity is considerable. The contribution of the carbonyl compounds is great in relation to their concentration. Nevertheless, the observation that three main carbonyl compounds could substitute for the whole carbonyl fraction may indicate the homogeneity of the carbonyl fraction. A fixed total quantity of pungent-smelling carbonyl compounds seems to be necessary for the complete whisky aroma regardless of which these carbonyl compounds are. In the ester fraction, altogether six main esters were needed to substitute for the whole ester fraction. This finding may indicate that the aroma of the ester fraction is richer and fuller than the carbonyl fraction with its sharp and pointed aroma. It may also be noted that those six esters constitute so great a proportion of the ester fraction, both quantitatively and from the standpoint of odor, that the effect of the other esters on the odor intensity necessarily remains rather small.

The combined contribution of alcohols and acids is rather low, amounting to less than 1/10 the total odor intensity, although the concentration of the alcohol fraction is fully 70%. Because the omission of the alcohol fraction produced no detectable difference to the total intensity of the whisky imitation, the contribution of isoamyl alcohol also remains low in spite of its high individual odor unit value. β-Phenylethyl alcohol has no noteworthy effect on the total odor intensity in the concentration used here, which usually corresponds to that in Scotch whiskies. The lowness of its contribution partly explains the findings of Sega et al. (1967) that variations in the amounts of β-phenylethyl alcohol had no effect on the flavor of beer. The same observation also applies to ethyl acetate in the ester fraction.

In the acid fraction, it is interesting that the contribution of the main acids (acid mixture III) to the total odor intensity is greater than that of the whole acid fraction. The contribution of these five acids thus decreases when they are mixed with the other minor acids. It is also noteworthy that these five compounds are the only acids present in the artificial beverage at concentrations above their threshold levels. Baker (1963) noticed synergism with an aqueous mixture of eight organic compounds on equivalent threshold levels, but Keith and Powers (1968) concluded that sub-threshold additive effects are not common. In view of these findings and the results of this study, it seems that suppression of olfactory stimulation may occur in the acid fraction, i.e., the odor of the combined components is less than that which would result from direct addition. With the mixtures of volatile aroma compounds whose concentrations exceeded their threshold levels, e.g., the carbonyl compound mixture II and ester mixture III, clear synergism was observed, while an additive effect seems more probable only in the mixtures composed of few components, e.g., the mixture of isobutyl and opt.act.amyl alcohols. The mixtures of the components with sub-threshold concentrations, especially the mixture of 13 minor acids (acid mixture I), show no additive effects but without exact threshold values it is difficult to say anything certain about possible suppression effects.

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Ms received 11/10/71; revised 2/16/72; accepted 2/24/72.

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BIOCHEMISTRY OF TEA FERMENTATION: PRODUCTS OF THE OXIDATION OF TEA FLAVANOLS IN A MODEL TEA FERMENTATION SYSTEM

INTRODUCTION

FRESH GREEN TEA leaf is converted to the black tea of commerce by a manufacturing process (Eden, 1965; Hainsworth, 1969; Harler, 1963) which depends on an endogenous enzymic oxidation of tea leaf constituents for the major chemical changes which take place (Roberts, 1962; Sanderson, 1972). While many of the relationships between the tea leaf flavanols and the pigments found in black tea extracts have now been accurately worked out (Sanderson, 1972), the reactions which lead to the formation of certain black tea pigments, especially the thearubigins which are the predominant pigments, remain uncertain. To clarify this situation, an investigation was carried out in which several combinations of tea flavanols were oxidized in a model tea fermentation system so that relationships between black tea constituents and their precursors in fresh tea leaf could be established. The results of this investigation corroborate and extend an earlier study carried out by Nakagawa and Torii (1965).

EXPERIMENTAL

Fresh tea leaf

Fresh tea leaf—i.e., rapidly growing shoot tips consisting of the apical bud, the first two leaves, and the included stems—was procured from tea plants growing in an experimental garden (Co and Sanderson, 1970).

Flavanols

ECG, EGC, and EGCG were extracted from fresh tea leaf material, separated by chromatography and purified by crystallization in our laboratory using previously reported procedures (Co and Sanderson, 1970). (Abbreviations used in this paper are summarized in Table 1.) EC and Cat were purchased from Mann Research Labs. and Pierce Chemical Co., respectively. These flavanols were all recrystallized from water and were found to be chromatographically pure before use.

All other chemicals were of A.R. grade.

Soluble tea enzymes preparation

This preparation was made according to procedures described by Co and Sanderson (1970) and Sanderson (1964).

Model tea fermentation system

The oxidations were carried out in jacketed reaction vessels held at 30°C by means of a circulating water bath. The reaction mixture was made up as follows: The tea flavanols were weighed into the reaction flasks in amounts which would produce a final total flavanol concentration of 0.05M. The flavanols were always at equal molarity when mixtures of flavanols were used as substrates. The flavanols were then completely dissolved in 10 ml of citrate-phosphate buffer, about 0.1M, pH 5.4 (Gomori, 1955). The oxidation reaction was started by adding 1.0 ml of the soluble tea enzymes preparation. The enzymic oxidation was continued for 30 min during which time the reaction mixture was aerated continuously by stirring vigorously with a magnetic stirrer. The reaction was stopped by adding 40 ml of methanol to the reaction mixture. Precipitation of the enzymes was ensured by placing the methanolic reaction mixture in an ice water bath for 10 min. Finally, the reaction mixture was clarified by centrifuging for 15 min at 12,000 × G at 0°C.

The same procedure was followed in the study of the dynamics of the oxidation of EC and/or EGCG except that the reaction was stopped after oxidation periods of varying lengths as indicated in Table 2.

Chromatography of reaction products

After methanol treatment and clarification, oxidized reaction mixtures were taken to dryness in a rotary vacuum evaporator at a temperature not exceeding 30°C. The residues were dissolved in 1 ml of water, 0.5 ml of butanol, and 0.5 ml of ethyl acetate. The organic layer of the two-phase system that forms contains most of the polyphenolic material present.

A 40 μl aliquot of the organic layer was

Table 1—Probable relationship between oxidation products described in tea fermentation oxidation products found in this investigation and those reported by Roberts (1962) and by Nakagawa and Torii (1965)

Name of compound	Designation this investigation	Roberts (1962)	Nakagawa and Torii (1965)
(−)-Epicatechin (I)	EC	2	
(−)-Epicatechin-3-gallate (II)	ECG	6	
(−)-Epigallocatechin (III)	EGC	1	
(−)-Epigallocatechin-3-gallate (IV)	EGCG	5	
(+)-Catechin (V)	Cat	4	
Theaflavin (VI)	TF	X	X
Theaflavin gallate A (VII)	TFG	Y	Y_2
Theaflavin gallate B (VIII)			Y_3
Theaflavin digallate (IX)			Y
Bisflavanol A (X)	A	A	A
Bisflavanol B (XI)	B	B	B,11(?)
Bisflavanol C (XII)	C	C	C
Gallic acid (XIII)	GA	7	7
Epitheaflavic acid (XIV)	ETA	Q	Q
3-Galloyl epitheaflavic acid (XV)	ETAG		
Ellagic acid	EA	–	–
Unknown P	37	P (tricetinidin?)	12,18,20,21
Unknown R	36	R (flavanotropolone?)	13,22
Unknown Z	35	Z (like ellagic acid)	14,23
Unknown D	16,17	D	15,16,17,24
Unknowns 15,30,31,33	15,30,31,33	–	
Thearubigins, R_f = 0–0.5	S_A	S_{IA}, S_I, and S_{II}	S
Thearubigins, R_f = 0–0.7	S_B		
Thearubigins, R_f = 0–1.0	S_C		
Thearubigins, R_f = 0.3–0.7	S_D		
Thearubigins, R_f = 0.5–1.0	S_E		
Thearubigins, R_f = 0.3–1.0	S_F		

spotted on Whatman No. 1 paper. The chromatograms were developed descendingly using the following solvent systems:

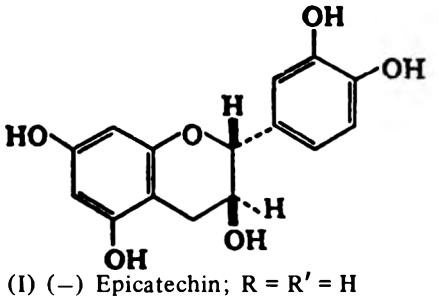
1st direction: butanol/acetic acid/water (4:1:2.2)

2nd direction: 2% acetic acid

The dried chromatograms were viewed under visible and ultraviolet light before and after fuming with ammonia vapors, and all detected spots were marked. Developed chromatograms were dipped in a solution of 0.25% ferric chloride–0.25% potassium ferricyanide after which they were washed twice in ~ 30 mM hydrochloric acid followed by a third wash in water. This process produced chromatograms on which the flavanols and their oxidation products were visualized as blue spots on a permanent white background.

RESULTS & DISCUSSION

VARIOUS COMBINATIONS of the major flavanols found in tea leaves—namely, (−)-epicatechin (EC, I), (−)-epicatechin-3-gallate (ECG, II), (−)-epigallocatechin (EGC, III), (−)-epigallocatechin-3-gallate (EGCG, IV) and (+)-catechin (Cat, V)—were oxidized in a model tea fermentation system to determine which of these compounds were precursors of the various polyphenolic compounds present in black tea. Oxidation products formed in this model tea fermentation system were determined by paper chromatography of the reaction mixtures. A composite diagrammatic representation of the chromatograms obtained is shown in Figure 1. The striking similarity between this composite chromatogram and similar chromatograms prepared of black tea extracts (see figures given by Roberts, 1962) suggests that the reactions studied in this investigation represent a significant portion of those taking place in tea leaf during the conversion of fresh tea leaf to black tea.

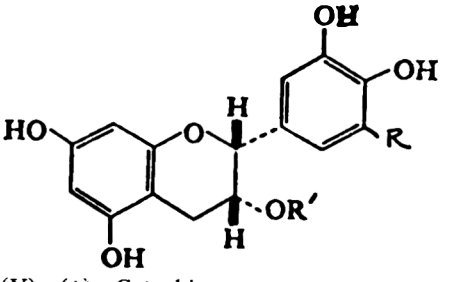


(I) (−) Epicatechin; R = R′ = H

(II) (−) Epicatechin gallate; R = H, R′ = 3,4,5-trihydroxybenzoyl

(III) (−) Epigallocatechin; R=OH,R′=H

(IV) (−) Epigallocatechin gallate;R=OH R′ = 3,4,5-trihydroxybenzoyl



(V) (+) Catechin

The results of these studies are summarized in Table 3, and the relationship between the substances identified in this investigation and investigations carried out by Roberts (1962) and by Nakagawa and Torii (1965) are shown in Table 1. The following generalizations which can be made on the basis of the results of this investigation are noteworthy: First, the epigallocatechins EGC and EGCG are more readily oxidized than the epicatechins EC and ECG in agreement with results reported earlier by Roberts and Wood (1950). In the experiments summarized in Table 3, the reaction period was chosen so that some of the substrate material would be remaining at the time the reaction was stopped. Under these conditions, the epigallocatechins completely disappeared, whereas residual amounts of the epicatechins remained unoxidized. However, as will be described below, it was shown in other experiments that the epicatechins were completely oxidized when the reaction period was extended.

Theaflavins (VI–IX) were found in oxidized model tea fermentation systems which had contained the appropriate flavanols. The results obtained in this inves-

Table 2—Summary of reaction products found[a] in a model tea fermentation system oxidizing EC and/or EGCG. (See Experimental section for conditions used)

Oxidation period (min)	Phenolic compounds present in reaction mixtures								
							Bisflavanols		
	EC	EGCG	GA	TFG	Thearubigins	Streaking	31	A	17
Experiment A: Substrate = EC only									
0	+++				−	−			
5	+++				+(S_A)	−			
10	++				+(S_A)	+			
15	++				+(S_A)	+			
20	+				+(S_A)	−			
40	−				+(S_A)	−			
60	−				++(S_A)	−			
140	−				++(S_A)	−			
Experiment B: Substrate = EGCG only									
0		+++	−		−	tr	tr	−	−
5		+++	+		+(S_C)	++	++	+	−
10		++	+		++(S_C)	+++	++	+	−
15		++	+		++(S_C)	+++	+++	++	+
20		+	++		+++(S_C)	+++	+++	+++	+
40		−	++		+++(S_C)	++	++	++	+
60		−	+		+++(S_B)	++	+	++	+
140		−	+		+++(S_B)	++	+	+	+
Experiment C: Substrate = EC + EGCG									
0	+++	+++	−	−	−	++	tr	−	−
5	+++	++	+	+	+(S_C)	+++	+	+	+
10	+++	++	++	++	+(S_C)	+++	++	++	+
15	++	+	+	+++	++(S_C)	+++	++	++	+
20	++	−	+	+++	++(S_C)	+++	−	++	+
40	+	−	+	++	+++(S_C)	++	−	+	−
60	+	−	−	+	+++(S_B)	++	−	−	−
140	−	−	−	−	+++(S_B)	+	−	−	−

[a]Quantitations indicated are estimates of spot intensities on paper chromatograms. Key to abbreviations used: +++ = dark spot; ++ = medium spot; + = light spot; tr = trace spot; − = no spot.

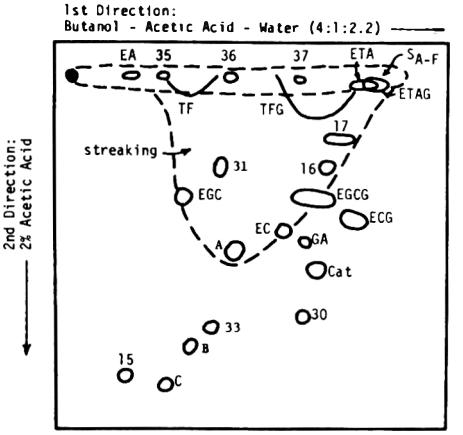


Fig. 1—Composite of chromatograms of oxidation products formed in model tea fermentation systems. (See text for experimental details. Key to abbreviations used is given in Table 1.)

Table 3–Oxidation products found in a model tea fermentation system containing various combinations of tea flavanols

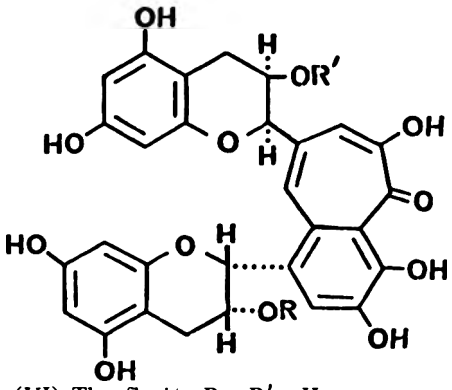
Model tea fermentation system no.	Flavanols[a] added to reaction mixture as substrates	Flavanols remaining in system at end of reaction period	New phenolic compounds formed					
			Theaflavins	Epitheaflavic acids	Bisflavanols	Others	Thearubigins	Streaking
1	Cat	+	–	–	–	16,17	S_B	–
2	EC	+	–	–	–	–	S_A	–
3	ECG	+	–	+	–	EC,GA,EA	S_C	–
4	EGC	–	–	–	C	15,30,36	–	–
5	EGCG	–	–	–	A	GA, 31	S_C	+
6	Cat EC	+ +	–	–	–	17	S_B	–
7	Cat ECG	+ +	–	+	–	EC	S_E	–
8	Cat EGC	+ –	–	–	C	15,17,30,36	S_D	–
9	Cat EGCG	+ –	–	–	A	EA,17,31	S_B	+
10	EC ECG	+ +	–	+	–	GA,EA	S_C	–
11	EC EGC	+ –	TF	–	C	15,30,36	S_A	–
12	EC EGCG	+ –	TF,TFG	–	–	GA	S_B	+
13	ECG EGC	+ –	TF,TFG	+	C	GA,EA,15,33,35	S_C	–
14	ECG EGCG	+ –	TFG	+	A	EC,GA,EA,31	S_C	+
15	EGC EGCG	– +	–	–	A,B,C	GA,15,31,33,36	S_D	+
16	Cat EC ECG	+ + +	–	+	–	EA,17	S_C	–
17	Cat EC EGC	+ + –	–	–	C	15,17,36	S_B	–
18	Cat EC EGCG	+ + –	TF	–	–	17	S_B	–
19	Cat EGC EGCG	+ – –	–	–	B,C	15,17,33,36,37	S_D	+
20	EC ECG EGC	+ + –	TF,TFG	+	C	GA,EA,15,33,35,36	S_F	–
21	EC ECG EGCG	+ + –	TFG	+	–	GA,EA,35	S_C	+
22	ECG EGC EGCG	+ – –	TFG	+	C	EC,GA,33,36	S_C	+
23	EGC EGCG EC	– – +	TF,TFG	–	B,C	GA,15,33,36	S_B	+
24	Cat ECG EGCG	+ + –	TFG	+	–	GA	S_C	+

[a]Key to abbreviations used is given in Table 1.

Table 3–Oxidation products found in a model tea fermentation system containing various combinations of tea flavanols (Continued)

Model tea fermentation system no.	Flavanols[a] added to reaction mixture as substrates	Flavanols remaining in system at end of reaction period	New phenolic compounds formed					
			Theaflavins	Epitheaflavic acids	Bisflavanols	Others	Thearubigins	Streaking
25	Cat	+	TF,TFG	+	C	15,17	S_D	–
	EC	+						
	ECG	+						
	EGC	–						
26	EC	+	TF,TFG	+	B,C	GA,15,33,36	S_F	+
	ECG	+						
	EGC	–						
	EGCG	–						
27	ECG	+	TF,TFG	+	B,C	EC,15,33,36	S_F	–
	EGC	–						
	EGCG	–						
	Cat	+						
28	EGC	–	TF	–	B,C	15,17,33,36	S_B	+
	EGCG	–						
	Cat	+						
	EC	+						
29	EGC	–	TFG	+	A,B,C	EC,15,31,33,36,37	S_F	+
	EGCG	–						
	Cat	+						
	ECG	+						
30	EGCG	–	TF,TFG	+	B,C	31,33,36,37	S_F	+
	EGC	–						
	ECG	+						
	Cat	+						
	EC	+						

[a]Key to abbreviations used is given in Table 1.



(VI) Theaflavin; R = R′ = H

(VII) Theaflavin gallate A; R=H;R′=3,4,5-trihydroxybenzoyl

(VIII) Theaflavin gallate B; R=3,4,5-trihydroxybenzolyl;R′=H

(IX) Theaflavin digallate; R = R′ = 3,4,5-trihydroxybenzoyl

tigation (Table 3) are consistent with the formation of theaflavins according to reactions (1) through (4) which are consistent with the reaction given for the formation of theaflavin by Takino et al. (1964):

(1) EC (I) + EGC (III) + 3/2 O_2 ⟶ Theaflavin (VI) + CO_2

(2) EC (I) + EGCG (IV) + 3/2 O_2 ⟶ Theaflavin gallate A (VII) + CO_2

(3) ECG (II) + EGC (III) + 3/2 O_2 ⟶ Theaflavin gallate B (VIII) + CO_2

(4) ECG (II) + EGCG (IV) + 3/2 O_2 ⟶ Theaflavin digallate (IX) + CO_2

The three theaflavin gallate compounds predicted by these reactions were resolved by Nakagawa and Torii (1965), and they have been isolated and fully characterized by Bryce et al. (1970) and by Coxon et al. (1970b).

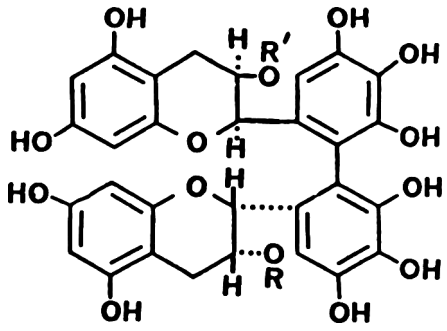
The bisflavanols A, B and C were formed (Table 3), as expected, according to reactions (5), (6) and (7) (Roberts, 1962; Vuataz and Brandenberger, 1961):

(5) 2 EGCG (IV) + 1/2 O_2 ⟶ Bisflavanol A (X) + H_2O

(6) EGCG (IV) + EGC (III) + 1/2 O_2 ⟶ Bisflavanol B (XI) + H_2O

(7) 2 EGC (III) + 1/2 O_2 ⟶ Bisflavanol C (XII) + H_2O

These bisflavanols have been fully characterized by Ferretti et al. (1968).

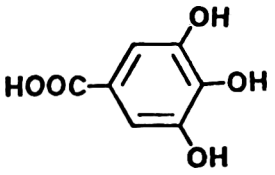


(X) Bisflavanol A; R = R′ = 3,4,5-trihydroxybenzoyl

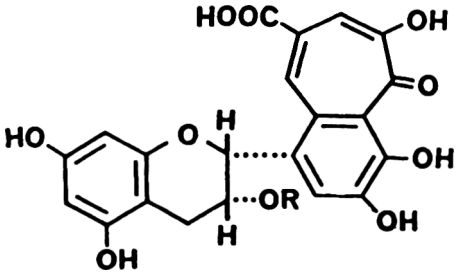
(XI) Bisflavanol B; R = 3,4,5-trihydroxybenzoyl; R′ = H

(XII) Bisflavanol C; R = R′ = H

Deesterification of the gallated flavanols, ECG and EGCG, in these model tea fermentation systems was evidenced by the detection of gallic acid (XIII) in most of the oxidized model systems which had contained one or both of these flavanols (Table 3): Similar results were obtained with model tea fermentation



(XIII) Gallic acid



(XIV) Epitheaflavic acid; R = H

(XV) 3-Galloyl epitheaflavic acid; R = 3,4,5-trihydroxybenzoyl

systems by Nakagawa and Torii (1965), and free gallic acid was shown to be formed in whole tea leaf systems undergoing tea fermentation by Roberts and Myers (1959). Accordingly, it was to be expected (Berkowitz et al., 1971) that epitheaflavic acids (XIV, XV; Coxon et al., 1970a; Bryce et al., 1970) would be formed when one of the epicatechins (either EC or ECG) and a gallated flavanol (either ECG or EGCG) were present in the model system according to the following reactions:

(8) EC (I) + GA (XIII) + 3/2 O_2 ⟶ Epitheaflavic acid (XIV) + CO_2

(9) ECG (II) + GA (XIII) + 3/2 O_2 ⟶ 3-Galloyl-epitheaflavic acid (XV) + CO_2

These reaction products have been fully characterized by Bryce et al. (1970) and by Coxon et al. (1970a). When no epitheaflavic acids were detected even though the potential to form these compounds existed in the model system, it was assumed that the epitheaflavic acids had been transformed to thearubigins (Berkowitz et al., 1971). The readiness with which the epitheaflavic acids are oxidized to thearubigins probably accounts for their presence in black tea at only low levels (Collier and Mallows, 1971; Bryce et al., 1972).

Thearubigins were formed (Table 3) in model tea fermentation systems by the oxidation of the individual tea flavanols (with the possible exception of EGC) and all combinations of these flavanols. These results show clearly that thearubigins are not necessarily formed from theaflavins as had been proposed earlier (Roberts, 1962). The structure of the thearubigins has not been accurately determined although it has been shown by Brown et al. (1969a, b) that the thearubigins are polymeric proanthocyanogens.

Streaking (Fig. 1, Table 3) was characteristic of all chromatograms of oxidized model tea fermentation systems which had contained EGCG. It is noteworthy that this streaking is also characteristic of black tea extracts.

Some "other" unidentified tea flavanol oxidation products were noticed (Table 3). Examination of the results summarized in Table 1 shows that the following relationships exist between these oxidation products and the tea flavanols:

(10) Cat. (V) ⟶ Unknowns 16, 17

(11) EGC (III) ⟶ Unknowns 15, 30, 34, 36

(12) EGCG (IV) ⟶ Unknown 31

(13) ECG (II) + EGC (III) ⟶ Unknown 35

(14) Cat. (V) + EGC (II) + EGCG (IV) ⟶ Unknown 37

The aforementioned results were extended in a study of the dynamics of the oxidation of EC and/or EGCG: These particular tea flavanols were chosen for their availability and their involvement in the formation of TFG, reaction (2). The results of this study are summarized in Table 2.

It was found that the exact position and extent of the thearubigins streak on paper chromatograms varied (Fig. 1, Table 1) depending on which tea flavanols were being oxidized in the model tea fermentation system when the oxidation period was fixed. However, these thearubigins streaks all intergraded, and they tended to merge into a single thearubigins streak with lower R_f values as the oxidation period was extended. In separate experiments with several of the model tea fermentation systems, it was shown that after very long oxidation periods, i.e., about 6 hr, the only extractable and chromatographable substances remaining in the reaction mixtures were thearubigins with R_f values ranging from 0 to < 0.1 in BAW solvent. It is noteworthy that the rate of change in these model tea fermentation systems declines sharply when the supply of unoxidized tea flavanols is depleted (Table 2) as has been shown previously (Coxon et al., 1970b).

The results of this investigation indicate that the thearubigins are a heterogeneous mixture of oxidation products derived from the oxidative condensation of all the tea flavanols present in the tea fermentation system. This view is consistent with the work on the chemical characterization of the thearubigins which has been carried out by Brown et al. (1969a, b). Further, the results of this investigation suggest that the thearubigins themselves undergo continuous further oxidation and polymerization as the tea fermentation process proceeds. The finding (Millin et al., 1969) that tea pigments increase in molecular weight on aging in solution is probably a reflection of these same changes in thearubigins.

It is noteworthy that virtually all of the known black tea pigments (Roberts, 1962; Sanderson, 1972) were found (Table 3) to be formed from the five tea leaf flavanols in the model tea fermentation system studied. In fact, it appears as though the only enzymic reaction which takes place during tea fermentation is the oxidation of the tea flavanols which leads to the formation of the black tea pigment system and modifies the taste of the tea leaf infusions (Roberts, 1962; Nakagawa and Torii, 1965; Berkowitz et al., 1971). The black tea flavor appears to be largely developed as a result of secondary oxidations involving amino acids, carotenes, and other, as yet unelucidated, flavor precursors and oxidized flavanol molecules which act as oxidizing agents (Co and Sanderson, 1970; Sanderson et al., 1971). Sanderson (1972) has recently reviewed the evidence for these reactions.

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Ms received 2/13/72; revised 2/22/72; accepted 2/24/72.

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THE VOLATILE CONSTITUENTS OF OIL OF THYME

INTRODUCTION

IN A SUMMARY of research into the identification of compounds present in thyme essential oil, Guenther (1949) credited Lallemand with the first work on the oil in 1853, in which thymol was identified as the main constituent. Guenther stated that further work on the oil was somewhat ambiguous because no clear distinction was made among thyme oil, origanum oil and other species, all of them being similar and being designated simply as "thyme oil." To alleviate confusion caused by lack of knowledge of the precise source of an oil, Guenther defined thyme oil, thymol type, as "oil containing 63–74% phenols which consist mostly of thymol." Later work by Messerschmidt (1964) concerning differences between the oils of *Thymus vulgaris* L. and those of *T. pulegioides* L. indicated that defining thyme oil in this manner was reasonable as only minor differences in the essential oils were observed, other than differences in total oil content.

Guenther also stated that the presence of the following compounds had been established: amyl alcohol, β,γ-hexenol, a terpene(?) with carrot-like odor, 1-α-pinene, β-pinene, camphene, p-cymene, γ-terpinene, linalool, 1-borneol, terpinen-4-ol, geraniol, thymol, carvacrol and a phenol(?), caryophyllene, a sesquiterpene and a sesquiterpene alcohol(?).

More recent studies have relied heavily on methods of identifying compounds. Ikeda et al. (1962) reported 12 monoterpene hydrocarbons present in thyme oil on the basis of retention data, and also gave their relative concentrations. Schratz and Wahlig (1965), in their comparisons of chromatograms of *Thymus serphyllum* samples prepared by distillation and by extraction methods, demonstrated the presence of many trace volatiles.

Messerschmidt (1964) identified 13 compounds on the basis of enrichment retention techniques and formation of chemical derivatives of compounds isolated from the oil. Messerschmidt (1965) also reported the identification of 11 other compounds on the basis of gas-liquid and thin layer chromatographic data. He found the distinguishing characteristic between the oils of *Thymus vulgaris* and *T. zygis* to be the thymol methyl ether content, the former species containing an appreciably larger proportion of this compound (1.4–2.5% vs. 0.3%).

This paper presents the results of an investigation of the volatile constituents of oil of thyme.

Table 1—Compounds identified in thyme essential oil

Peak no.[a]	Compound	Identification method[b]
1	tricyclene	IR
2	α-pinene	IR
3	camphene	IR
4	β-pinene	RT
6	myrcene	IR
7	α-terpinene	IR
8	limonene	IR
9	1,8 cineole	IR
10	γ-terpinene	IR
11	p-cymene	IR
15	trans-sabinene hydrate	MP, IR
16	linalool	IR
17	fenchyl alcohol	IR
18	pinocarvone	MS, IR, SYN
19	1-terpinen-4-ol	IR
20	carvacrol methyl ether	MS, IR, SYN
21	caryophyllene	IR
22	trans-pinocarveol	IR
23	α-terpineol	IR
24	borneol	IR
25	geranyl acetate	IR
27	thymol	IR
28	carvacrol	IR

[a]Peak number of component in chromatogram as shown in Figure 2
[b]IR = Infrared spectrum; MP = Melting point; MS = Mass spectrum; UV = Ultraviolet spectrum; SYN = Synthesis; RT = Retention time and enrichment

EXPERIMENTAL

Apparatus

Initial separation of components for identification was made using a Varian Aerograph 1520 chromatograph equipped with dual 10 ft × 0.375 in. OD glass columns packed with 3% (w/w) Carbowax 20M on 60–80 mesh Chromosorb G. A thermal conductivity detector and helium flow rate of 40 ml/min were used. Final separations, unless otherwise indicated, were made using a Varian Aerograph Series 200 instrument equipped with a thermal conductivity detector and a 10 ft × 0.375 in. OD glass column packed with 3% (w/w) SF96(50) on 60–80 mesh Chromosorb G operated with helium flow rate of 60 ml/min.

Tricyclene and α-pinene were separated using a 25 ft × 0.125 in. OD glass column packed with 3% (w/w) Carbowax 20M on 60–80 mesh Chromosorb G in the Varian Aerograph. A helium flow rate of 25 ml/min was used and the oven temperature was held isothermally at 55°C.

Limonene and 1,8-cineole were separated on a 20 ft × 0.125 in. glass column packed with 2.5% (w/w) Apiezon L on 60–70 mesh, DMCS-treated, A/W Chromosorb G. The column was fitted into a modified Beckman thermotrac instrument equipped with Carle Micro-thermistor detectors. A similar column packed with 2.5% (w/w) Carbowax 20M on 60–70 mesh Chromosorb G column was used as the reference column. The helium flow rate was 25 ml/min and the oven temperature was 100°C.

Eluted fractions were trapped in thin-walled glass capillaries as described by Jennings et al. (1964) and infrared spectra were taken on thin films between NaCl plates on a Perkin-Elmer Model 257 Infrared Spectrophotometer fitted with a 5× beam condenser. Ultraviolet spectra were measured with a Beckman DB-G Spectrophotometer, using 95% ethanol as the solvent. Mass spectra were obtained with a Varian M66 Mass Spectrometer by inserting the capillary collection tube used for collecting the samples directly into the sample inlet system. Accurate mass determinations were made using a Varian V-5560 reference kit.

Materials and procedures

Components identified were all isolated

from thyme essential oil, except sabinene hydrate. A sample of thyme essential oil was obtained from Fritzsche, Dodge and Olcott, New York. Collections were made in thin-walled capillary tubes, using 25 μl injections of the oil and linear programming at 2°C/min from 75–225°C. Programming was begun immediately after injection. Attenuation on the instrument was ×16.

Further separations were made by repassing each fraction through at least one other column. Approximately 8 μl $MeCl_2$ were used to transfer the fractions from the capillary tube to the column. The instrument was programmed at 2°C/min from 50–150°C, or until the peak pattern indicated that all the components in the fraction had been eluted.

Identifications were made on the basis of retention data, mass spectral, ultraviolet and infrared spectroscopic data, and physical properties. Infrared spectra of all of the compounds identified except β-pinene and sabinene were compared with published reference spectra as a means of final identification. Mass spectral and UV data were used in the process of identification to indicate possible structures. Retention data were used to indicate the presence of β-pinene, as its low concentration in the oil precluded infrared spectroscopic confirmation. Melting point and retention data were used along with IR spectra to identify trans-sabinene hydrate (Russell and Jennings, 1970).

Samples of methyl ethers of thymol and carvacrol synthesized using microscale equipment, provided authentic materials for use in identifying components by infrared spectroscopy and retention data. The ethers were prepared using thymol and carvacrol which had been collected from the essential oil using the Carbowax 20M preparative column. Approximately 30 μl of the phenol were reacted with 1.5 ml $(CH_3O)_2SO_2$ in concentrated KOH. After the mixture was refluxed 30 min, the methyl ether was extracted with 2 ml pentane. A 50-μl portion of the pentane solution was injected onto the 10 ft × 0.375 in. OD SF96(50) column and the reaction product was collected.

Pinocarvone was synthesized by reacting a few milligrams of α-pinene oxide (1, Aldrich) with a 25% solution of Tibal (Tri-isopropyl aluminum, Texas Alkyl) in hexane, under dry nitrogen. After 1 hr, the reaction was quenched by the addition of ice. The hexane layer was reacted at ambient temperature overnight with MnO_2 to oxidize the carveols (2) to pinocarvone (3), (see Fig. 1). The product was collected from the reactant mixture by preparative gas chromatography and IR, MS, and UV spectra were obtained for comparative purposes.

RESULTS & DISCUSSION

Identification of volatile constituents

Figure 2 shows a chromatogram of 0.7 μl of thyme essential oil on the Carbowax 20M glass column in a Varian Aerograph 1200 instrument with a flame ionization detector. The compounds identified are listed in Table 1.

Peaks 1 and 2 were identified as tricyclene and α-pinene, respectively. Tricyclene was identified by comparison of its infrared spectrum with that obtained from an authentic sample of tricyclene (Aldrich Co.) and by comparison with the infrared spectrum of Takeshita and Kitajima (1956). α-Pinene was identified by comparison of its infrared spectrum with that of Mitzner et al. (1965).

β-Pinene (peak 4), a minor component, was tentatively identified by retention data. The presence of α-pinene indicated

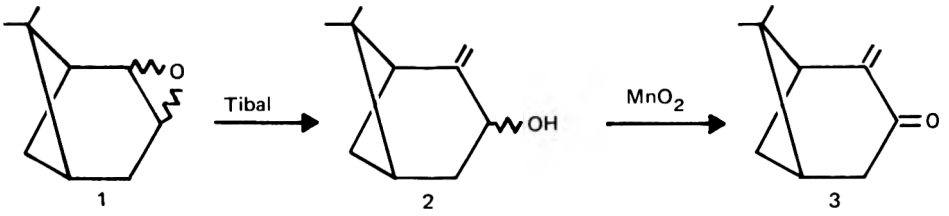

Fig. 1—Synthesis of pinocarvone.

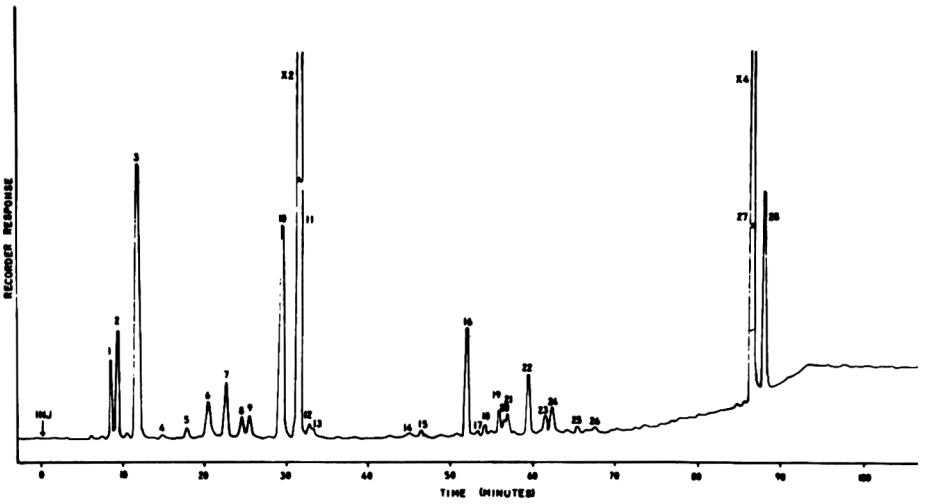

Fig. 2—Gas chromatogram of volatile components of thyme essential oil.

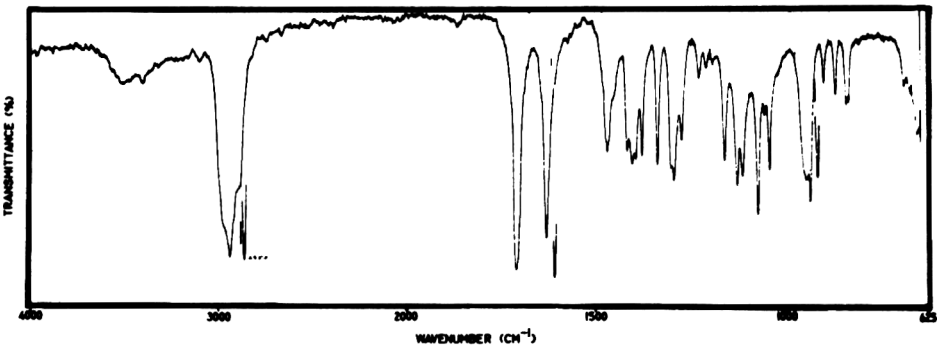

Fig. 3—Infrared spectrum of pinocarvone.

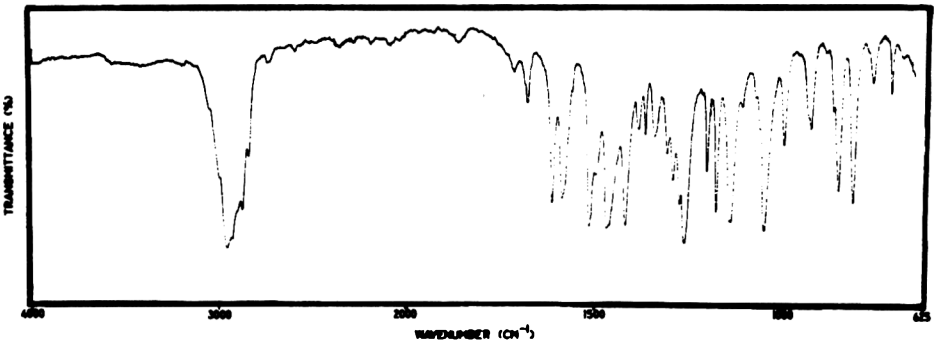

Fig. 4—Infrared spectrum of carvacrol methyl ether.

the possible presence of β-pinene in the oil; and identical retention data on dissimilar columns, and enrichment from an authentic source (K&K Lab) were used for confirmation of its presence.

The compounds in peaks 6 and 7 gave spectra matching those reported by Mitzner et al. (1965) for myrcene and α-terpinene, respectively.

Peaks 8 and 9 were identified by comparing the IR spectra with reference spectra. Peak 8 was identified as limonene (Mitzner et al., 1965) and peak 9 as 1,8 cineole (Mitzner and Mancini, 1969).

Peaks 10 and 11 were identified as γ-terpinene and p-cymene, respectively, by comparing the infrared spectra of the isolated compounds with those presented by Mitzner et al., 1965. In the process of identifying these compounds, the formation of p-cymene from γ-terpinene was observed. This was previously reported to occur in *Thymus vulgaris* by Granger et al. (1964). These observations indicate that some of the p-cymene present may be due to artifact formation.

Component 15 from the essential oil was present only in trace amounts. However, a compound with identical retention time was present in larger amount in an extract of dried thyme leaf. This compound, which was a white solid with melting point of 60–61°C, was identified as trans-sabinene hydrate by comparison of these physical properties with those previously reported by Russell and Jennings (1970).

Peak 16 was identified as linalool by comparison of its infrared spectrum with that of reference spectra (Mitzner et al., 1968). A minor component, peak 17, was identified similarly as fenchyl alcohol (Mitzner et al., 1968). Identification of peak 18 was not so forthright, as the infrared spectrum (Fig. 3) did not match published reference spectra. Mass spectral analysis indicated a molecular weight of 150, and accurate mass determination yielded a molecular formula of $C_{10}H_{14}O$. A UV λ-max was observed to be 241 nm. The component was identified as pinocarvone by comparing UV and infrared spectroscopic data with the findings of Moore and Fisher (1956), Erskine and Waight (1960) and by matching spectra from the synthesized product.

The main component of peak 19 was identified as 1-terpenen-4-ol on the basis of its infrared spectrum (Mitzner et al., 1968). The isolated component of peak 20 was subjected to mass spectral analysis in addition to infrared characterization. The mass spectrum gave an accurate mass of 164.1263, indicating a molecular formula of $C_{11}H_{16}O$. The presence of thymol methyl ether (mol. wt. 164, $C_{11}H_{16}O$) in *Thymus vulgaris* was previously reported by Messerschmidt (1965). However, published infrared spectra as well as spectra from the synthesized thymol methyl ether were not in agreement. Thymol methyl ether was not found in our samples and synthesis confirmed the structure of peak 20 to be carvacrol methyl ether (IR spectrum shown in Fig. 4). This compound had been previously reported in a natural product in the work of Barreto and Enzell (1961) on *Cupressus torulosa* Don.

Peak 21 was identified as caryophyllene by comparison of the IR spectrum of the isolated component with reference spectra (Wenninger et al., 1967). The main component of peak 22 was identified as trans-pinocarveol by comparing its infrared spectrum with that reported by Arbuzov et al. (1965).

Peaks 23 and 24 were identified as α-terpineol and borneol, respectively, by comparison of their infrared spectra with those reported by Mitzner et al. (1968).

Peak 25 was identified as geranyl acetate by comparison of its spectrum with that of an authentic sample (K&K Lab) and with that of Mitzner and Mancini (1969). Peak 27 was identified as thymol and peak 28 as carvacrol by comparing the infrared spectra of the isolated compounds with reference spectra (Mitzner et al., 1968; Sadtler Reference Spectrum #2470, *Sadtler Standard Spectra*, 1959, respectively).

Further work will be reported comparing volatile constituents from extracts of thyme leaf samples, effects of varying temperature and pH and the influence of these parameters on sensory properties.

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Ms received 1/6/72; revised 2/22/72; accepted 2/24/72.

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A PRECISE METHOD FOR THE DETERMINATION OF DIMETHYL SULFIDE IN PROCESSED FOODS

INTRODUCTION

DIMETHYL SULFIDE (DMS), a degradation product of an S-methyl-methionine sulfonium salt (Challenger and Hayward, 1954; McRorie et al., 1954), has been isolated and identified in a number of processed foods (Bailey et al., 1961; Self et al., 1963). Patton et al. (1956) reported the flavor threshold of dimethyl sulfide in water to be 12 ppb while Guadagni et al. (1963) reported its odor threshold in water to be 0.33 ppb. Since DMS does have low threshold values, it appears likely that the level in processed foods can be important in consumer acceptance. Bills and Keenan (1968) observed that a sample of frozen sweet corn containing an exceptionally low level of 0.3 ppm DMS had a flavor much like pumpkin or squash. Guadagni et al. (1968) reported that a DMS level of 0.5–2 ppm in tomato juice produced the most desirable aroma quality.

It is reasonable to assume that optimal levels of DMS in the finished product can be achieved either by selecting proper processing conditions according to the DMS generating potential of the raw material (precursor level) or by blending of raw material or finished product. Quality control of this type requires the existence of an analytical procedure for the determination of DMS of greater speed and precision than is offered by present methods (Miers, 1966; Bills and Keenan, 1968; Nelson and Hoff, 1968).

The present method, which fulfills these requirements, was developed by adapting and modifying a procedure described by McAullife (1971) for the determination of low concentrations of hydrocarbons in aqueous solutions.

EXPERIMENTAL

Preparation of standard curve

A 100 ml volumetric flask containing 99 ml of ethylene glycol was cooled to 4°C. 1 ml of dimethyl sulfide (Matheson, Coleman and Bell), also at 4°C, was added to the flask and then mixed until the DMS was completely dissolved. The tightly stoppered flask containing the mixture (solution I) was then allowed to reach room temperature (24°C ± 1°).

1 ml of solution I was added to 99 ml of distilled water and mixed. This solution contained 100 ppm DMS with a 1% ethylene glycol base. Aliquots of 1, 5 and 10 ml were transferred to 3–100 ml volumetric flasks and brought to volume with an aqueous solution of 1% ethylene glycol. These solutions contained 1, 5 and 10 ppm DMS, respectively. All concentrations were calculated on a volume basis.

A 50 ml Yale Luer-lok hypodermic syringe with a gas-tight Luer-lok two-way valve was used for all analyses. A 15 guage pipette needle attached to the valve was used to draw exactly 25 ml of standard solution into the syringe. After closing the valve, the pipette needle was removed and the syringe inverted. Helium gas (25 ml) was added and the syringe valve was again closed. The sample was mechanically shaken for 3 min at 190 cycles/minute. The syringe was connected to a sample loop (approximately 1.7 ml) attached to the gas chromatograph by means of a gas sampling valve. With the syringe inverted, 15–18 ml of headspace gas was forced through the sample loop, and the 1.7 ml sample flushed onto the column by the nitrogen carrier gas. Care was taken to flush the sample loop for 30 sec with helium between equilibrations to remove any residual DMS in the valve system. The peak heights (mm) of the 1, 5 and 10 ppm DMS standard solutions were plotted against concentration (ppm) using simple linear regression to provide a standard curve for DMS.

Analysis of processed foods

Three cans of commercially processed peas, beets, vegetable juice, kraut juice, and four cans each of tomato juice and yellow sweet corn were analyzed. Samples were prepared by pipetting 25 ml of the liquid product (filtered through cheesecloth in the case of beets, peas and corn) into a 100 ml volumetric flask. The sample was brought to volume with an aqueous solution of 1.5% ethylene glycol containing a drop of G.E. Antifoam 60. From each diluted product sample, a 25 ml aliquot was withdrawn with a 50 ml syringe, analyzed and the peak height recorded as previously outlined. The concentration of DMS in the sample was obtained from the standard curve. The value obtained was multiplied by a factor of 4 since the original product had been diluted four times.

Identity of DMS peak

Retention time and solubility in concentrated sulfuric acid (Miers, 1966; Nelson, 1967) were used to identify the DMS peak. A 2 ml gaseous sample was withdrawn from an equilibrated sample using a 2 ml syringe containing 10 µl of concentrated sulfuric acid deposited as a film on the inner surface of the syringe barrel. After 60 sec, the 2 ml sample was injected into the gas chromatograph through the sample port. The disappearance of the DMS peak was noted. 10 µl of distilled water were next added to the 2 ml syringe. After 60 sec, the remaining vapors were injected into the gas chromatograph. Confirmation of the peak as DMS was noted by the reappearance of a peak at the retention time of the DMS standard. Each processed food was analyzed in this manner.

Gas chromatographic conditions and equipment

A gas chromatograph equipped with a flame ionization detector and stainless steel column (6 ft × 1/4 in.) packed with 15% Ucon nonpolar (LB1715) on 60/70 mesh Chromosorb W was used. The carrier gas was nitrogen with a flow rate of approximately 50 ml/min maintained at 50°C. A gas sampling valve in the nitrogen line was used to divert the carrier gas through the sample loop.

RESULTS & DISCUSSION

IF A VOLATILE substance is brought to equilibrium between a liquid phase and a gas phase, the concentration in the gas phase will constitute some measure of the concentration in the liquid phase and thereby also of the total concentration in the liquid before equilibration took place. The present method is based on the assumption that Henry's law applies to the system in the syringe, that there exists a proportionality between the concentrations of DMS in the gas phase and in the liquid phase.

The validity of this assumption is demonstrated in Figure 1. Here, the logarithm of the peak heights from four successive equilibrations were plotted against equilibration number. The procedure was the same as previously outlined except that after expulsion of the gas phase from one equilibration, the syringe was refilled with an additional 25 ml of helium and shaken to obtain the gas phase of the next equilibration. This was repeated four times. The slope of the resulting line equals $-\log(k+1)$, where k is Henry's constant (McAullife, 1971). The linearity of this plot therefore provides evidence of adherence to Henry's law.

When samples of different composition are to be analyzed, it is reasonable to expect that the distribution of DMS between the two phases would be dependent on the solute concentration of the samples. McAullife illustrated this in extracting hydrocarbons from water containing various concentrations of sodium chloride. As outlined in the procedure, all samples were diluted four times by the addition of 3 vol of an aqueous solution of 1.5% ethylene glycol in an effort to minimize such differences. The parallelity between lines obtained from preparations of different composition (Fig. 1) demon-

strates that Henry's constant was essentially identical for all the diluted samples and independent of their origin when run under the conditions specified in the procedure. Ethylene glycol has the further effect of rendering DMS more soluble in the aqueous media, thus minimizing losses in transfer operations by escaping gas phase. On the other hand, it has to be realized that dilution and addition of ethylene glycol will reduce the sensitivity of the method. We found this not to be a serious problem in analyzing horticultural products. The detector signal was most often attenuated 300-fold during these analyses. On the other hand, if dairy products are to be analyzed, where DMS concentrations may be lower by several orders of magnitude, then the wisdom of dilution and ethylene glycol might be questioned. The most important reason for introducing ethylene glycol was to obtain convenience and precision in preparing standard DMS solutions. Dimethyl sulfide is highly soluble in this solvent, and its use makes it possible to prepare by sequence dilution a series of diluted solutions with good precision.

Several DMS standards and 20 processed food samples of unknown DMS concentrations were prepared and analyzed. Concentrations of the original undiluted samples were obtained (Table 1) by means of a standard curve of peak heights vs. DMS concentration and multiplied by 4 (dilution factor). Only the liquid or serum portion of a sample was used on the reasonable assumption that the concentration of DMS in the serum was in equilibrium with that of the aqueous phase in the solid portion of the food. Canned tomato juice had DMS concentrations from 5.9–10.2 ppm. Miers (1966) reported that commercially canned tomatoes and tomato juice contained 1.6–7.9 ppm dimethyl sulfide. Processed corn contained from 10.1–16.0 ppm which corresponds with values reported by Bills and Keenan (1968). To our knowledge, dimethyl sulfide has not been reported in beets. Beets contained very large quantities of DMS: 14.1, 27.9 and 43.6 ppm in the three cans analyzed. Self et al. (1963) reported frozen peas to contain medium concentrations of DMS. Our data showed processed peas to contain approximately 5.0 ppm. Vegetable juice and kraut juice contained levels of 1.6–2.2 ppm and 2.4–3.8 ppm DMS, respectively.

Four equilibrations should in theory yield results of higher precision than results obtained from only one equilibration as specified in the procedure. In order to evaluate this, the data in Figure 1 and Table 1 were treated by extrapolation to yield peak heights at "zero" equilibration (antilog of y-intercept). However, when the results are compared with those based only on the first equilibration (Table 1), it is evident that very little is gained by this additional effort.

To demonstrate the reproducibility of the procedure, a series of processed food samples and standard solutions were prepared as outlined earlier. A 25 ml subsample was analyzed from each 100 ml sample or standard solution. Immediately following the analysis of the first aliquot, a second 25 ml aliquot was analyzed in the same manner. Similarly, two standard curves were obtained using the peak heights of the DMS standards from the

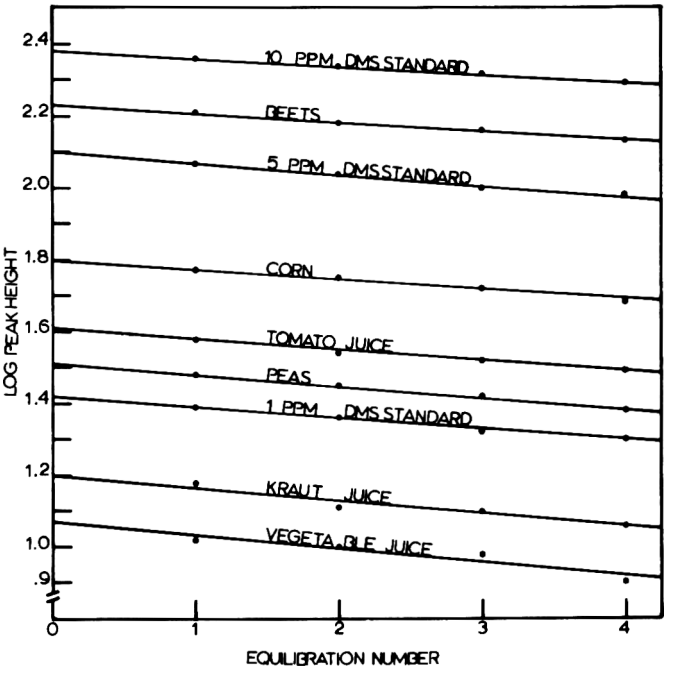

Fig. 1–Equilibration curves of DMS standards and DMS in processed food samples.

Table 1–Dimethyl sulfide (ppm) in 20 commercially processed foods

	Product					
Can	Veg. juice	Kraut juice	Peas	Tomato juice	Yellow corn	Beets
1	2.2 (2.2)[a]	3.8 (3.8)	5.1 (5.2)	7.4 (7.4)	12.2 (12.1)	43.6 (42.8)
2	2.1 (2.0)	3.2 (3.2)	4.9 (5.0)	10.2 (10.0)	16.0 (16.0)	14.1 (14.2)
3	1.6 (1.6)	2.4 (2.3)	5.0 (5.0)	5.9 (6.0)	11.6 (11.8)	27.9 (28.0)
4				6.4 (6.3)	10.1 (10.2)	

[a]Concentration obtained using four successive equilibrations on the same 25-ml aliquot and determining the peak height at "zero" equilibration number (Fig. 1).

Table 2–Concentration (ppm) of dimethyl sulfide as determined from two consecutive subsamples and two standard curves

	Product					
	Beets	Corn	Tomato juice	Peas	Kraut juice	Veg. juice
A Sample Aliq. #1 vs. Std. Curve #1	27.9	10.1	6.4	5.0	2.4	1.6
B Sample aliq. #2 vs. Std. Curve #1	27.7	9.7	6.1	4.8	2.2	1.6
C Sample aliq. #2 vs. Std. Curve #2	28.0	10.0	6.2	4.9	2.3	1.7
| A–B |, %	.7	4.0	4.7	4.0	8.3	0.0
| A–C |, %	.4	1.0	3.1	2.0	4.2	5.9

first and second aliquots respectively. As is shown in Table 2, the reproducibility depends on which standard curve is used for the second aliquot, illustrating the importance of using volumetric containers of equal volumes for preparing solutions and the necessity of taking the headspace volume in these containers into consideration. As the first aliquot is removed, a certain portion of the DMS in solution enters the headspace and the concentration in solution decreases correspondingly. The second aliquot will therefore contain less DMS than the first. When processed foods were opened for sampling, greater precision resulted when 25 ml samples to be diluted were promptly removed. The standard errors for five replicate samples prepared from each of three processed foods with mean DMS concentrations of 3.0, 6.1 and 15.1 ppm were ± .10, ± .24 and ± .29 ppm, respectively.

Conceivably in foods one might expect dimethyl sulfide to exist as a solute dissolved in the aqueous phase or adsorbed onto solid food particles dispersed in the aqueous phase. If the latter was occurring to any great extent, it would be questionable that an equilibration procedure as described would measure the total concentration of DMS in a food sample. The fact that the peak heights after successive equilibration of a sample (Fig. 1) lie on a straight line and that the slopes of the DMS standards and food samples are equal, suggests that adsorption phenomena do not occur. To further support this, a 25 ml aliquot containing an equal volume (12.5 ml) of a standard solution and a food sample was equilibrated. If no adsorption of DMS was taking place, then one would expect a peak height equal to the mean of those peak heights of both solutions when a 25 ml aliquot was equilibrated separately. This was in fact the result obtained. Therefore, one may conclude that the procedure as outlined is measuring a quantity of DMS in the gaseous phase proportional to the total concentration present in the liquid phase with no interference of other solution phenomena.

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Ms received 1/14/72; revised 3/16/72; accepted 3/16/72.

Journal Paper No. 4656 of the Purdue Agricultural Experiment Station, Lafayette, IN 47907.

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ECONOMIC APPLICATIONS OF SWEETNESS SCALES

INTRODUCTION

THE PRESENT STUDY concerns the economics of sweetness mixtures, in which pairs of sweeteners are used in conjunction in order to increase the overall sweetness of a product. The different costs of materials such as sucrose, glucose (dextrose), saccharin and cyclamate provide possibilities for considering mixtures in terms of a model that minimizes overall cost while maintaining sweetness, or maximizing sweetness while maintaining cost.

The tastes of sweetener mixtures have been studied by food scientists and psychologists for the past five decades. Early work by the sugar industry focused on the possibility that inverting sugar (sucrose) to a mixture of glucose and fructose would enhance sweetness, and thus enzymatic changes of sucrose could provide greater sweetness at the same cost (Sale and Skinner, 1922). At about the same time in Germany a number of studies on the taste of mixtures of the artificial sweeteners, saccharin and dulcin (Paul, 1922) were underway as a result of a shortness of sucrose. The results of these experiments indicated that mixtures were significantly sweeter than their components alone.

The method for determining how sweetnesses 'add' together in a mixture has been elucidated by Cameron (1943; 1944; 1945; 1947). Cameron asked his subjects to taste two solutions of different sugars and then to select the concentration of sucrose that matched the sweetness of each sample (sucrose equivalent). He subsequently mixed together the sugars and repeated the experiment. The results were expressed as three 'sucrose equivalents,' two for the unmixed components and one for the mixture. Additivity of sweetness occurred when the 'equivalent' for the mixture equalled the arithmetic sum of the unmixed 'equivalents.' In a large series of experiments Cameron demonstrated that additivity occurred for pairs of sugars, but only when glucose, maltose or lactose was used as the reference sugar. Expressing sweetness in sucrose or fructose equivalents did not result in additivity of sweetness.

These early studies by Paul and Cameron lacked a true measure of subjective sweetness, and relied on the concentration of glucose or sucrose as the implicit sweetness unit. Recent work by Stevens (1953; 1960; 1969) demonstrated that subjects may be directed to give numerical judgments in proportion to subjective magnitude, taste intensity included, and that these numerical estimates, called 'magnitude estimates,' provided meaningful ratio measures of taste intensity. For example, a sweetness judgment of 20 means twice the sweetness of a judgment of 10, and eight times the sweetness of a judgment of 2.5. Moskowitz (1970a; 1970b; 1971a) reported a series of sweetness scales for several dozen different sugars and several artificial sweeteners. Details of the experimental technique are provided by Moskowitz (1970a).

A convenient and systematic outcome of these direct scaling studies was the finding that the numerical judgments of sweetness could be related to molar or percentage concentration by the simple power function $S = kI^n$. That is, S represents the sweetness judgment and I represents molarity. The exponent n and the intercept k may be obtained from the straight line that results when the power function is plotted in log-log coordinates, to yield the equation $\log S = n \log I + \log k$. The slope of the line provides the exponent, and the intercept provides the value for log k.

The exponent n is the critical parameter for the sweetness equation because it governs the rate at which sweetness increases with concentration. It appears to exceed 1.0 for sugars (Stevens, 1969; Moskowitz, 1970a; 1970b; 1971a), but is less than 1.0 for saccharin and cyclamate. When n exceeds 1.0 sensory magnitude accelerates or grows more rapidly than molar concentration, whereas for n less than 1.0 the opposite occurs and sensory magnitude grows less rapidly. Very low values of n would indicate that large increments of concentration scarcely produce any changes in perceived sweetness. The intercept, or multiplier, k, depends upon the size of numbers selected by the subject and upon the measure of concentration selected. However, when several sugars are rated for sweetness in the same session and their exponents n are made equal either experimentally or by subsequent statistical analysis, then k reflects the ratio of sweetness among different sweeteners (Moskowitz, 1970a). This is because k reflects the relative distance in logarithmic values (i.e., ratio) of two parallel lines.

Recent work in sweetness has attempted to combine power functions of sweetness in order to predict mixture sweetness. Papers by Stone and Oliver (1969), Stone et al. (1969) and Yamaguchi et al. (1970) have tried various combinations of sweetness functions. Usually, however, some multiplicative constant is needed to account for the often-observed result that there is 'synergism,' so that the sweetness of the mixture exceeds the predicted sweetness.

Because of the synergistic effect in mixtures, a combination of power functions and an associated multiplier to handle the effect is shown below:

$$\text{Sweetness } S_a = k_1 C_a^m; \text{ Sweetness } S_b = k_2 C_b^n$$

$$\text{Mixture sweetness } S_{a,b} = k_3 (k_1 C_a^m + k_2 C_b^n)$$

Empirical studies of mixtures (Stone and Oliver, 1969; Stone et al., 1969; Moskowitz, 1971b) suggest that the values of k_3 for synergistic mixtures are moderately greater than 1.0, e.g., about 1.4–1.8, so that the actual prediction made by summing simple power functions is an underestimate. For convenience in simulation we may assume that k_3 remains unchanged across most of the sweetness range and may be viewed simply as a 'change-of-scale.' The form of sweetness summation is unaffected if k_3 is permitted to vary to correct the under- or overpredictions.

EXPERIMENTAL

Procedure

In three experiments glucose was evaluated for sweetness in conjunction with fructose, sodium cyclamate and sodium saccharin. In each experiment subjects received seven solutions of glucose, seven solutions of the second sweetener, and 34 mixtures of the two sweeteners in varying ratios and dilutions. Samples were served to the subjects in small, ¾ oz paper cups and maintained at the room temperature (22°C). The solutions were made up three days prior to the experiment and stored under refrigeration, to permit both mutarotation to an equilibrium mixture of isomers and to prevent mold growth. S's were instructed to judge only the sweetness of the solutions, and for the simple unmixed sweeteners power functions of the form $S = kI^n$ were fitted to the median judgments of 24 S's. Because of experi-

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mental variation the exponents and intercepts of the glucose function varied across experiments (as shown later by the 'generating functions' in the figures), although in each instance the glucose exponent was higher than 1.0 (about 1.3–1.6). Power functions also described the sweetness of fructose. Power functions were forced to fit the saccharin and cyclamate functions, even though they demonstrated significant nonlinearity in log-log coordinates. A revision of the simple summation model may be made to account for quadratic and cubic terms in the saccharin and cyclamate functions. For ease of computation, however, only their linear portions (i.e., simple power functions) were used.

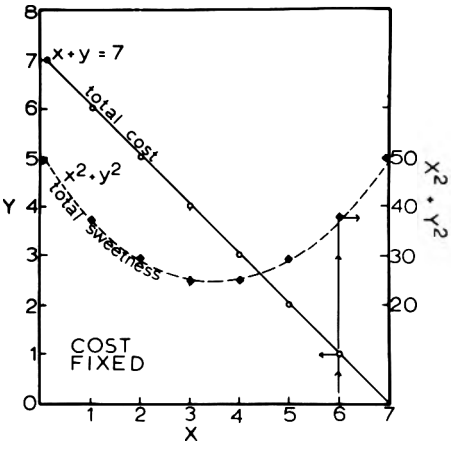

Fig. 1–A hypothetical iso-cost function in which X and Y are two sweeteners, each having unit cost. Total cost = $7. Sweetness is assumed to be equal to the square of molar concentration (total sweetness = $X^2 + Y^2$). All points on the solid line satisfy the equation $X + Y = 7$. All points on the dashed curve satisfy the function $F(X,Y) = X^2 + Y^2$, or $G(X) = X^2 + (7-Y)^2$. To obtain a value for Y for any X, use the solid line. The total 'sweetness' is given by the value of the right-hand ordinate, labelled $X^2 + Y^2$.

For each sweetener the cost of the mixture was ascertained from the prices of the ingredients. The cost per mole was obtained from the 1971 catalog of the Sigma Chemical Co. and reflects the cost of reagent-grade material. For each mixture of two ingredients, therefore, there are two associated values: a total cost obtained from a simple linear sum of independent costs and a total sweetness based upon the addition of two power functions.

Other pairs of sweeteners were also investigated, but only by computer simulation. For these 'hypothetical' mixtures the individual power functions relating sweetness to concentration were obtained from Moskowitz (1971b). For both the empirical and the hypothetical mixtures the value of k_3, which accounts for synergistic effects was arbitrarily set at 1.0 to facilitate computation and to permit comparison of the various mixtures with each other.

Types of simulation

Two theoretical problems were considered: maximization of sweetness subject to maintaining a fixed cost and minimization of total cost subject to maintaining constant sweetness. Initial approaches were to solve analytically these problems with the help of Lagrange multipliers (Taylor, 1955) and with the appropriate cost and sweetness functions. In all cases where the exponents were not small whole numbers the analytic solution did not work, and thus a computer simulation was needed.

In the computer simulation the overall cost of the mixture was first fixed, and a large number of pairs of concentrations were then computed that satisfied the cost constraint. A smooth curve was drawn showing the relative amounts of the two sweeteners whose overall cost was the desired amount. For each pair the sweetness was then calculated. Then a curve was drawn showing the sweetness of the mixture at the different levels of sweeteners for the fixed total cost. With this method several different overall costs were scanned in order to produce different cost functions (iso-costs), and their corresponding contours were computed. In the second part of the simulation the overall sweetness was fixed and a large number of pairs of concentrations were determined by solving the sweetness equation. The total cost of each mixture was then calculated. Again, several different levels of total sweetness were scanned in order to determine the sweetness contours, and the costs of these mixtures were subsequently calculated.

RESULTS

FIGURE 1 illustrates a straight line (total-cost contour) for a hypothetical pair of sweeteners X and Y. The 'cost' of 1 mole of X and of Y is 1 unit respectively for each. For each value of X there is

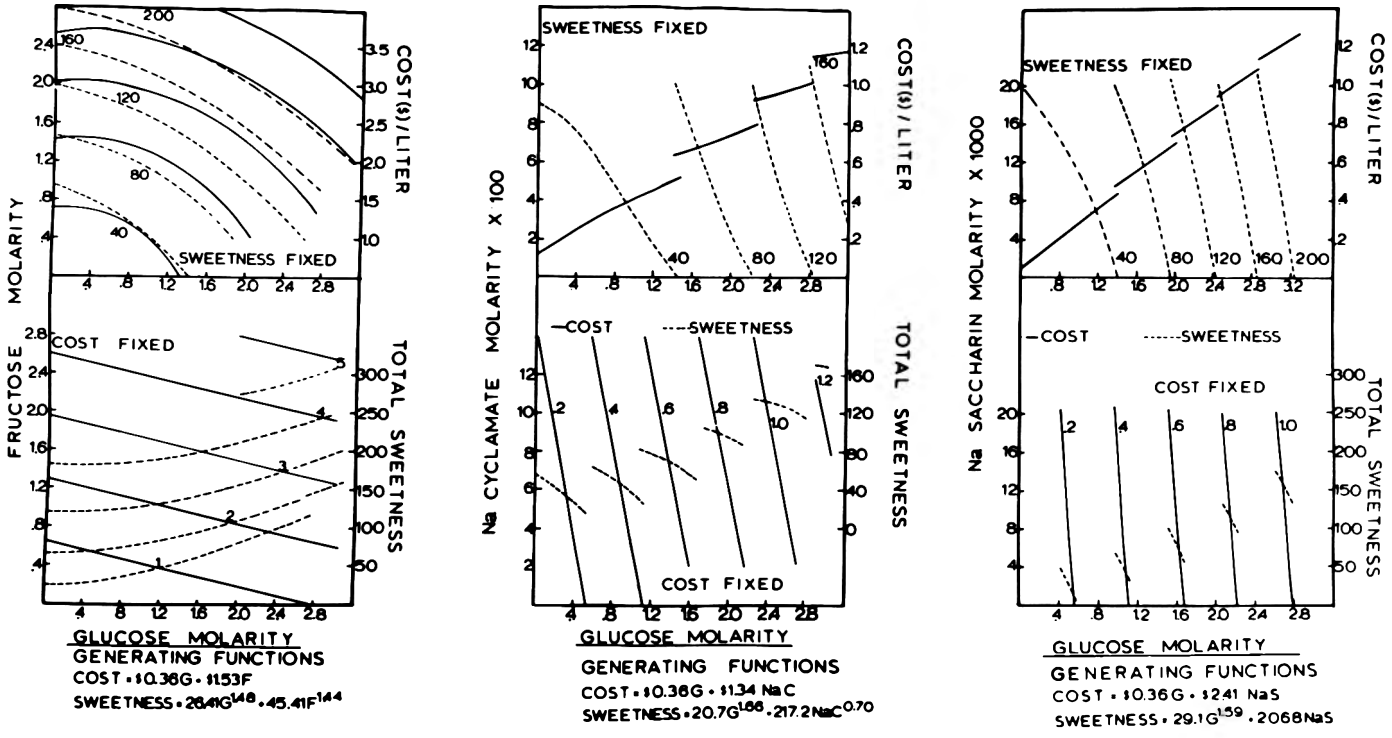

Fig. 2–Mixtures between glucose and fructose, Na cyclamate and Na saccharin. The generating equations are shown below each pair of graphs. Numbers on the top figure in each pair indicate the overall, fixed sweetness level, whereas those in the lower portion indicate overall, fixed cost. All pairs in this figure were investigated in actual experiments, although the curves are idealized versions of the empirical mixture data. Cost is given in $ per mole weight of the mixture.

only one value of Y that satisfies the cost constraint $X + Y = 7$. Only positive values of X and Y are shown, since negative values indicate that a concentration must be subtracted from the mixture. Sweetness is assumed in this example to be represented by the square of molar concentration (so that a 4:1 increase in molarity would lead to an increase of 16:1 in sweetness). In addition, the sweetnesses of the components X and Y are assumed to add algebraically. Thus, the curve represented by $X^2 + Y^2$ represents the overall sweetness of the mixture, and its numerical value may be obtained from the vertical axis at the right of Figure 1.

For any value of X, the corresponding value of Y can be found that satisfies the constraint, and the overall sweetness of the mixture can be calculated. In fact, both variables, Y and $(X^2 + Y^2)$, are uniquely determined for any value of X. The sweetness curve may thus be considered either as a function of both X and Y (i.e., $X^2 + Y^2$) or as a function of X alone [i.e., $X^2 + (7-X)^2$]. This unique determination of the sweetness function arises from the cost constraint, which makes Y directly depend upon X. A similar figure may be constructed for the dual problem, of computing overall cost when sweetness is maintained at a constant value (e.g., $X^2 + Y^2 = 10$). Total cost in the dual problem would be given by the equation $X + (10-X^2)^{.5}$.

Figure 2 shows the mixture functions obtained from three empirical studies. Below each part of the figure are the sensory functions that were used to generate sweetness values, as well as the cost functions used to compute the cost per mole of hybrid mixture. The overall sweetness of the mixtures was fixed at five different values: 40, 80, 120, 160 and 200. In the present system the sweetening power of 0.5M glucose (9%) has been assigned a sweetness value of 10. Because of experimental variations, the sweetness functions for glucose, as shown in the bottom equations of Figure 2, differ slightly among themselves so that the exponent varies between 1.3 and 1.6.

For each of the three mixture-sets in Figure 2, the horizontal axis represents the molarity of glucose. The computer analysis scanned a large number of concentrations between 0.0 and 3.0 moles. The corresponding molarity of the second sweetener satisfying either the cost or the sweetness constraint is shown at the left hand side of the vertical axis.

In order to use the figures, one must first locate the contour that is of interest. For example, consider the mixture whose overall sweetness is 40. A large number of glucose concentrations satisfy this requirement, and for each concentration a value for fructose may be found. One possible pair is 0.4 moles of glucose and 0.8 moles of fructose (approximately). The overall cost of these two sweeteners may be obtained by first extending a vertical line upwards from the horizontal axis (at 0.4 molar glucose). The cost contour (solid curve) intercepts this vertical straight line at a value given at the right hand side of the figure. The analogous reasoning is applicable to situations in which the total cost of the mixture is fixed at a single level and sweetness is to be found.

Two primary results merit discussion. First, there are different 'feasible ranges' of mixtures that satisfy the constraints of sweetness or cost. For example, mixtures of sugars (e.g., fructose and glucose) provide large ranges of concentrations with which to work, whereas markedly narrower ranges are found when a sugar is mixed with an artificial sweetener of greater potency. Second, there are different 'cost ranges.' Sugar mixtures are significantly more expensive than mixtures of sugar with artificial sweeteners (in some cases 4x more expensive). Therefore, when sweetness of the product is the major consideration, artificial sweeteners provide reduced costs.

When the overall cost of the mixture is maintained at a fixed value (the opposite problem to maintaining overall sweet-

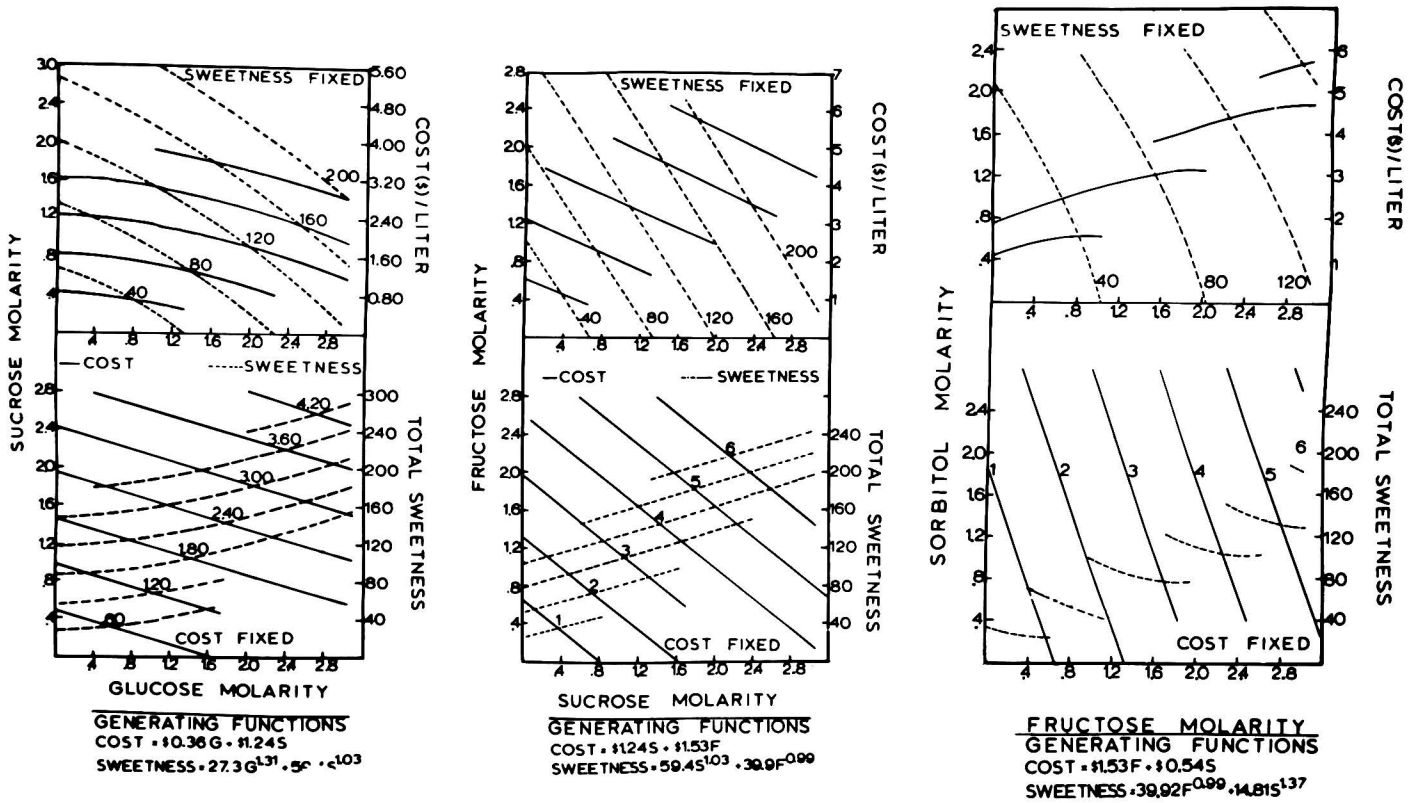

Fig. 3–Mixtures of glucose, sucrose, fructose and sorbitol, obtained from computer simulation of mixture equations. Sweetness power functions were obtained from Moskowitz (1971a).

ness), the mixtures again show different behaviors. For fixed total cost, glucose-fructose mixtures become sweeter with increasing amounts of glucose, a result that is due to the smaller cost of glucose. That lower cost of glucose is sufficient to outweigh the greater sweetening power of fructose. On the other hand, mixtures of glucose with cyclamate or saccharin become less sweet (for fixed cost) as glucose predominates in the mixture. This latter result is obtained because a great deal of sweetness can be obtained for relatively small amounts of the artificial sweetener.

Figure 3 presents the results of computer simulation of mixtures between glucose, sucrose, fructose and sorbitol. These four sugars are commonly used by the food industry to provide sweetness and represent a relatively moderate variation of costs and relative sweetness.

The sweet functions were obtained from estimates provided by Moskowitz (1971a). Sucrose and fructose are the sweetest of the two sugars and grow more slowly in sweetness than either glucose or sorbitol (Cameron, 1947; Moskowitz, 1970a; 1971b). The sweetness curves of glucose and sorbitol are parallel in log-log coordinates, as are the curves for sucrose and fructose. Below each set of functions are the generating equations that were used in the simulation.

Glucose-sucrose and fructose-sucrose mixtures follow similar contours. For example, at a fixed sweetness when the concentration of glucose is increased, the overall cost of the mixture decreases. This occurs since glucose is far less costly than sucrose. For fixed costs the cheapness of glucose far outweighs the sweetness advantage of sucrose. Similar arguments may be made for mixtures of fructose and sucrose wherein fructose is more expensive than sucrose.

When fructose is combined with sorbitol, however, the factors of cost and of sweetness compete against each other. Sorbitol is less expensive than fructose but fructose is much sweeter. The result is a slight increase in the sweetness of a mixture with increases in fructose content when the cost is held constant. As a general rule then, mixtures of this type in which the cost favors one material and the sweetness favors another, material will tend to have flatter contours. This is especially true if costs and sweetness ratios are approximately commensurate and counterbalance each other. Steep contours will occur when one dimension (cost or sweetness) markedly overrides the other.

Figure 4 shows a series of sorbitol-glucose mixtures in which the sweetness was fixed at one of four values (40, 80, 120, 160). For each of several prices of sorbitol (e.g., \$0.10 per mole) the cost function was traced out. The result is a series of cost contours for each sweetness level. The shape of the contour changes as the price of sorbitol is systematically increased. At low sorbitol costs (e.g., \$0.10 per mole) increases in glucose, the more expensive sugar raises the cost of the mixture. At intermediate sorbitol costs (e.g., \$0.30 per mole) there are two optimum points, either very high concentrations of sorbitol or very high concentrations of glucose. Finally, with high sorbitol costs (e.g., \$0.50), the price of sorbitol militates against using it, and the best strategy is to use only glucose. This approach to tracing out the several possible contours elucidates the type of strategy that might be used when a single ingredient systematically varies in cost, but can be replaced by another material possessing many of the same properties.

Finally, Figure 5 illustrates the contours that are obtained when cost is maintained at a given level (either \$0.40, \$0.80 or \$1.20 per liter of sweetener mixture) and the price of sorbitol systematically varies. The order of the cost functions in the figure is maintained for the sweetness function, so that the uppermost cost function corresponds to the uppermost sweetness function. Large changes in sorbitol, when it is inexpen-

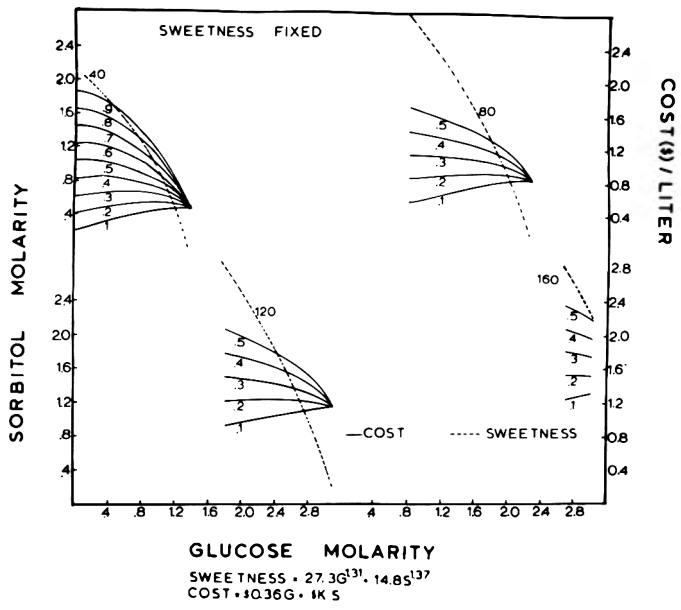

Fig. 4—Contours for glucose and sorbitol when overall sweetness of the mixture was fixed at four values. Each of the solid lines reflects the overall cost of the mixture when the cost of sorbitol is varied at 10:1 range (from \$0.10—1.00 per mole).

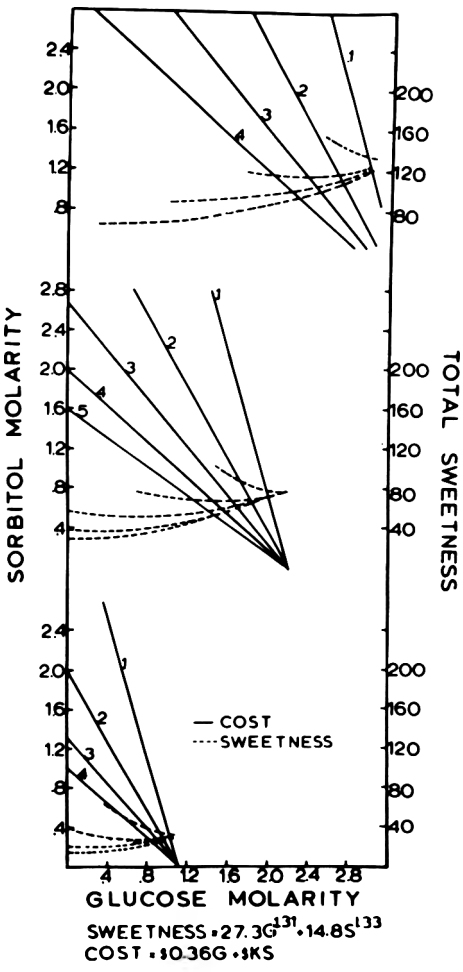

Fig. 5—Contours for glucose and sorbitol when the overall cost of the mixture is fixed at one of three values. For each total cost, sorbitol costs have been varied over a 5:1 range. Each cost of sorbitol yields a new contour for total cost (solid line) and for each contour of total cost there is a comparable contour for total sweetness.

sive, can be tolerated and offset by small changes in glucose without affecting the cost function. The optimum then is to use all sorbitol, with concomitant increases in sweetness. Intermediate prices of sorbitol for fixed total cost reduce the high sweetness when large amounts of sorbitol are used (since the prices are commensurate for the two sugars but glucose is sweeter). For high costs of sorbitol, both the price and the low sweetness militate against producing a high degree of sweetness when much sorbitol is used, and mixtures tend to have less sweetness with more sorbitol.

DISCUSSION

THE PRESENT STUDY concerns a model system in which the sweetener is sampled in aqueous solution. Similar data should be generated by experimental means to test the approach in specific food products. The technique of magnitude estimation permits this approach to be used with relative rapidity and little expense, and may be applied in actual product development. In addition, the fact that a multiplicative correction must be used to account for synergistic effects in mixtures is not a serious detriment, for it requires only a change-of-scale for sweetness. The values corresponding to psychological sweetness may be multiplied by a correcting factor, so that they represent the actual sweetness levels relative to a standard, or a computer program can be written to account for the multiplier. Many other factors besides overall sweetness enter into the selection of an appropriate sweetener and concentration for a given food. Mixtures that maintain sweetness at a fixed level may not be equally acceptable to the consumer, and specific parameters of each food have to be considered before selecting any one mixture. Hence, the present study provides only two constraints for mixtures: the levels of sweetener that sum to a given cost and to a given sweetness. Other constraints may be the acceptability to the consumer, weight of sweetener and perhaps even caloric value.

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Ms received 12/26/71; revised 2/13/72; accepted 2/14/72.

The computer program SWEETSUM was developed at the U.S. Army Natick Labs. for the GE 225.

JFS APPLIED SCIENCE and ENGINEERING

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IMMERSION FREEZING OF FISH IN DICHLORODIFLUOROMETHANE

INTRODUCTION

INTEREST in immersion freezing of food has led to the investigation of several freezants for this purpose. Cryogenic liquids were thought to be promising initially, but the cost of these liquids, such as carbon dioxide and nitrogen, limited their use for freezing food. Approval of dichlorodifluoromethane (DDM) (Federal Register, 1967) for use on food makes possible the use of this material, which has most of the characteristics of the cryogenic fluids, but at a much lower cost. The reduced cost is possible because the DDM vapor is condensed and recycled, instead of being lost.

Several DDM freezers are in commercial operation for processing fruits and vegetables as described by Smith and Robe (1969). These freezers provide a brief immersion in DDM for shape-setting followed by a series of overhead sprays. Use of a small DDM freezer for processing shrimp was described by Lawler (1969).

The properties of DDM demonstrate its suitability for freezing food. Its boiling point of −21.6°F makes it possible to condense the vapors, using conventional refrigeration equipment. DDM absorbs 76 BTU/lb upon evaporation. Baker and Charm (1969) reported a heat transfer coefficient of 38.7 BTU/hr ft^2 °F, which tends to offset the temperature difference advantage of liquid nitrogen, which they reported to have a heat transfer coefficient of 16.7 BTU/hr ft^2 °F.

Economic factors ultimately determine which freezing method will be employed. DDM freezing requires mechanical refrigeration for condensation of the vapor and compares with conventional methods of freezing in this cost, but has an added cost due to small losses of DDM vapor. Although direct comparison of freezing costs is difficult, DDM appears to have very low dehydration losses, on the order of 0.25% or less (R. Britton, personal communication). This could be a strong factor in selection of a freezing method, especially for a product of high value. In a study of freezing fruits by Wolford et al. (1968) it was suggested that DDM frozen fruit was of sufficiently higher quality than fruit frozen by other methods that it might create new markets.

Work previously reported on the use of DDM for immersion freezing of fish was concerned with freezing whole tuna. Crawford et al. (1969) concluded that there were "definite but not vast" advantages with DDM. Quality assessments were made after thawing and canning.

This study was concerned with salmon steaks frozen and held frozen until cooked for consumption. Freezing times were studied and presented as a function of thickness in nomographic form. This means of expression of freezing times avoids making unreasonable assumptions, such as steady-state heat transfer, in attempting to estimate freezing times by conventional means.

EXPERIMENTAL

Freezing fish in DDM

Fresh troll-caught king salmon (*Oncorhynchus tschawytscha*) were purchased from a commercial source. The fish, averaging 10 lb in weight, were cut into steaks approximately ¾ in. thick, weighing about 200g. Immediately before freezing samples were individually weighed and placed in a nylon mesh-covered basket. One steak in five in the sample container was fitted with a thermocouple in the center. Samples were immersed in DDM at its boiling point (−21.6°F) and frozen in 6–8 min. On reaching a center temperature of +15°F the sample basket was removed from the freezant. Steaks were reweighed, then wrapped drugstore-fashion (the manner in which meat was wrapped by butchers before the advent of transparent films for prepackaged meats) in Saran film and placed in a freezer for storage.

Control samples were frozen by two commercial processes, air blast and liquid nitrogen (LN)-boosted air blast. Samples were prepared in the same manner as for DDM freezing, weighed and laid unwapped on the wire shelves of the cart. A sheet of polyethylene was laid on each shelf to prevent the salmon steaks from sticking to the wire and interfering with the weight loss determination. Air blast freezing was performed at −30°F and LN-boosted air blast freezing at −70°F, with an air velocity of about 300 ft/min in both instances. Time for air blast freezing was 90 min and for LN-boosted air blast freezing 25 min. After freezing to +15°F, each group of samples was placed in a frozen storage room. Samples were removed five at a time for reweighing and wrapping in Saran film. All three groups of samples were placed in storage at +5°F.

Quality assessment

Samples for chemical evaluation and a taste panel were taken from storage at 1 wk after freezing, 2 wk, 1 month, and each month thereafter for a total of 6 months. The taste panel was supplemented by chemical determinations for rancidity performed by the 2-thiobarbituric acid method. Peroxide determinations were run by the AOAC (1965) method, with extraction of fat by the method of Bligh and Dyer (1959). One modification was made to the AOAC method to permit visibility of the end point in the presence of the pink astaxanthin pigment. The acetic acid-chloroform mixture employed was 1:3 instead of 3:1, giving a two-phase system with the pigment in the chloroform layer. The 2-thiobarbituric acid (TBA) method for measurement of malonaldehyde was performed by the method of Yu and Sinnhuber (1957, 1958), with modifications suggested by Palmateer (1960).

Table 1—Tenderness score averages for salmon steaks[a]

Weeks after freezing	Air blast	LN-boosted air blast	DDM
1	5.60	6.40[b]	5.30
2	5.21	5.71[b]	5.00
5	5.17	5.17	4.42
9	4.43	4.43	5.00
13	4.44	5.25[c]	4.00
17	4.29	3.86	4.29
21	3.50	3.67	3.39
27	4.15	4.40	4.20

[a]Scoring was on a 7-point scale with 7 indicating very tender and 1 indicating very tough

[b]Significantly higher than the other two scores at the 95% confidence level

[c]Significantly higher than the DDM score

[1] Present address: Wm. Wrigley Jr. Co., 3535 S. Ashland Ave., Chicago, IL 60609

Table 2—Rancidity score averages for salmon steaks[a]

Weeks after freezing	Air blast	LN-boosted air blast	DDM
1	6.10	6.50	6.10
2	6.43	6.21	6.36
5	5.33	5.25[b]	5.75
9	5.14	4.57	5.29
13	5.39	4.89	4.78
17	4.71	5.07	4.86
21	3.94	4.22[c]	3.22
27	5.35	5.20	5.40

[a]Scoring was on a 7-point scale with 7 indicating no rancidity and 1 extreme rancidity.
[b]Significantly lower than the DDM score
[c]Significantly higher than the DDM score

Table 3—Overall desirability score averages for salmon steaks[a]

Weeks after freezing	Air blast	LN-boosted air blast	DDM
1	5.80	6.00	5.75
2	6.00	5.36	5.57
5	5.33	5.17	5.33
9	4.86	3.43[b]	4.86
13	4.89	4.89	4.44
17	4.43	4.36	4.79
21	3.33	3.44	2.89
27	4.70	4.60	4.85

[a]Scoring was on a 7-point scale with 7 most desirable and 1 least desirable.
[b]Significantly lower than the other two scores

Drip losses were determined by unwrapping and weighing the samples, rewrapping, and thawing for 4 hr at room temperature, unwrapping and reweighing. Thawing the samples wrapped prevented collection of condensed moisture. Drip loss was expressed as a percentage of the original (frozen) weight lost during thawing.

Taste panel members were presented with one sample of salmon frozen by each of the three methods. Samples were prepared by wrapping in aluminum foil and cooking for 15 min in live steam. Panelists were asked to rate each sample for tenderness, rancidity, and overall desirability on 7-point scales, 1 being lowest or least desirable, and 7 being highest, or most desirable. Six to ten panelists participated at each session. Results were analyzed for statistical significance by calculation of fiducial limits as suggested by Snedecor (1946).

Freezing time determinations

Measurement of freezing times was performed in the following manner: thickness of a fillet was measured by placing it on a flat, level surface, leveling a ruler on top of the thickest part and measuring the distance from the ruler to the surface with a scale calibrated in decimal inches. A thermocouple was inserted in the thickest part at the center. The fillet was placed in a nylon mesh-covered basket which closed with a clip. Simultaneously with immersion in the freezant, timing was started on a stopwatch.

Different varieties of fish were used, including halibut (*Hippoglossus stenolepis*), rockfish (*Sebastoides spp.*), ling cod (*Ophiodon elongatus*), and sole (*Pseudopleuronectidae spp.*), in order to sample a range of thicknesses. Thicknesses ranged from 0.3–1.4 in.

Determination of DDM residues

Samples of sole, ling cod and king salmon were frozen in DDM and held for intervals up to an hour. Analysis for DDM was performed by the method described by Crawford et al. (1969), except that the sample was sealed in a can containing 155 ml carbon tetrachloride and a 0.040 ml sample was taken for chromatography.

RESULTS & DISCUSSION

TASTE PANEL results for tenderness, rancidity and overall desirability are shown in Tables 1, 2 and 3.

Examination of results shown in these tables indicates the overall similarity of quality with the three freezing methods. Generally the results showed a gradual decline in quality, except for an unexplained drop at the 21st wk, followed by a rise at the last taste panel. Instances of "statistically significant" results occurred infrequently and did not establish a trend. From this overall view it is concluded that no major differences in quality were shown by the three freezing methods employed. Fish frozen by immersion in DDM were equivalent in quality to fish frozen by established methods.

Rancidity assessments by the taste panel (Table 2) were supplemented by TBA determinations for malonaldehyde and peroxides (Fig. 1 and 2). Results indicated that there were no significant differences between the three freezing methods. Rancidity was regarded as a storage problem by Love (1966), preventable by proper packaging and storage. Thus, the freezing method should not affect rancidity, except perhaps brine immersion freezing, where a brine residue can accelerate rancidity. The lipophilic nature of DDM raises the question of whether DDM would affect the highly unsaturated, unstable fats of fish. No observable tendency for DDM freezing to accelerate rancidity was noted from this study.

Drip losses, shown in Figure 3, are essentially the same for salmon steaks frozen by all three methods. These results further indicate the overall similarity between the three freezing methods with respect to quality.

The three freezing methods used for study of quality provide an opportunity to examine the effect of freezing time on quality. DDM freezing of salmon steaks for the quality study required 6–8 min, air blast freezing took 90 min and LN-boosted air blast took 25 min. Dyer (1969) in a review of quality of fish as a

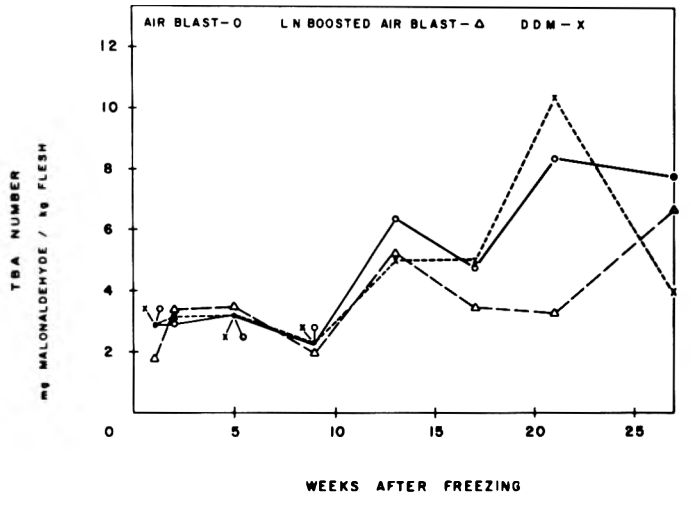

Fig. 1—TBA numbers for king salmon frozen by three methods.

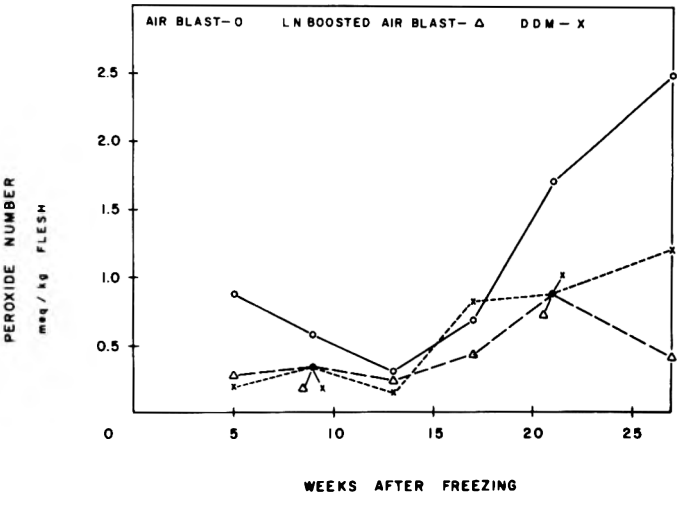

Fig. 2—Peroxide numbers for king salmon frozen by three methods.

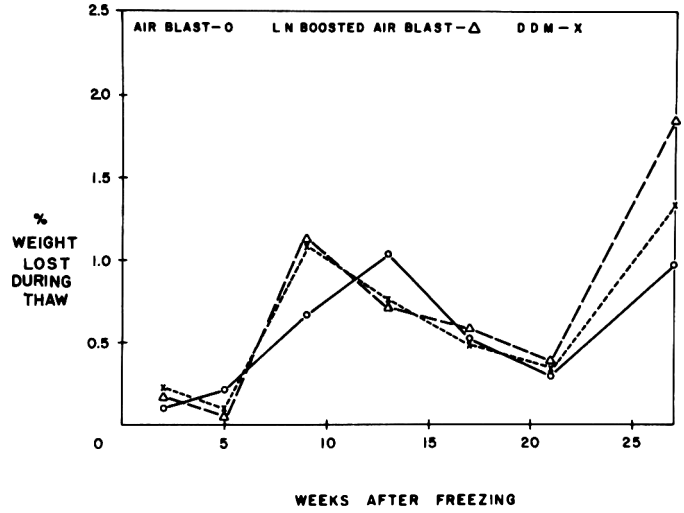

Fig. 3–Drip losses for king salmon steaks frozen by DDM immersion, air blast and LN-boosted air blast.

Table 4–Dehydration during freezing

	Air blast	LN-boosted air blast	DDM
Loss of weight during freezing	1.2%	0.8%	0.11%
No. of samples	55	43	30

Table 5–DDM residues after freezing

Min after freezing	Sole ppm w/w	Ling cod ppm w/w	King salmon ppm w/w
0	3281	2402	3268
10	241	916	692
20	123	266	300
30	138	210	271
60	174	144	240

function of freezing rate, indicated that although differences in quality do exist, freezing quality has been overemphasized. A similar conclusion can be made for this study, that no quality differences attributable to the speed of freezing were shown.

Dehydration losses shown in Table 4 are considerably higher for air blast and LN-boosted air blast frozen fish than for DDM frozen fish. DDM is a hydrophobic material, while in air blast freezing water may be evaporated (or sublimed) due to the vapor-holding capacity of air and the vapor pressure of water in food. In commercial practice DDM freezing is performed in a static atmosphere to reduce DDM vapor losses, thus inherently minimizing water evaporation from the product. The low losses in DDM freezing could be of considerable importance.

The freezing time nomographs in Figures 4 and 5 present measured data that can be used in the estimation of freezer size. Methods of estimating freezing times based on the material being at the freezing point initially and steady-state beat transfer conditions during freezing are clearly unsuitable for use with a freezing method as fast as DDM. Baker and Charm (1969) found that by using the Nagaoka equation (a modification of the Plank equation) to calculate freezing times of fish in liquid nitrogen and liquid DDM, there was a large discrepency between measured and calculated times.

Examination of DDM residue determinations after freezing in Table 5 indicates that there is little difference in the tendency for DDM to remain associated with high-fat fish when compared with low-fat fish. No residue limits were set when approval was granted (Federal Register, 1967). Crawford et al. (1969) reported levels less than 1 ppm after cooking.

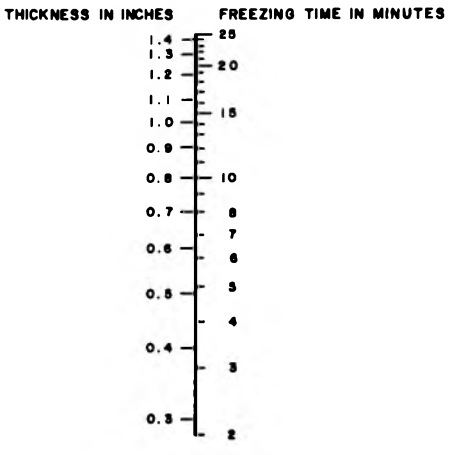

Fig. 4–Freezing time nomograph for unpackaged fillets of low-fat, white-fleshed fish in liquid DDM.

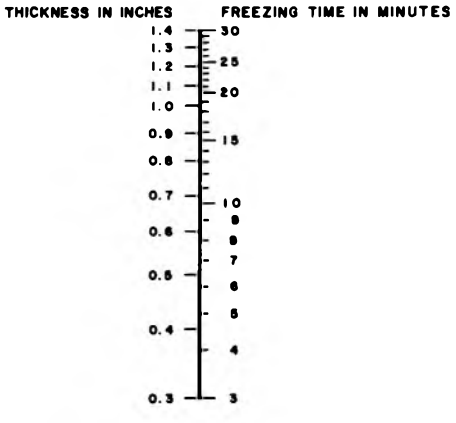

Fig. 5–Freezing time nomograph for packaged fillets of low-fat, white-fleshed fish in liquid DDM. (Packaging was heat-shrunk cryovac film which conformed closely to the surface of the fish.)

CONCLUSIONS

THE RESULTS of this work demonstrated that the salmon steaks frozen by DDM immersion were comparable in quality to the salmon steaks frozen by methods representing good commercial practice. Direct contact of the liquid freezant did not degrade the quality of the salmon in any observable way. Results of the quality study indicated that this method of freezing should be useful for other types of fishery products as well.

The lipids showed no change in tendencies toward rancidity, indicating that DDM residue did not affect highly unsaturated fatty acids. Furthermore, the data on the decay of DDM residues showed that DDM had no greater tendency to remain on high-fat content fish flesh than on lean fish.

Freezing time nomographs gave explicit representation of empirically obtained data which will be valuable in DDM freezer design. These nomographs presented freezing times which included prefreezing cooling from 50°F and postfreezing cooling to approximately 0°F. A designer, therefore, may refer to the nomograph without making further approximations to cope with the assumptions of methods of calculating freezing times.

Of considerable economic importance is the low weight loss in DDM immersion freezing compared with air blast methods. This difference could be sufficient to offset the slightly higher cost of DDM freezing, depending upon the value of the product, and make it directly competitive

with conventional methods. The rapidity of DDM freezing makes continuous, in-line freezing possible with lower labor requirements than batch processes and should be of interest to processors of fishery products.

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Ms received 10/16/71; revised 2/13/72; accepted 2/14/72.

Our thanks to Dr. John J. Daly of E.I. duPont de Nemours Company who generously provided a laboratory freezer, supplies of dichlorodifluoromethane freezant (duPont Freezant 12) and analysis of samples for residual freezant.

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DEVELOPMENT OF IRRADIATION STERILIZED CODFISH CAKES

INTRODUCTION

HIGH PROTEIN FOODS (particularly meat, poultry and seafoods) that are stable without refrigeration are of vital importance to the Armed Forces (Joint Committee on Atomic Energy, 1966). Sterilization of fish steaks and fillets by thermal or radiation processing has not been too successful because these types of processing cause severe degradation of textural characteristics and appearance (Learson et al., 1969). Sinnhuber et al. (1966) produced irradiation sterilized codfish cakes and halibut cakes that were acceptable shortly after irradiation and after 12 months storage at 21°C; however, some darkening occurred. Sinnhuber et al. (1968) conducted another study that involved the use of antioxidants for controlling browning. The results again showed the fish cakes were acceptable for the duration of the study (12 months) but none of the antioxidants studied seemed to improve flavor scores significantly or to slow down the browning reaction. Yu et al. (1969) showed that the browning was probably due to monosaccharides in the fish muscle and that the browning could be reduced by leaching the ground fish in water and by the addition of antioxidants and SO_2. This leaching, however, also removed much of the flavor from the fish cakes.

Using procedures similar to those developed by Sinnhuber et al. (1966), experiments were conducted to confirm and extend their work in the development of process criteria for producing a shelf stable, highly acceptable fish item that can be used in military and civilian feeding systems.

EXPERIMENTAL

Formation of codfish cakes

The codfish cakes used in these studies were made from frozen codfish fillets (*Gadus morhua*). Thawed fillets were ground in a meat grinder using a 0.635-cm plate and to each 100 parts (100 lbs) of ground fish was added 4 parts (4 lb) white corn meal, 1.5 parts [1-½ lb gelatin (Viscomix, Swift & Co., Kearney, N.J.) and 0.5 parts (½ lb) salt (NaCl)]. The fish and additives were well mixed in a mechanical mixer and stuffed into 10.16 cm diam, pre-stuck, regenerated cellulose meat casings (Visking Div., Union Carbide Co., Chicago, Ill.). This product was then heated in a boiling water bath (to inactivate the autolytic enzymes) until a center temperature of 80–85°C was reached and then cooled overnight in a 4°C refrigerator.

Table 1—Proximate analysis of irradiation sterilized codfish cakes[a]

Chemical characteristic	Percent
H_2O	72–75
Protein	20–23
Salt (NaCl)	0.3–0.6
Carbohydrates	3.0–6.0
Fat	0.2–0.4
Ash	1.2–1.8

[a]3.2 Mrad at −30 ± 10°C

Table 2—Effects of irradiation temperature on irradiation sterilized codfish cakes

Irradiation Dose Mrad	Irradiation Temp °C	Storage temp °C	Mean scores for 7 or 8 technical panelists Time in storage 1 mo	4 mo	9 mo
			Preference[a]		
3.2	ambient	21	7.4[c]	6.2[d]	6.7[e]
3.2	−30 ± 10°	21	7.6	7.1	7.4
3.2	−80 ± 10°	21	7.7	7.6	7.3
none	–	−29	7.7	7.6	7.6
			Discoloration[b]		
3.2	ambient		1.3	1.6	2.4[c]
3.2	−30 ± 10°		1.4	1.7	1.8
3.2	−80 ± 10°		1.3	1.6	1.7
none	–		1.1	2.2	1.1
			Off-odor[b]		
3.2	ambient		1.4	1.0	1.1
3.2	−30 ± 10°		1.3	1.1	1.1
3.2	−80 ± 10°		1.4	1.0	1.0
none	–		2.0	1.4	1.0
			Irradiation-flavor[b]		
3.2	ambient		1.5	1.1	1.2
3.2	−30 ± 10°		1.3	1.1	1.0
3.2	−80 ± 0°		1.0	1.1	1.0
none	–		1.5	1.6	1.0
			Off-flavor (other than irradiation)[b]		
3.2	ambient		1.1	1.0	1.2
3.2	−30 ± 10°		1.1	1.0	1.1
3.2	−80 ±		1.0	1.0	1.4
none	–		1.1	1.4	1.3
			Mushiness[b]		
3.2	ambient		1.5	1.1	1.8
3.2	−30 ± 10°		1.6	1.1	1.9
3.2	−80 ± 10°		1.5	1.1	1.6
none	–		1.9	1.3	1.3

[a]1 = dislike extremely; 9 = like extremely
[b]1 = none; 9 = extreme
[c]Significantly different (95% confidence level) from the other ambient samples
[d]Significantly different (95% confidence level) from the other 4-month samples
[e]Significantly different (95% confidence level) from the other 9-month samples

After removing the casing, the codfish rolls were cut into 1.25 cm thick slices weighing approximately 114g. Most of the samples were packed in metal cans with an epoxy-phenolic enamel lining. Some of the slices were packed in laminated (Mylar/aluminum foil/medium density polyethylene) pouches, one slice per pouch. A porous packet containing 3g of activated charcoal was added to all cans prior to closing them under vacuum (48 mm Hg). The pouches were also closed under vacuum (gauge 48 mm Hg) using an impulse heat-sealing machine. After closure all samples were frozen (−20°C) (−4°F) and part of these were used as nonirradiated frozen controls.

Table 3—Consumer evaluations of irradiation sterilized codfish cakes

Type panel	No. of panelists	Dose Mrad[a]	Storage Time	Storage Temp	Mean preference Scores[b]
Military	531	4.5	3 mo	21–27°C	5.4[c]
Military	528	None	3 mo	−29°C	6.3
Military	403	4.5	9 mo	21–27°C	5.4
Military	448	None	9 mo	−29°C	5.4
Civilians	32	3.2	10 mo	21°C	6.8
Civilians	32	None	10 mo	−29°C	6.4

[a]At −30 ± 10°C
[b]1 = dislike extremely; 9 = like extremely
[c]Significantly different (99% confidence level) from the frozen nonirradiated control

Table 4—Effects of storage temperature on irradiation sterilized codfish cakes

Irradiation Mrad at −30 ± 10°C	Storage temp °C	Mean scores for 7 or 8 technical panelists: Time in storage 10 days	1 mo	4 mo	9 mo	16 mo	20 mo	25 mo
		Preference[a]						
3.2	21	6.8	6.9	6.9	7.3	6.4	6.9	6.9
3.2	38	6.0	5.8	6.6	–[c]	–[c]	–[c]	–[c]
3.2	−29	6.2	6.4	6.0	–[d]	6.2	6.7	6.7
none	−29	7.0	6.6	6.9	7.4	7.0	7.0	7.1
		Discoloration[b]						
3.2	21	1.4	1.1	1.3	1.3	1.4	1.8[e]	2.0[e]
3.2	38	2.0	1.4	1.3	–[c]	–[c]	–[c]	–[c]
3.2	−29	1.3	1.3	1.1	–[d]	1.2	1.3	1.7
none	−29	1.5	1.4	1.3	1.1	1.3	1.0	1.7
		Off-odor[b]						
3.2	21	1.4	1.1	1.0	1.1	1.8	2.1	1.0
3.2	38	2.0	1.8	1.1	–[c]	–[c]	–[c]	–[c]
3.2	−29	1.2	2.1	1.0	–[d]	1.8	1.3	1.7
none	−29	1.5	1.8	1.0	1.1	1.1	1.3	1.0
		Irradiation flavor[b]						
3.2	21	1.1	1.1	1.4	1.3	1.0	1.2	1.0
3.2	38	1.8	1.0	1.1	–[c]	–[c]	–[c]	–[c]
3.2	−29	2.1	1.3	1.4	–[d]	1.3	1.8	1.8
none	−29	1.4	1.1	1.0	1.1	1.2	1.2	1.1
		Off-flavor (other than irradiation)[b]						
3.2	21	1.3	1.4	1.9	1.2	1.8	1.3	1.4
3.2	38	2.1	2.2	1.1	–[c]	–[c]	–[c]	–[c]
3.2	−29	1.9	1.8	1.3	–[d]	1.6	1.2	1.8
none	−29	1.1	1.8	1.2	2.1	1.2	1.0	1.3
		Mushiness[b]						
3.2	21	1.1	1.5	1.2	1.6	1.3	1.2	1.8
3.2	38	2.1	1.8	1.4	–[c]	–[c]	–[c]	–[c]
3.2	−29	1.9	1.8	1.1	–[d]	1.4	1.3	1.9
none	−29	1.2	1.5	1.2	1.1	1.3	1.2	1.8

[a]1 = dislike extremely; 9 = like extremely
[b]1 = none; 9 = extreme
[c]Rejected for subjective evaluation due to discoloration.
[d]Sample lost
[e]Significantly different (95% confidence level) from the other 21°C stored samples.

Irradiation and storage

The experimental samples were irradiated at the U.S. Army Natick Labs. using either the 1.2 megacurie Cobalt-60 source or the 10 MeV electron linear accelerator. The irradiation doses, as so stated, are minimum doses and may range up to a maximum dose of 120% of the minimum dose. Most of the samples were irradiated in the frozen state at −30 ± 10°C (−22 ± 18°F) and were given 3.2 Mrad. This is the established 12-D dose as determined by inoculated pack studies for codfish cakes when irradiated at −30°C (Anellis, 1971, personal communication). A few of the cans were given 4.5 Mrad. After irradiation, the samples were stored at 21°C, 38°C or −29°C, which were used as irradiated frozen controls.

Evaluations

Proximate analyses were done on representative samples from several different lots using the standard methods of the AOAC (1970).

Samples from all experimental lots were evaluated by seven or eight trained expert panelists for the following sensory characteristics: discoloration, off-odor, irradiation flavor, off-flavor (other than irradiation), mushiness and an indication of preference. A 9-point intensity scale was used for evaluating the effects of the various treatments on the sensory characteristics with "1" signifying "none" and "9" indicating "extreme." A 9-point hedonic scale (Peryam and Pilgrim, 1957) was used for preference evaluations.

For serving, the fish cakes were breaded, deep-fat fried (177°C) until golden brown (approximately 1 min), and served hot. Some of these samples were also evaluated for preference by 32-member consumer-type panels, and two lots, which were given 4.5 Mrad at −30 ± 10°C, were evaluated by troops. Troop evaluations were done using the same technique used by Hembree and Burt (1965).

All samples used in sensory evaluations were tested to ascertain the absence of preformed toxin of *Clostridium botulinum*, types A and B. All data were statistically analyzed using analysis of variance and the Duncan multiple range test.

RESULTS & DISCUSSION

Appearance

The condition and appearance of the irradiated samples upon opening the cans or pouches were similar to the nonirradiated controls except for color. The irradiated samples, including those stored at −29°C, were darker, and this darkening was particularly noticeable in the samples stored at 38°C for 4 months or longer. In some instances the 38°C-stored samples were so dark they had to be rejected for organoleptic testing. All samples were moist and there was little, if any, liquid in the containers. They were firm in texture and could be easily handled without breaking during the breading and deep frying. The composition of the products is shown in Table 1. Yield, based on the weight of the thawed fillets, was prac-

tically 100%. These high yields were probably due to the addition of gelatin.

Effects of irradiation

The influences of irradiation on product quality must be considered in relation to many factors including total dose, irradiation temperature, type of irradiation and storage conditions after irradiation. Results of many experiments on codfish cakes have shown that, as with meat (Wadsworth and Shults, 1966) radiation induced changes in the sensory characteristics are increased as the dose is increased and are decreased as the irradiation temperature is decreased.

Table 2 contains results of a typical storage study involving variations in irradiation temperature. Although all samples were scored in the acceptable range, those that were irradiated at subzero temperatures were preferred and more stable than those irradiated in the nonfrozen state. In this study, and it has been observed in others, the benefits of reduction of irradiation temperature from −30°C to −80°C are marginal. Irradiation, regardless of temperature, seems to have very little effect on any of the sensory characteristics studied except color, and products irradiated in the nonfrozen state seem to darken more rapidly during storage.

The data in Table 3 show the results of some consumer evaluations of codfish cakes irradiated at two dose levels: 3.2 Mrad and 4.5 Mrad. These data, and they are consistent with results of other similar studies, show that the fishcakes are acceptable and relatively stable at room temperature storage. The data also show that product acceptability is related to irradiation dose.

Effects of storage

Several experiments have been conducted in which the irradiated test samples were stored at various temperatures. Table 4 contains results of one of these studies that lasted 25 months. The data show that the irradiated fish cakes are very acceptable and stable when stored at 21°C. At elevated temperatures (38°C) the fish cakes become unacceptable some time between the 4th- and 9th-month evaluation. This deterioration is primarily due to discoloration as the products are usually so "brown" at the 9th-month withdrawal they could not be served to the panelists. Sinnhuber and his associates (1966, 1968) had similar results, reporting that although there was some discoloration, the test samples scored in the acceptable range throughout the study. This discoloration is probably due to the Maillard-type nonenzymatic browning reaction (Yu et al., 1969; Learson et al., 1969) and unrelated to the irradiation as browning has been frequently observed in heated fish muscle (Van den Brock, 1965). It is interesting to note that storage had very little effect on the other sensory characteristics studied.

An evaluation of all the data clearly points out that a slight discoloration had no material effect on acceptance but strong discoloration, which occurred in the samples stored at 38°C, markedly affected acceptability.

Effects of type of irradiation

On numerous occasions codfish cakes that had been packed in flexible bags and sterilized with either gamma rays from the Cobalt-60 source or by electrons generated by the linear accelerator were compared. In no instance have there been any detectable differences in acceptance that could be attributed to the type of irradiation.

CONCLUSIONS

RESULTS of these studies and those conducted elsewhere show that high quality, well accepted codfish cakes, that will remain stable at room temperature (21°C) for long periods of time can be produced. These fishcakes were sterilized by ionizing radiation and given a 12-D sterilizing dose (3.2 Mrad at −30 ± 10°C). They had a tendency to darken when held in storage, particularly when storage temperatures were elevated. The irradiated fishcakes stored at 38°C, although darker than those stored at lower temperatures, were scored in the acceptable range up to 4-months storage, but were considered unacceptable after 9-months storage. No differences in product quality could be detected due to the type of irradiation (gamma vs. electrons).

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Ms received 12/1/71; revised 2/28/72; accepted 3/4/72.

Presented at the 31st Annual Meeting of the Institute of Food Technologists in New York City.

This paper reports research undertaken at the U.S. Army Natick (Mass.) Labs. and has been assigned No. TP 1126 in the series of papers approved for publication. The findings in this report are not to be construed as an official Department of the Army position.

The authors acknowledge the assistance of Dr. E. Wierbicki, Irradiated Food Products Div., Food Lab., U.S. Army Natick Labs., Natick, Mass. and Mr. L.J. Ronsivalli, U.S. Dept. of Commerce, Atlantic Fishery Products Technology Center, Gloucester, Mass. for their contribution in this investigation.

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EFFECT OF HYDROGEN PEROXIDE ON THE COLOR, COMPOSITION AND NUTRITIVE QUALITY OF FPC (FISH PROTEIN CONCENTRATE)

INTRODUCTION

COLOR is an important quality attribute of any food because acceptance or rejection of a product is usually based on impression gained from the appearance of the food material.

The color of fish protein concentrate (FPC) varies from light gray to creamy, depending on the type of fish used, method of extraction and also the particle size. The importance of the color of FPC becomes more critical when it comes in contact with water and/or oil because it darkens, which obviously is not desirable.

The color of FPC has two origins: (1) the melanins present in scales, which are insoluble in alcohol; and (2) the red pigment of the blood (hemoglobin) in combination with protein which is mostly alcohol soluble (Brown, 1957). During the manufacture of FPC, blood pigment is mostly removed by centrifugation; however, the melanin from the scales remains in the final product.

In recent years the practice of adding a variety of oxidizing agents to food materials for the purpose of pasteurization, as well as decolorization, is well recognized (McDonough et al., 1968; Yatsu et al., 1970; Pack et al., 1968; Fox and Kowsikowski, 1967; and Subramanian and Olson, 1968). It has been shown that there was no major change in the proteins and nutritional value of cheddar cheese made from hydrogen peroxide-treated milk (Tepley et al., 1958). Grindard and Nickerson (1967) studied the effect of hydrogen peroxide on the electrophoretic pattern of individual protein from skim milk. They reported nonprotein nitrogen increased slightly as a function of hydrogen peroxide concentration and time. Hydrogen peroxide did not induce complex formation between β-lactoglobulin and K casein. Fish and Mickelsen (1967) reported that hydrogen peroxide decreased denaturation of skim milk after heating for 35 min at 85°C.

The objective of this study was to determine if the color of FPC could be improved by treatment with small quantities of hydrogen peroxide followed by spray drying without adversely affecting chemical composition and nutritional value.

Table 1—Average color values of FPC-flour, paste and cookies measured by Hunter color meter after being treated with different levels of hydrogen peroxide and then spray dried

	% level of hydrogen peroxide					
	0.00	0.30	0.60	1.25	2.50	5.00
			Dry FPC			
L[f]	63.2[a] ± 0.45	63.2[ab] ± 0.94	66.7[c] ± 0.30	68.6[d] ± 0.40	69.3[e] ± 0.13	72.0[f] ± .50
a[g]	.79[a] ± 0.04	1.3[b] ± 0.02	1.1[c] ± .04	1.3[bd] ± .13	1.4[b] ± .10	1.2[cd] ± .09
b[h]	15.9[a] ± .26	16.3[b] ± .27	16.5[cb] ± .21	17.0[d] ± .30	18.1[e] ± .33	18.1[e] ± .20
			FPC paste			
L	48.8[ab] ± .39	49.2[a] ± .13	51.0[b] ± .81	52.4[c] ± .28	55.4[d] ± .25	57.2[e] ± .22
a	1.7[a] ± .04	1.7[a] ± .03	2.2[b] ± .14	2.5[c] ± .08	2.5[c] ± .12	2.5[c] ± .09
b	16.2[a] ± .18	16.5[b] ± .20	17.4[c] ± .27	18.4[d] ± .28	19.2[e] ± .19	20.3[f] ± .16
			FPC cookies			
L	63.2[a] ± .12	62.2[b] ± .26	63.4[ad] ± .30	63.9[c] ± .40	64.0[cd] ± .70	66.6[e] ± .70
a	1.5[a] ± .08	2.9[b] ± .28	3.5[c] ± .07	3.3[cb] ± .30	3.8[dc] ± .53	1.6[a] ± .26
b	16.4[a] ± .32	17.9[b] ± .16	19.3[c] ± .10	19.3[dc] ± .16	19.4[dc] ± .04	18.6[e] ± .49

[a—e]Means in the same row bearing different superscripts are significantly different by at least $P < 0.05$.
[f]L = lightness
[g]a = greenness or redness
[h]b = yellowness or blueness

MATERIALS & METHODS

Materials

FPC was prepared from Atlantic menhaden (*Brevoortia tyrannus*) by countercurrent extraction with 91% isopropyl alcohol (Bureau of Commercial Fisheries, 1966). After drying, the FPC was milled in a Rietz disintegrator.

For bleaching, five levels of hydrogen peroxide were used. These levels, expressed as percent of the weight of the dried FPC, were as follows: 0.00, 0.30, 0.60, 1.25, 2.5, and 5.0%. Two liters of distilled water (50 ± 2°C) were added to each 454-g sample of FPC. The sample was blended in a stainless steel container with the aid of an electric stirrer. The calculated amount of hydrogen peroxide was added gradually to the slurry and the sample was stirred for 15 min. The temperature of the sample was kept constant (50 ± 2°C) during bleaching using a water bath. After blending, an additional 1 liter of hot water (50 ± 2°C) was added for each pound of FPC and the sample was mixed for 5 min. The final slurry contained a ratio of (w/w) 1:6:6 FPC to water. The slurry was spray dried at 60 lb/in.^{2} pressure in a laboratory spray drier. The inlet temperature was 280 ± 5°F and the outlet temperature was 190 ± 5°F.

Methods of evaluation

The color of the treated samples was determined with a Hunter Color Meter (Model 25). Samples were evaluated in the dry form, as a wet paste, and in cookies in which 10% of the flour was replaced by FPC (AACC, 1962). The paste was prepared by blending a 29-g sample of FPC with 65 ml of distilled water. A uniform paste with no syneresis was obtained with this combination. To evaluate the samples, the color meter was standardized with a white ceramic tile plate No. 2880. The values for this tile were as follows: L (lightness) = 83.3; a (redness) = −3.3; and b (yellowness) = ± 26.4. The values for the experimental samples were read directly from the color scales.

Sensory evaluations were also made on the treated cookie samples. Cookies were evaluated for appearance, texture and flavor by a 10-member taste panel. A 9-point hedonic scale (1 = least acceptable; 9 = most acceptable) was used to measure preference. The standard had a value of 5 (Peryam and Pilgrim, 1957).

Samples were analyzed for total amino acids with an automatic amino acid analyzer by the method described by Moore et al. (1958). Available lysine was analyzed as described by Carpenter (1960). Tryptophan was analyzed by the method of Spies and Chambers (1949).

The nutritive quality of the samples was determined in a rat-feeding study. Diets were prepared that contained 10% protein from each of the FPC samples. A diet containing 10% protein from casein was used as a reference

standard. The composition of the basal diet has been reported by Stillings et al. (1969).

Male weanling rats of the Sprague Dawley strain were obtained at 22 days of age. During the first 2 days they were fed a basal diet containing 15% casein. They were then assigned to groups of 10 on the basis of body weight, and the groups were randomly assigned to the experimental diets. The rats were fed ad libitum for 4 wk. Feed consumption was recorded three times each week, and the gains in weight were determined once each week. At the end of the experiment, the PER (protein efficiency ratio) was determined by dividing the gain in weight by the weight of protein consumed. Data were evaluated statistically and differences between the means were determined by Tukey's procedure as described by Steel and Torrie (1960).

Table 2—Average score of a 9-point hedonic scale for sensory evaluation of the cookies containing 10% FPC treated with varying levels of hydrogen peroxide

% level of peroxide	Appearance		Texture		Flavor	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Control (0.0)	3.36 ± 1.36[a]		5.91 ± 1.38		5.00 ± 1.00	
0.3	4.36 ± 1.50[abc]		5.36 ± 1.03		4.90 ± 1.04	
0.6	4.36 ± 0.80[ab]		6.18 ± 0.75		4.72 ± 0.90	
1.25	4.45 ± 1.21[abc]		5.64 ± 1.21		4.63 ± 1.20	
2.50	4.72 ± 1.10[bc]		6.00 ± 1.61		5.09 ± 1.57	
5.00	5.63 ± 1.50[c]		5.45 ± 1.29		4.63 ± 1.12	

[a,b,c]Means in the same column bearing different superscripts are significantly different by at least $P < 0.05$.

Table 3—Amino acid composition of Atlantic menhaden FPC samples before and after treating with different levels of hydrogen peroxide (grams of amino acid/16g nitrogen)

Amino acid	% level of hydrogen peroxide					
	0.0	0.30	0.60	1.25	2.5	5.0
Lysine	7.40	7.30	7.70	7.60	7.15	7.30
Available lysine	6.70	7.15	7.20	7.90	7.35	7.20
Histidine	2.15	2.10	2.22	2.20	2.00	2.00
Arginine	6.00	5.90	6.00	6.20	6.10	6.20
Threonine	3.65	3.70	3.80	3.80	3.60	3.80
Valine	4.60	4.80	4.85	4.85	4.55	4.80
Methionine	2.60	2.70	2.65	2.65	2.40	2.40
Isoleucine	3.75	3.95	3.90	3.95	3.75	3.95
Leucine	6.50	6.80	6.70	6.75	6.40	6.70
Tyrosine	2.70	2.80	2.75	2.70	2.60	2.55
Phenylalanine	3.40	3.50	3.60	3.60	3.20	3.50
Tryptophan	1.20	1.22	1.10	1.10	1.00	1.10

Table 4—Effect of hydrogen peroxide treatment on the nutritive quality of fish protein concentrate

Dietary protein source	Average daily weight gain (g)	Average daily feed intake (g)	Protein efficiency ratio[a]
Casein	4.60 ± 0.29[b]	13.12 ± 0.52	3.30 ± 0.10
FPC Peroxide level[c]			
0.0%	4.70 ± 0.17	13.41 ± 0.34	3.36 ± 0.07
0.3%	5.21 ± 0.30	14.78 ± 0.65	3.39 ± 0.09
0.6%	5.02 ± 0.35	14.67 ± 0.66	3.30 ± 0.10
1.25%	4.25 ± 0.20	13.78 ± 0.40	2.92 ± 0.08
2.50%	3.69 ± 0.16	12.56 ± 0.31	2.84 ± 0.06
5.00%	3.74 ± 0.27	12.17 ± 0.59	2.91 ± 0.12
Tukey's ω ($P < 0.05$)	1.13	2.27	0.39

[a]Calculated by dividing the total weight gained by the protein consumed.
[b]Standard error of the mean
[c]Hydrogen peroxide level was calculated as a percentage of the weight of the FPC.

RESULTS & DISCUSSION

TABLE 1 shows the effect of varying levels of hydrogen peroxide on color of treated FPC samples. Overall results showed that the color of the spray-dried FPC, based on the L values, was lightened considerably with increasing concentrations of hydrogen peroxide. The L values for the FPC paste were all lower than those for the dry FPC. Incorporating the hydrogen peroxide-treated FPC samples into cookies lightened the color, compared to the color of those containing nontreated FPC, but the differences were much smaller than those for the dry and wet FPC.

Peroxide treatment decreased the degree of redness (a value) in dry FPC, but it increased the redness in the wet FPC and the cookies containing FPC, with the exception of the highest treatment. Peroxide-treated samples had a higher degree of yellowness (b value) in all cases for dried FPC's, slurries, and cookie samples.

Table 2 shows the results of the sensory evaluation of cookies containing the experimental FPC's. Cookies containing FPC treated with 2.5 and 5.0% hydrogen peroxide were significantly lighter than the control sample. No significant differences were noted in the flavor and texture of the various samples.

Table 3 shows the results of amino acid analysis of the treated samples. The hydrogen peroxide treatments had a negligible effect on the amino acid composition of the samples. A slight decrease was noted in the amounts of methionine and tyrosine in samples treated with 2.5 and 5.0% hydrogen peroxide.

Table 4 shows data on the nutritive quality of the samples, which were evaluated as the sole source of protein in diets containing 10% protein. The use of 0.30 and 0.60% peroxide had no effect on the nutritive quality. Samples treated with 1.25, 2.50 and 5.0% hydrogen peroxide had slightly lower PER values.

In general, these studies showed that the color of FPC and of products containing FPC can be improved by the use of small amounts of hydrogen peroxide. The optimum level of peroxide was 0.6%, which improved the color of the FPC samples without decreasing the nutritive quality. Higher levels of peroxide gave further improvements in color, but the nutritive quality of the FPC was lowered slightly. This was possibly due to the breakdown of cystine, which was not measured in this experiment. Also, there was a slight decrease in the methionine content of the FPC samples when 1.25%

or more hydrogen peroxide was added. This indicates that possibly at higher levels of peroxide, methionine sulfoxide was formed which lowered the PER values (Miller et al., 1970).

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Ms received 10/16/71; revised 2/26/72; accepted 3/4/72.

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PHOSPHOLIPID CHANGES IN MUSCLE FROM FROZEN STORED LAKE MICHIGAN COHO SALMON

INTRODUCTION

FROM STUDIES of the different lipid fractions in fish muscle during frozen storage, it has been ascertained that increases in free fatty acid (FFA) content are primarily due to hydrolysis of the phospholipids catalyzed by phospholipases in the muscle and release of FFA. The FFA could then cause denaturation of proteins in the flesh (Lovern et al., 1959; Olley and Lovern, 1960; Bligh, 1961; Jonas and Tomlinson, 1962). Frozen storage of cod muscle at −12°C for 9 months resulted in an increase of the FFA content from 5–325 mg/100g flesh due to the hydrolysis of phosphatidylethanolamine (PE) and phosphatidylcholine (PC); PE hydrolysis ceased after storage for 4 months, whereas PC hydrolysis continued thereafter at a slower rate (Bligh and Scott, 1966). Storage of frozen herring for up to 12 wk at −15°C resulted in an increase of the FFA content from 50–1000 mg/100g flesh in dark muscle and from 17–280 mg/100g flesh in white muscle (Bosund and Ganrot, 1969). This FFA increase in the white and dark muscle was due to the hydrolysis of PC, PE and to a varying extent, also of triglycerides. Additional evidence for enzymatic phospholipid hydrolysis in stored fish was reported when the formation of glyceryl-phosphorycholine and choline was observed in cold-stored rainbow trout muscle (Jonas and Bilinski, 1967).

Other research has shown that phospholipid hydrolysis in frozen muscle during storage is not confined to fish. During storage of chicken muscle at −10°C, decreases in PC and PE and increases of lysophosphatidylcholine (LPC) and FFA were observed; these results suggested that, since lipid hydrolysis occurred throughout frozen storage, lipid hydrolysis and protein denaturation may be interdependent phenomena (Davidkova and Khan, 1967).

A slight decline in the total phospholipids in frozen bovine muscle was evident during the first two weeks of storage, then the phospholipid loss was high between the second and fifth weeks; a net increase in the FFA content was also observed (Awad et al., 1968).

The purpose of the study was to examine changes in the phospholipid fraction of the stored, frozen muscle and relate these changes to specific hydrolysis of these lipids by lipolytic enzymes present in the tissue.

EXPERIMENTAL

Fish

Lake Michigan Coho salmon were obtained from the Michigan State Dept. of Natural Resources in Sept., 1968 and July, 1969. These fish were kept on ice for approximately 15 hr and then frozen and stored whole in a blast freezer at −20°C. Fish used for analyses were kept in separate polyethylene bags and, when needed, steaks were sawed vertically from the Dorsal fin region of the frozen fish (Gruger et al., 1964; Braddock and Dugan, 1969). The skin, belly flap and dark muscle were discarded. Whenever it was necessary to compare component changes before and after storage, muscle was used from the same fish. The 1968 salmon had an average weight of 7–8 lb and a total lipid content of 22% (g fat/g dry weight flesh), while the 1969 salmon were 4–5 lb and approximately 11% fat. The fish were not differentiated on the basis of sex.

Fatty acid analysis

Total lipid samples from the salmon were obtained using a method in which the tissue was homogenized with chloroform and methanol in such proportions that a miscible system was formed (Bligh and Dyer, 1959). Neutral lipid and phospholipid fractions were then separated by thin-layer chromatography (TLC) on silica gel G (petroleum ether:ethyl ether:acetic acid, 70:30:2). The phospholipids remained at the origin on the plate, while the glycerides, free fatty acids and other neutral lipids migrated. The band containing the triglycerides (TG) was identified by chromatographing corn oil (Mazola), spraying with 2',7'-dichlorofluorescein and observing the plate under ultraviolet (UV) radiation. This band was scraped from the plate directly into 30 ml Et_2O and saved for methyl ester preparation. All TLC was performed under a CO_2 atmosphere produced by placing a piece of solid CO_2 in the TLC solvent tanks prior to development of a plate.

The phospholipid band from the plate was eluted on a sintered glass funnel with three 15 ml portions of chloroform:methanol (4:1), concentrated under a nitrogen stream and rechromatographed in $CHCl_3$:MeOH:H_2O (65:25:4). The phospholipids were separated into PC, PE, LPC and PLE. The bands were identified through the use of standards, ninhydrin and molybdate spray reagents (Dittmer and Lester, 1964) and scraped into Et_2O for preparation of methyl esters.

Methyl esters were prepared from the TG and phospholipid fractions by a low temperature trans-esterification method (Zook, 1968).

The methyl esters were identified and determined using a Beckman GC-5 gas chromatograph equipped with dual flame ionization detectors. Resolution of the methyl ester peaks was achieved at 190°C using a 6 ft × 1/8 in. stainless steel column packed with 20% DEGS-chromosorb W 80/100 mesh.

Silica gel G plates sprayed with 20% silver nitrate in 90% ethanol and activated at 110°C for 1 hr in absence of light were used to separate the methyl esters according to the total number and geometry of double bonds in the molecules. The solvent system used was petroleum ether:HOAc:H_2O, 90:10:1 by volume. Each band was scraped from the plate, eluted with Et_2O, and injected into the gas chromatograph.

Fatty acid methyl ester standards used for comparison in all of the TLC and GLC work were obtained from Supelco. Use was also made of Supelco PUFA mixture #7033, which contained the methyl esters $C_{16:1}$, $C_{18:1}$, $C_{20:1}$, $C_{20:5\omega3}$ and $C_{22:6\omega3}$.

Additional techniques which aided in identification of the fatty acids included the following: (1) Reference to previously published research concerning identification of fatty acids from Pacific Coast Coho salmon (Braddock and Dugan, 1969); (2) Comparisons of the GLC retention times of known fatty acid methyl esters with unknown peaks obtained from Coho salmon fatty acid methyl esters and graphs of log retention time vs. carbon chain length for each series of fatty acid methyl esters (Horning et al., 1964; Hofstetter et al., 1965).

Phosphorus analysis

Phospholipid bands consisting of PE, PC, LPE and LPC were scraped from the TLC plates as described above and placed into micro-Kjeldahl flasks for digestion with perchloric acid following previously described procedures (Harris and Popat, 1954). After digestion, the samples were cooled and 5 ml distilled water was used to rinse the neck of each flask. Then 1 ml each of 2.5% ammonium molybdate and 10% ascorbic acid were added, followed by rinsing with 2 ml distilled water. The samples were placed in a boiling water bath for 5 min to allow the color to develop, centrifuged at 3000 × G for 5 min and the absorbance read at 820 nm. For quantitative purposes, absorbance of samples was compared with standard phosphorus solutions prepared from potassium dihydrogen phosphate.

RESULTS & DISCUSSION

THE DIVERSITY and quantity of lipids in many species of fish have contributed to numerous technical problems for the scientists and processors concerned with maintenance of high quality products for the consumer. One of these problems, the contribution of lipid hydrolysis and FFA production in frozen fish to protein

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denaturation and quality losses was mentioned above. The following discussion will be concerned primarily with this lipid hydrolysis of the phospholipid fraction in frozen stored fish.

The complexity of the fatty acids in the neutral and phospholipids of the Coho salmon samples used in this study can be seen by examination of Table 1 and a recent publication (Braddock and Dugan, 1969). Duplicate samples were analyzed from three different fish and averaged to obtain the values in Table 1. The same spectrum of fatty acids was found in both the triglycerides and the phospholipids analyzed. Quantities of specific fatty acids were different, however. For instance, when comparing the percentage composition of the fatty acids from the triglycerides and total phospholipids of fresh frozen Coho salmon in Table 1, it is apparent that the same fatty acids were present in both lipid fractions. There were slightly greater percentages of the $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ and lesser percentages of $C_{16:1}$, $C_{20:1}$, $C_{18:2}$ and $C_{20:5}$ fatty acids in the phospholipids than in the triglycerides. After storage for 1 yr at −20°C, the difference between the triglyceride and phospholipid fatty acids is much greater, and is reflected by lesser amounts of $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3\omega3}$ and a greater concentration of $C_{18:0}$, $C_{20:3}$, $C_{20:4}$, $C_{20:5}$, $C_{22:4\omega3}$, $C_{22:5\omega3}$ and $C_{22:6}$ in the remaining phospholipid fraction. The increased differences between triglyceride and phospholipid fatty acids that occurred during frozen storage were caused by large changes in phospholipid fatty acids and small changes in triglyceride fatty acids.

It has generally been agreed that increases in the FFA during frozen storage of fish come from the phospholipids in the flesh; however, recent evidence indicates that FFA production during frozen storage may also take place in some species through the hydrolysis of triglycerides (Awad et al., 1969; Bosund and Ganrot, 1969). In this study, there were only minor changes in the FFA composition of the triglycerides during a storage period of over 1 yr at −20°C (Table 1).

Comparison of the percentage composition of the fatty acids in the fresh frozen and frozen stored salmon phospholipids presented in Table 1 reveals that the fatty acids $C_{20:4\omega3}$, $C_{20:5}$, $C_{22:5}$ and $C_{22:6}$ are more concentrated in the phospholipids remaining after frozen storage for 1 yr at −20°C, while the fatty acids $C_{16:0}$ and $C_{18:1}$ are less concentrated. These changes reflect losses of specific fatty acids from the phospholipid fractions, either through enzymatic hydrolysis or oxidative reactions, or both, and will be discussed later.

Early research reported only trace amounts of the lyso-derivatives in fish muscle phospholipids and no increase during cold storage (Lovern et al., 1959). However, losses of phospholipids and increases of the lyso-compounds during frozen storage have been reported for chicken muscle (Davidkova and Khan, 1967). In this study, a decrease in the total phospholipid content with increasing storage time of frozen salmon was apparent. Data are presented in Table 2 showing that significant decreases in PE, PC and LPC occurred after 6 months frozen storage. (Analyses which yielded the data for Tables 2 and 3 were made using duplicate samples from the same two fish.) Accuracy better than 1% of value was achieved from the phosphorus determination (Table 2) and 10% for fatty acid analyses by GLC (Table 3). There were increases in the LPE fraction and an unidentified phosphorus-containing fraction.

If one computes from Table 2 the percentage decreases of PC (35%) and PE (20%) during frozen storage, it is apparent that the PC had decreased the greater amount. This is consistent with the observations of Bosund and Ganrot (1969) who showed that in frozen stored Baltic herring, the hydrolysis rate of PC was faster than that of PE in both white and dark muscle. Also, in light of recent discoveries in the authors' laboratory concerning oxidative changes in frozen salmon and systems containing lipid and protein, reactions of the PE amino group with carbonyl constituents known to be present in the flesh may account for additional losses of PE and LPE.

As another explanation for the increased amount of LPE shown in Table 2, it is suggested that the release of specific fatty acids during hydrolysis may inhibit enzymatic activity resulting in incomplete hydrolysis and a buildup of the LPE. A suitable fatty acid to participate in this

Table 1—Percentage composition of the fatty acids in the triglycerides and phospholipids of fresh frozen and frozen stored Lake Michigan Coho salmon

	Percent (GLC peak area)[c]			
	Triglycerides		Total Phospholipids	
Fatty acid[a]	Fresh	Frozen 1 yr[b]	Fresh	Frozen 1 yr[b]
14:0	3.2	5.4	3.0	1.8
15:0	0.4	1.1	0.5	0.4
16:0	10.3	7.9	15.6	6.0
17:0	0.8	1.8	0.9	0.6
18:0	3.5	3.7	4.7	5.5
20:0	0.2	0.4	0.1	0.1
22:0	0.5	0.7	0.5	0.4
14:1ω6	0.3	1.4	0.4	0.5
15:1ω6	0.2	0.8	0.2	0.6
16:1ω7	10.7	8.8	8.9	7.0
18:1ω9	21.2	19.4	25.3	14.1
20:1ω9	3.4	3.6	2.3	1.2
22:1ω9	0.5	0.6	0.6	1.5
24:1ω9	0.8	tr	0.5	tr
16:2ω4	1.1	1.6	1.1	0.6
18:2ω6	5.6	5.5	3.6	1.9
20:2ω6	1.3	1.3	0.6	0.7
22:2ω6	0.1	tr	0.3	0.7
16:3ω4	2.0	2.2	1.9	1.3
18:3ω6	0.5	0.4	0.5	0.6
18:3ω3	2.9	3.2	2.6	1.8
20:3ω6	2.5	2.5	2.7	4.4
18:4ω3	0.8	1.0	0.7	0.6
20:4ω6	1.6	1.9	1.3	1.0
20:4ω3	0.7	0.7	0.5	3.1
22:4ω6	1.6	1.6	0.9	0.8
22: ω3	1.5	1.2	1.4	2.4
20:5ω3	4.8	4.7	3.7	8.4
22:5ω6	1.8	1.4	0.6	2.0
22:5ω3	4.4	5.2	4.1	6.0
22:6ω3	10.6	9.9	9.9	24.0

[a]The number after the ω denotes the position of the ultimate double bond relative to the terminal methyl group.

[b]Storage temperature was −20°C.

[c]Variability of GLC method for fatty acid determination was less than 10% of the value.

Table 2—Phospholipid content of fresh frozen Coho salmon and after 6 months storage at −20°C

Phospholipid	Phosphorus content (mg P/g flesh) Fresh	Stored
Total phospholipid	18.5	16.0
PC	9.9	6.4
PE	3.4	2.7
LPC	2.9	1.9
LPE	1.1	1.9
X[a]	1.2	3.1

[a]Unidentified phosphorus-containing material

Table 3—Percentage composition of the fatty acid methyl esters from the phospholipids of Coho salmon frozen at −20°C for 6 months

Fatty acid[a]	Percent (GLC peak area)[c] PC	PE	LPC	LPE	LPE[b]
16:0	13.4	7.6	11.0	24.7	47.2
16:1ω7	3.7	8.6	4.8	5.3	7.6
18:0	4.7	4.8	5.5	1.9	5.1
18:1ω9	10.7	21.7	8.6	7.4	12.8
18:2ω6	5.1	5.1	3.2	1.7	3.7
18:3ω3	1.8	2.2	1.3	1.0	1.8
20:4ω6	2.6	1.3	2.9	2.9	1.4
20:5ω3	22.8	26.5	29.5	22.2	4.3
22:2ω6	1.7	5.7	4.3	2.0	0.6
22:4ω6	2.7	1.9	5.9	1.6	0.3
22:5ω6	2.0	0.5	1.1	2.2	0.9
22:5ω3	3.8	2.4	5.6	2.7	0.8
22:6ω3	24.9	11.7	16.3	24.3	13.5

[a]The number after the ω denotes the position of the ultimate double bond relative to the terminal methyl group.

[b]The values in this column were obtained following treatment of Coho salmon PE with porcine pancreatic lipase, in which the phospholipase A activity had been suppressed.

[c]Variability of method was less than 10% of value.

reaction would be oleic acid, which has been shown to be in unusually high quantities in the Coho salmon used in these studies, and which has been extensively hydrolyzed from the PE, as indicated by its decrease in the residual LPE as shown in Table 3. Yurkowski and Brockerhoff (1965) have shown that oleic acid has a strong inhibiting effect on the lyso-lecithinase of cod muscle.

Additional data concerning lipid hydrolysis in stored, frozen salmon are presented in Table 3, which lists the proportions of certain fatty acids for different phospholipids. Changes in the fatty acids of the PC and LPC fractions were different from changes in the PE and LPE fractions. Most notable were the changes in the PE and LPE which showed increased concentrations of the $C_{16:0}$ and $C_{22:6}$ acids and a major loss of the $C_{18:1}$ acid in the remaining LPE. Smaller decreases were also observed in the amounts of the $C_{16:1}$, $C_{18:0}$, $C_{18:2}$, $C_{20:5}$ and $C_{22:2}$ acids present in the LPE. Phospholipid composition analyses performed on fresh frozen Coho salmon showed very little difference in the fatty acids of PC and PE when compared before and after 6 months frozen storage.

The data in Table 3 imply a preferential hydrolysis of PE containing $C_{16:0}$, $C_{18:1}$ and $C_{22:6}$ acids. A recent paper by Olley et al. (1969) imply a preferential hydrolysis of phospholipids containing $C_{16:0}$, $C_{18:1}$ and $C_{20:5}$ acids. They reported that $C_{16:0}$, $C_{18:1}$ and $C_{20:5}$ acids became a greater proportion of the FFA and the $C_{18:0}$ and $C_{22:6}$ acids were concentrated in the remaining phospholipid. In this study, the fact that the $C_{16:0}$ and $C_{22:6}$ acids were greatly concentrated and the $C_{18:1}$ acid was much less concentrated in the remaining LPE is evidence for a slower rate of hydrolysis of PE molecules containing $C_{16:0}$. The situation is not the same for the PC and LPC phospholipids, both of which show similar proportions of fatty acids, implying a generally non-preferential hydrolysis.

The position of the $C_{16:0}$ in the PE molecule may be important to the hydrolysis. For this reason, pure PE from Coho salmon was allowed to react for 20 min with porcine pancreatic lipase at pH 9.5 in a 10% sucrose-bile salt buffer. These conditions were found to repress phospholipase A activity in the pancreatic lipase preparation. The result of this hydrolysis was to hydrolyze the fatty acid from the α′-position of the PE, leaving the β-acyl-LPE.

Recently, Slotboom et al. (1970) proved that lipase preparations from hog pancreas hydrolyze exclusively the fatty acid ester bound at the 1-position of all common types of phosphoglycerides, regardless of the nature and distribution of the fatty acid constituents. Since the $C_{22:6}$ acid is concentrated and the $C_{16:0}$ acid is very much more concentrated in the remaining LPE (Table 3), these acids must predominate at the β-position in the PE, and the hydrolysis in the frozen fish must largely take place at the α′-position of the PE. This is not necessarily true for the PC, however, which may reflect more hydrolysis at both positions. Time of storage may be an important factor also, since it has been shown that the proportion of $C_{16:0}$ and $C_{18:0}$ in the fatty acids liberated by phospholipase A from the β-position of lecithin was high in the early stages of the reaction, and as hydrolysis time increased, the concentration of $C_{18:1}$, $C_{18:2}$ and $C_{20:4}$ increased (Moore and Williams, 1961).

It should be recognized that pH and other conditions can regulate both the extent and rate of enzymatic hydrolysis of lipids in the muscle and these may be different for PE and PC. For example, it has been shown that rat intestinal phospholipase A and liver phospholipase B both have a pH optimum of 6.5, with markedly reduced activity below pH 6.1 and above pH 6.9 for the former and below pH 5.8 and above pH 7.2 for the latter (Epstein and Shapiro, 1959; Marples and Thompson, 1960). Such conditions, which may occur in the tissue during frozen storage of the fish, may be the regulating factor governing the hydrolysis.

Thus, apparently the $C_{16:0}$ and $C_{22:6}$ fatty acids at the β-position of the PE from Lake Michigan Coho salmon play a role in the preferential hydrolysis by phospholipases of this phospholipid during frozen storage. Evidence does exist that, for some fish triglycerides and phospholipids, the α′-position contains saturate and monoenoic fatty acids and the β-position has the long chain polyunsaturated fatty acids (PUFA) and palmitic acid (Brockerhoff et al., 1968). Also, the hydrolytic enzymes could be affected by many factors, such as the buildup of substrates, oxidative reactions resulting in inhibition and concentration of solutes during freezing and frozen storage. These factors may affect the mode of attack or the orientation of substrates, which, in turn, affect the selectivity and rate of hydrolysis.

This study shows that chemical changes detrimental to the maintenance of original characteristics are occurring in a complex system such as frozen fish. Concurrent with the enzymatic changes resulting in the production of FFA from the phospholipid fractions in the flesh were autoxidative changes in the frozen fish. The effect of these autoxidative reactions on the enzymatic hydrolysis as well as means to control the reactions and hydrolysis in relevant food systems are to be the subject of further studies.

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Ms received 12/28/71; revised 3/1/72; accepted 3/4/72.

Michigan Agricultural Experiment Station Publication No. 5765.

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PRESSURE FREEZING-AIR DRYING: A NEW TECHNIQUE TO REDUCE DETERIORATION IN DRYING TISSUE

INTRODUCTION

FREEZE DRYING preserves the size of tissues and foods, in contrast to other methods of drying which yield shrivelled products; however, freeze drying is a very expensive and slow process.

We found (Haas, 1970, 1971) that it is possible to approach freeze-dried quality by freezing under gaseous pressure and subsequent air drying. The concept of pressure freezing was based on observations by Hedén (1964) that freezing of bacteria under pressure produced less cell wall destructure than anticipated. Recently Ahlgren and Blackshear (1970) reported reduced hemolysis by pressure freezing.

Pressure-frozen produce per se is softer at any given temperature than regular frozen material. When pressure freezing is followed by freeze drying, the resulting food in most cases is considerably larger in volume than pieces freeze dried without pressure.

EXPERIMENTAL

Pressure freezing process

Gaseous pressurization in a Parr Bomb (Fig. 1), freezing at −20° to −25°C, followed by pressure release and subsequent drying in an air oven was the method used.

Only certain gases could be employed: e.g., methane, nitrogen, carbon monoxide, air, Freon® 13 ($CClF_3$) and ethane; other gases such as carbon dioxide, nitrous oxide and helium were ineffective and did not give any protection from shrivelling during drying. It appears that the gases which do not prevent shrivelling are too soluble in water (CO_2, N_2O) and/or too diffusive (CO_2, N_2O and He). Of course, gases which become liquid at too low a pressure, such as ethylene oxide, also cannot be used in this process.

The useful gases differed in their efficacy at a given pressure. For example, Freon 13 and methane retained a larger structure than nitrogen. Yet we employed nitrogen for most of our work because of its nontoxicity, low cost, protection of the food from oxidation and availability. The amount of pressure is an important factor. The effect first becomes noticeable at 50 psig for nitrogen and increases with increasing pressures. Pressures up to 1500 psig were used. The rate of pressurization and length of holding time are not important except that there must be sufficient time for both saturation and freezing.

Pressure without subsequent freezing is without any effect on final product volume. Thawing under pressure nullifies the effect. Thawing must take place after pressure release; delay between thawing and drying is not critical. The temperature should be such that the product will freeze thoroughly. We did experiments with celery at −10°C and this

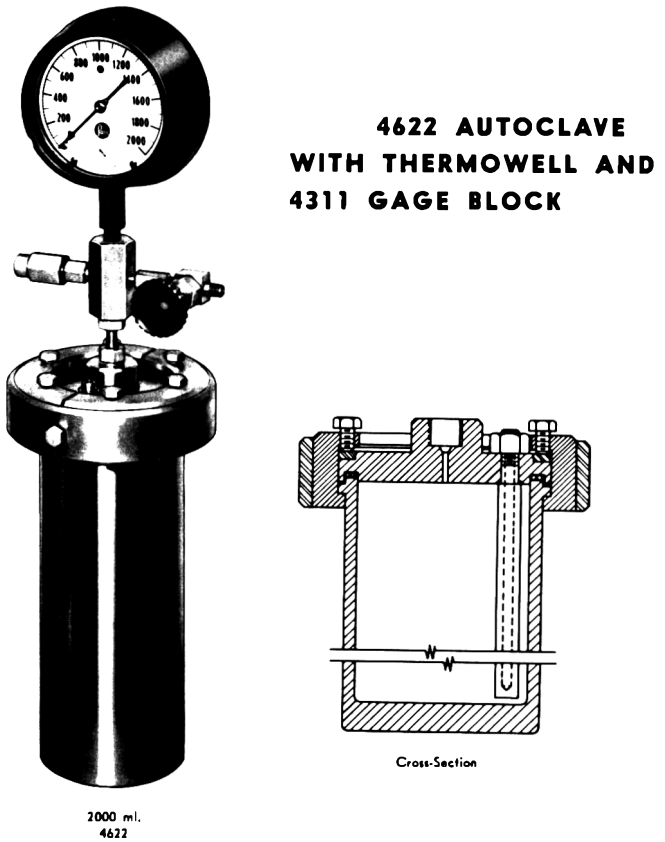

Fig. 1—Parr Bomb (2000 ml).

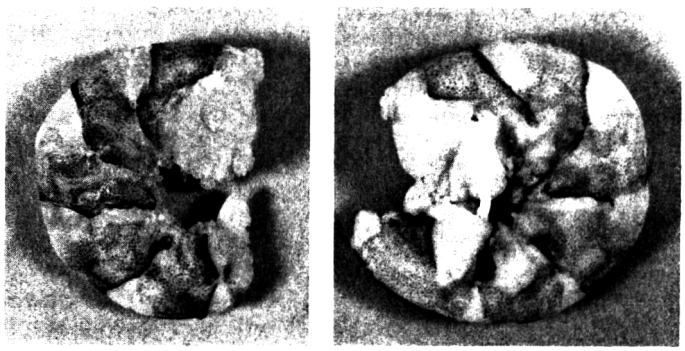

Fig. 2—Color and size comparison of frozen (left) vs. pressure-frozen (right) shrimp.

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temperature was inadequate for optimum effect; −20° to −25°C was our temperature of choice. Cooling to −60°C gave no further benefit; in fact cooling to this temperature made the product brittle and considerable shattering occurred in the frozen state.

Immersion pressure freezing (pressurization combined with immersion freezing) with orange sections, tomatoes, strawberries and mushrooms was tried using a cage held high in the Parr Bomb by an outside magnet. The bomb and the sample in the cage were pressurized with nitrogen to 1000 psig. The lower part of the bomb containing the liquid (propylene glycol or Freon 12) was cooled to −25°C. The magnet was then removed and the cage and the sample plunged into the freezing liquid. After air drying, with most samples, no beneficial effect over regular pressure freezing-air drying was observed.

Table 1—Persistence of gases in pressure-frozen celery

Gas	Days storage 0	1	5
cc methane/g celery	3.9	2	2.2
cc methane/g celery after 45 min air drying		0.94 (wt loss 59%)	0.69 (wt loss 56.5%)
cc CO_2/g celery	2.2	0.47	0.67
cc CO_2/g celery after 45 min air drying		0.1 (wt loss 35%)	0.09 (wt loss 35%)

Table 2—Effect of pressure freezing at 1000 psig with N_2 then air drying for 1 hr at 65°C and 35°C until dry

Products where dry volume of pressure frozen sample was at least double that of the control[a]		
Vegetable	Fruits	Meat/Fish
Asparagus	Blueberries	Chicken, boiled
Beans, string	Cantaloupe	Codfish, boiled
Carrots, blanched	Cherries	
Carrots, raw	Pears	
Celery	Pineapple	
Corn, boiled	Plums	
Cucumbers	Raspberries	
Onions	Strawberries	
Peas, blanched		
Peas, raw		
Peppers, green		
Potato dice, boiled		
Rice, boiled		
Products where no improvement was achieved		
Chives	Honeydew melon	Boiled ham
Mushrooms, raw	Oranges	Clams
	Raisins	Shrimp

[a]In most cases more than double

Mechanism

Upon thawing tissues which have been pressure-frozen, a large number of gas bubbles are visible in the tissue. When viewed under the microscope the pressure-frozen material is more opaque than the tissue frozen at atmospheric pressure; this opacity is probably caused by many tiny gas bubbles. It appears that after pressure freezing the gas stays in the tissue cells and does not leave upon thawing. This gas then remains within the tissue during drying and prevents collapse which otherwise occurs due to capillary forces.

This tenet was confirmed with the mushroom, *Agaricus campestris*, which shrivels as much as the control when pressure frozen-air dried from the fresh state. When the cells are examined after thawing, we do not find an accumulation of bubbles such as we find in other tissues which do not shrivel upon subsequent drying.

The presence of gas in a sample at different stages of drying was determined after liberation by heating in a saturated salt solution and measured by mass spectrometry in a celery sample pressure frozen with methane (Table 1). The reason some gases are ineffective is probably due to their greater water solubility (such as with CO_2 and N_2O) which lets the gas escape too rapidly from the moist tissue after freezing, or because of high diffusion rates which are a property of these gases and of helium. The difference in permeation of the two groups of gases was also shown qualitatively by passage through artificial membranes. Results of these experiments were reported separately (Haas and Prescott, 1972). The permeability of some films to gases has been reviewed by Sacharow (1965).

We do not know why the gas once in the cells can no longer escape. Obviously the gas dissolves at the elevated pressure up to its limit

Table 3—Rehydration[a] of air-dried products (N_2 1000 psig)

Product	Water rehydration temp	% Wt gain at 5 min control[b]	% Wt gain at 5 min sample[c]	% Wt gain at 15 min control[b]	% Wt gain at 15 min sample[c]
Peas, blanched	Boiling	27	53	51	193
Beans, green	Boiling	496	814	550	940
Potatoes, boiled	Boiling	168[d]	400[d]	–	–
Mushrooms, blanched	Boiling	97	112	142	161
Celery	Cold[e]	77	273	182	486
Peppers, green	Cold[e]	129	478	225	586
Pears	Cold[e]	16	76	66	104
Strawberries	Cold[e]	24	88	41	136
Codfish	Boiling	108	278	180	300
Chicken	Boiling	28.5	74	–	–
Shrimp	Cold[e]	72	90	125	180

[a]Rehydration was carried out without agitation. During hot rehydration the water was kept boiling. Weighing was done after removing surface water by blotting with cheese cloth.
[b]Control is frozen at −20°C at atmospheric pressure then air dried.
[c]Sample is pressure frozen-air dried.
[d]1-min rehydration
[e]26°C

Table 4—Effect of pressure freezing at 1000 psig with N_2 and subsequent freeze drying

Products with obvious benefits[a] vs. conventionally freeze-dried controls		
Vegetables	Fruits	Meat/Fish
Beans, string	Bananas	
Carrots	Pineapple	
Celery	Strawberries	
Peas		
Peppers, green		
Radishes		
Products with no obvious benefits		
Lettuce	Apples	Shrimp
Mushrooms, raw	Tomatoes	

[a]Faster rehydration in water, more rapid dehydration and somewhat larger size than controls (for strawberries, 5% larger than fresh vs. 7% smaller for conventional process)

of solubility. Upon freezing, the gas is frozen out of solution and must go to other locations within the plant cells. Here some of the gas must get trapped in such a way or be present in such a physical form that it cannot leave the tissue easily upon thawing after the pressure is released. This must be due to the lower solubility of the gas in water at normal pressure, because if a sample is thawed under high pressure before pressure release, no unusual pressure-freezing effects are observed.

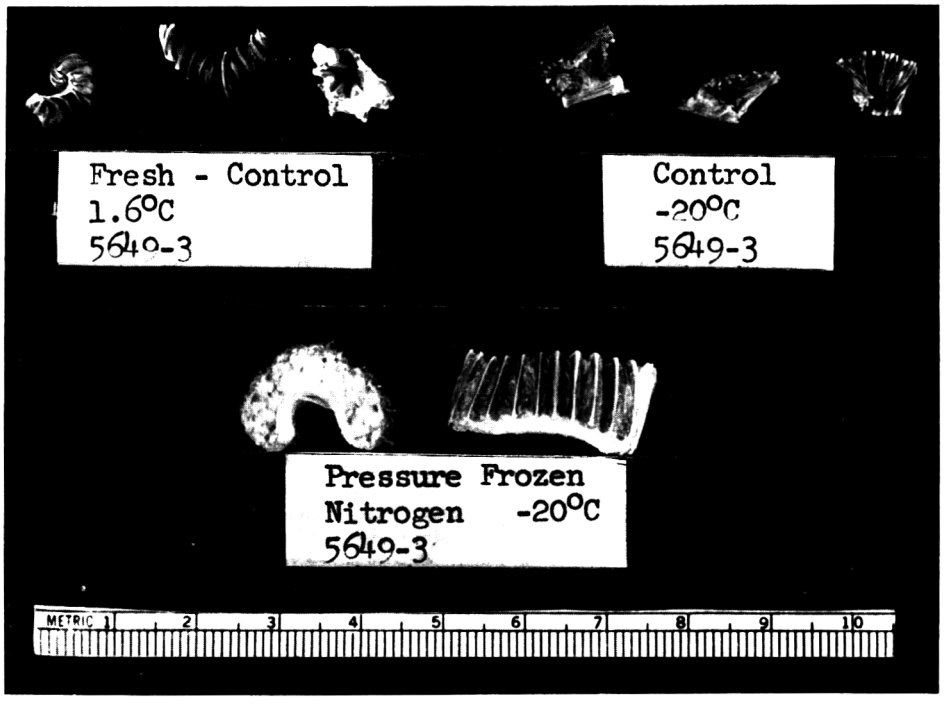

Fig. 3–Celery sample pressure frozen-air dried vs. air-dried control

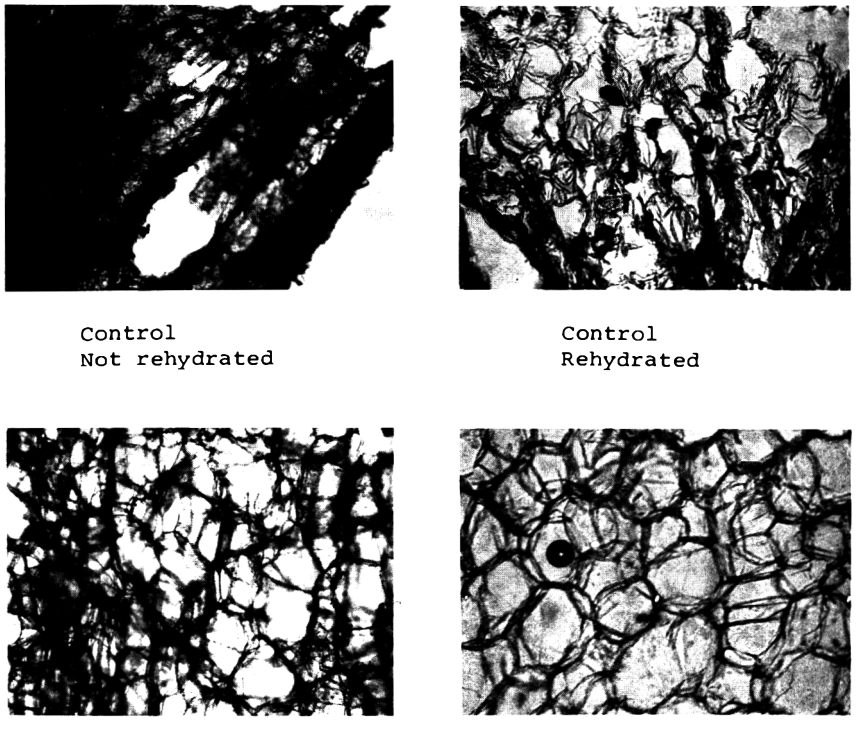

Fig. 4–Celery pressure frozen-air dried vs. control. Photomicrograph of tissue damage (magnification ≈40X)

RESULTS & DISCUSSION

PRESSURE FREEZING was tried on a large variety of foods and subsequently the products were either evaluated as-is or after subsequent air drying or freeze drying.

Evaluation in frozen state

Fruits such as strawberries, pineapple and banana were evaluated in the pressure-frozen state and in each case a softer product was obtained (when tested at the same temperature). Therefore, it is possible to prepare frozen products which can be eaten without thawing. Shrimps were also evaluated; they have the added advantages of somewhat larger size and particularly good color contrast (Fig. 2), only red and white without any greys.

The color always appears somewhat lighter in the frozen state. This was particularly noticeable for chlorophyll-containing materials. We tested for chlorophyll using acetone extraction and found that there is no difference in amount; it appears lighter because of the gas bubbles and greater bulk of piece.

Pressure freezing-air drying

The largest amount of our experimental work was in the area of pressure freezing followed by air drying. The wide range of products investigated is tabulated in Table 2.

In general, the advantages of pressure freezing-air drying are: less textural damage; larger size–less to no shrivelling; more rapid dehydration; more uniform rehydration; and in some cases better color. For green bell peppers this volume increase obtained upon pressure freezing at 1000 psig and then air drying was five- to sixfold compared with the control, and for strawberries, threefold. There are, in some cases, also disadvantageous effects. At high pressures with some types of produce, there was a loss of solids due to increased drip losses, and with celery and peppers a somewhat pulpy mouthfeel was observed. Results were excellent for boiled chicken, cod fish, potato, corn, rice, carrots, string beans and many other foods. More work should be done by the user of this process for a definite foodstuff on determining the optimum pressure for the product he desires.

The dramatic effect on structure may be seen from Figures 3 and 4. The effect on rehydration rate may be seen from Table 3.

Air-drying rate is also increased: with dehumidified air (dewpoint −43°C) at 54°C and 50 feet/min velocity, green bell peppers pressure frozen at 1000 psig nitrogen lost 95% of their original moisture in 115 min. An unfrozen control

Table 5—Rehydration[a] of freeze-dried products (N_2 1000 psig)

Product	Water rehydration temp	% Wt gain at 5 min		% Wt gain at 15 min	
		control[b]	sample[c]	control[b]	sample[c]
Carrots, blanched	Boiling	118	842	344	1058
Strawberries	Cold[d]	76	218	190	234

[a]See footnote Table 3.
[b]Control is frozen at −20°C atmospheric pressure then freeze dried.
[c]Sample is pressure frozen-freeze dried.
[d]26°C

took 240 min for the same water loss to occur.

Costs, which are very much dependent on quantities processed, were calculated as considerably below freeze-dried cost.

Pressure freezing-freeze drying

When freeze drying follows pressure freezing, we get the following advantages: larger size, more rapid and uniform rehydration, more rapid dehydration and in some cases more flavor.

Produce investigated is listed in Table 4. The effect on rehydration may be seen from Table 5.

Freeze-drying rate was increased about 20% after using nitrogen at 1000 psig. Orange segments, which are very difficult to dry by conventional freeze drying, can be freeze dried in 40 hr after pressure freezing vs. 50 hr after regular freezing. Freeze-drying runs were performed with a maximum platen temperature of 52°C and drying time was measured as the period required to remove 90% of initial moisture present.

There was no advantage in pressure-freezing solutions. The gas was lost rapidly, and when dehydration followed pressure freezing, no increase in bulk was observed. Certain gels, particularly gelatin and agar gels, however, acted more like cellular materials and gave an expanded dry product. Foams collapsed upon pressure-freezing.

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Ms received 12/10/71; revised 1/31/72; accepted 2/3/72.

Presented at the American Chemical Society Meeting, Div. of Agric. & Food Chemistry, Washington, D.C., Sept. 15, 1971.

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REFRIGERATED APPLE SLICES: PRESERVATIVE EFFECTS OF ASCORBIC ACID, CALCIUM AND SULFITES

INTRODUCTION

AT PRESENT the fresh, refrigerated apple slices as supplied to bakers have a comparatively short life (usually of the order of 2–3 wk). This limits inventories and distances over which the fruit can be shipped and requires frequent intermittent processing of relatively small lots.

An earlier paper (Ponting et al., 1971) reported that an alkaline sulfite treatment was more effective than an acidic sulfite treatment for preserving a firm texture in refrigerated apple slices. It was also reported that calcium was very effective in increasing firmness of apple slices treated in an alkaline sulfite bath, and somewhat less effective in an acidic sulfite bath. This report is an extension of the previous paper to include results of experiments on preserving overall quality, especially color, by means of both sulfite-containing and sulfite-free treatments. The latter consist of combinations of ascorbic acid and calcium at various concentrations. It will be shown that such combination treatments make it possible to preserve fresh apple slices for many weeks in refrigerated storage without the use of sulfur dioxide; or, if sulfur dioxide is used, a very low concentration in combination with calcium is required to preserve color.

MATERIALS & METHODS

TWO VARIETIES of apple, Golden Delicious and Newtown Pippin, were obtained from commercial controlled atmosphere storage. Golden Delicious (3 in. diam) and Newtown Pippin (2-3/4-in. diam) apples were used which had been stored 4–6 months. Apples were peeled, cored and sliced radially into twelfths; then 200-g samples were dipped for 3 min in 1 liter of treating solution, drained 1 min, packaged in heat sealed polyester bags and stored at 34°F. The treating solutions consisted of combinations of ascorbic acid (0, 0.5 and 1.0%), calcium (0, 0.05 and 0.10%) and sulfur dioxide (0, 0.01, 0.03, 0.05 and 0.10%). Chemically pure calcium chloride was used as a source of calcium, since it did not appear to impair flavor in our experiments, although Archer (1962) found that calcium lactate gave a somewhat better flavor in canned apples. Chemically pure sodium bisulfite or sodium sulfite was used as a source of sulfur dioxide, depending on the pH desired. Two series of samples were processed, one at the natural pH of the apples (pH 3.5) and one at pH 7.0. The dipping solutions were adjusted to the proper pH with 6N hydrochloric acid or 6N sodium hydroxide. In addition, for the pH 7 series 1% bicarbonate was added with 0.5% ascorbic acid and 2% sodium bicarbonate with 1% ascorbic acid.

At intervals of 1 or 2 wk the samples were evaluated by two observers for color, flavor and texture. Combinations showing the best quality characteristics after a storage period of several weeks were evaluated objectively at a fixed storage time by measuring reflectance with a Gardner Automatic Color Difference meter and comparing this to the reflectance of freshly sliced apples of the same lot. Photographs were also made of these samples. The reflectance of other similarly treated samples was measured at intervals over a time period of several weeks to show the rate of darkening. Reflectance measurements were made by reading total reflectance, R_d, from two-slice samples rotated to three positions about 120° apart, on a thin glass plate over a 2-1/4-in. light aperture. These three readings were averaged for each sample and the average of three samples was used for the final reflectance value. The percent loss in reflectance compared to fresh apple slices was calculated from R_d values; the instrument was calibrated against a white porcelain plate. Loss in total reflectance, R_d, correlated better with subjective evaluation of color than changes in

Table 1—Effect of calcium and ascorbic acid dipping treatments on reflectance of Newtown Pippin apple slices stored 11 wk at 34°F

Treatment[a] (3 min dip)	pH 3.5 Total reflectance, R_d	pH 3.5 Decrease in R_d vs. fresh apple slices, %	pH 7.0 Total Reflectance R_d	pH 7.0 Decrease in R_d vs. fresh apple slices, %
Fresh slices	52.9	–	50.2	–
Control–water dip	19.9	62.5	21.2	57.9
0.05% Ca (as $CaCl_2$)	39.8	24.8	32.0	36.3
0.1% Ca	40.6	23.3	41.3	17.7
0.5% AA	22.3	57.9	44.1	12.2
0.5% AA + 0.05% Ca	38.7	26.9	38.8	22.8
0.5% AA + 0.1% Ca	40.1	24.2	45.2	10.0
1.0% AA	39.4	25.5	44.3	11.8
1.0% AA + 0.05% Ca	42.1	20.5	45.8	8.8
1.0% AA + 0.1% Ca	50.7	4.2	46.2	8.0

[a]AA = Ascorbic acid

Table 2—Effect of calcium and ascorbic acid dipping treatments on reflectance of Golden Delicious apple slices stored 9 weeks at 34°F.

Treatment[a] (3 min dip)	pH 3.5 Total Reflectance, R_d	pH 3.5 Decrease in R_d vs. fresh apple slices, %	pH 7.0 Total reflectance, R_d	pH 7.0 Decrease in R_d vs. fresh apple slices, %
Fresh slices	57.5	–	57.5	–
Control–water dip	22.7	60.5	27.4	52.4
0.05% Ca (as $CaCl_2$)	23.7	58.9		
0.10% Ca	28.1	51.2	46.6	19.0
0.5% AA	23.5	59.2		
0.5% AA + 0.05% Ca	29.9	48.0		
0.5% AA + 0.10% Ca	42.8	25.6		
1.0% AA	31.3	45.6	32.2	44.0
1.0% AA + 0.05% Ca	35.0	39.2		
1.0% AA + 0.10% Ca	47.7	17.0	48.3	16.0

[a]AA = Ascorbic acid

"a" or "b" readings of the meter, since there was little change in hue except in sulfited apples. Enzymatic darkening was the main color change causing loss in reflectance.

RESULTS & DISCUSSION

RESULTS are presented in Tables 1–3 and Figures 1 and 2. Tables 1 and 2 and Figure 1 illustrate the effectiveness of various combinations of ascorbic acid and calcium in maintaining the natural color of sliced apples during refrigerated storage. It may be seen that neither ascorbic acid alone nor calcium alone, in the concentrations used, was effective in preventing discoloration of the apple slices, but the combination treatments with 1.0% ascorbic acid and 0.1% calcium (as $CaCl_2$) was very effective. A combined treatment with 0.5% ascorbic acid and 0.05% calcium was effective with Golden Delicious apples at pH 7 but not sufficient for these apples at pH 3.5 or for Newtown Pippin apples at either pH. Lower concentrations can perhaps be used in some cases for short storage periods, although it appears that if the treatment is sufficient for protecting the color well for short periods it will also prevent browning over longer times. In fact, the color may actually lighten after some storage, as shown in Table 3. This shows the change in color (reflectance) with time of storage at 34°F.

Sulfur dioxide treatment is, like ascorbic acid treatment, synergistic with calcium treatment. This is illustrated in Figure 2. It is noteworthy that calcium added to very low concentrations of sulfur dioxide makes an effective treatment; this is especially so at pH 7 where the calcium-SO_2 treatment is effective at any concentration of SO_2 above 0.01% (Fig. 2). At pH 3.5 the calcium is effective at the low concentrations of SO_2 (0.01 and 0.03%) but less so at higher concentrations. As SO_2 concentration is increased above 0.1% the color can be maintained but the apple slices become very soft, especially at the natural pH (Ponting et al., 1971). A treatment with 0.03% SO_2 and 0.1% calcium is sufficient to protect the color of Golden Delicious apples for several weeks at either pH 3.5 or 7, but the color is better at pH 7. Flavor and texture are also better at pH 7. A dip in 0.03% SO_2 plus 0.1% calcium also gives excellent protection to the color of Newtown Pippin slices, as shown in Table 3. Although this table indicates a considerable decrease in reflectance with time for all treatments, the color quality to the eye is quite acceptable, as can be seen in Figures 1 and 2.

The other general quality factors, texture and flavor, parallel color retention in apple slices treated with ascorbic acid or SO_2 combined with calcium. However, calcium treatment alone results in a poor color but a good flavor, as in untreated slices, while treatment with ascorbic acid alone, SO_2 alone, or combinations of these seem to affect apple flavor adversely. Sulfur dioxide in higher concentrations also adds its own obnoxious flavor. Therefore it is desirable to use the least amount of ascorbic acid or SO_2 required to maintain color if the best flavor is to be retained. Calcium ion has the value of allowing color stability at low concentrations of these antioxidants, especially of SO_2; thus the slices retain a good flavor for a long period. There appears to be a

Table 3–Effect of time of storage at 34°F. on darkening of apple slices.

Treatment[a] (3 min dip)	Percent loss of reflectance compared to fresh slices					
	23 days		37 days		50 days	
	Newtown Pippin	Golden Delicious	Newtown Pippin	Golden Delicious	Newtown Pippin	Golden Delicious
Control–water dip	36.0	23.5	70.0	40.0	72.0	50.8
1.0% AA, 0.10% Ca, pH 7	21.2	13.2	30.0	10.7	29.8	8.7
0.5% AA, 0.05% Ca, pH 7	28.3	10.5	31.0	4.8	32.1	14.2
1.0% AA, 0.05%, Ca, pH 7	20.4	5.7	25.8	1.5	43.1	6.5
0.03% SO_2, 0.10% Ca, pH 7	14.8	12.8	18.3	7.8	36.3	15.9
0.5% AA, 0.10% Ca, pH 7	23.0	12.5	33.3	6.3	31.9	8.2
1.0% AA, 0.1% Ca, pH 3.5	31.2	9.2	39.0	13.3	34.4	21.7

[a]AA = Ascorbic acid

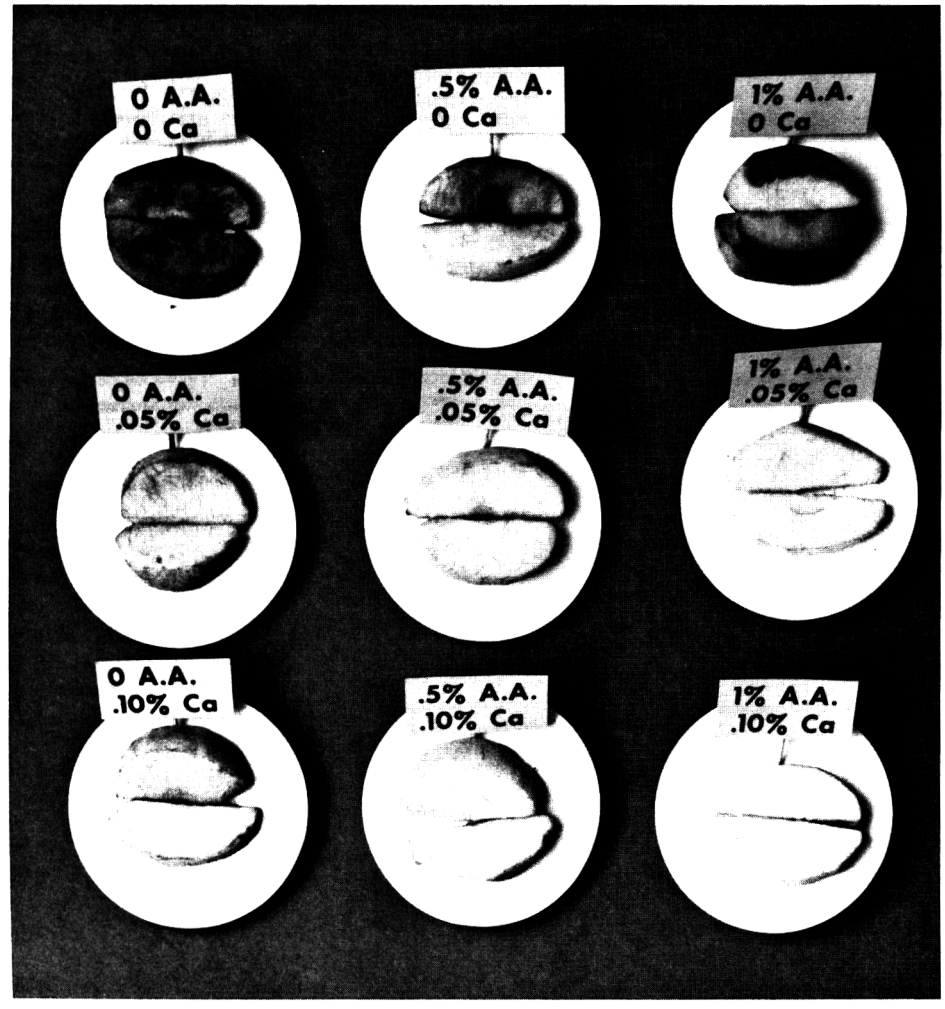

Fig. 1–Newtown Pippin apple slices stored 11 wk at 34°F after dipping in a solution at pH 7 containing ascorbic acid and calcium at the concentrations shown.

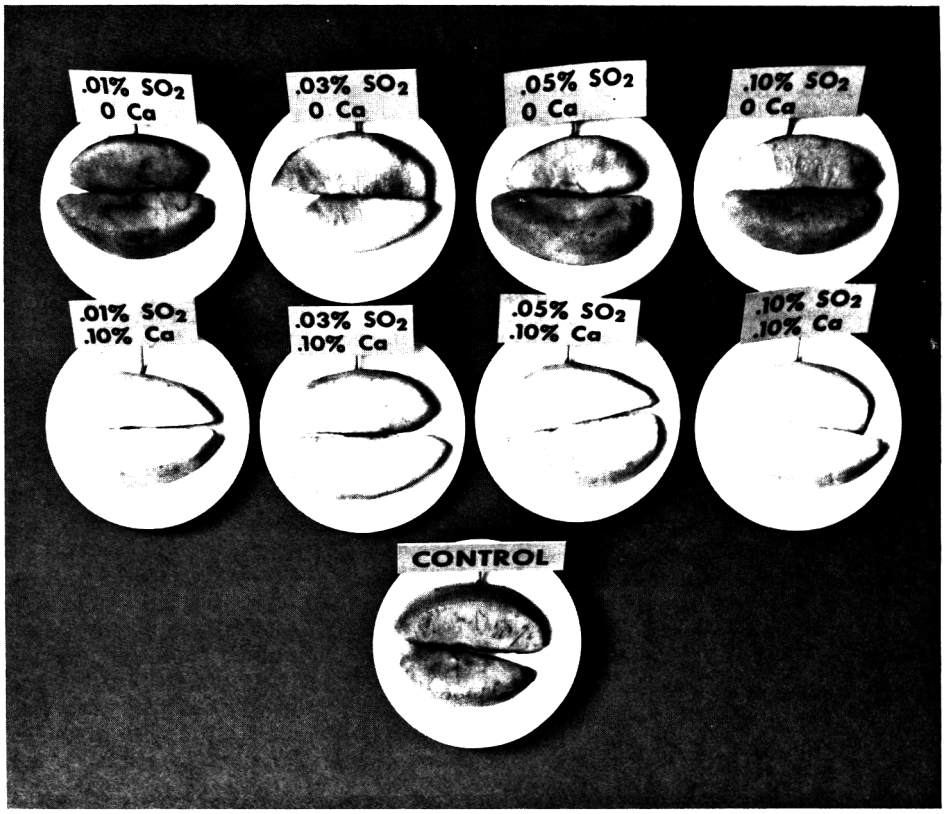

Fig. 2–Golden Delicious apple slices stored 9 wk at 34°F after dipping in a solution at pH 7 containing sulfur dioxide and calcium at the concentrations shown.

slow loss of apple flavor in storage but this does not seriously decrease quality.

Calcium-treated apple slices are more resistant to microbial spoilage, as well as enzymic browning, than those without such treatment. This may be the main reason for the improvement of storage life with calcium treatment, since storage life is limited by microbial and perhaps physiological breakdown if enzymic browning is prevented in the early storage period. Microbial damage is indicated by the spotty deterioration of color in Figures 1 and 2. It has been suggested by Coggins (1971) that mechanical hardening of the surface of oranges protects them from penetration of molds. Apple slices may likewise be protected by the firming of the surface by calcium, since the longest-lived slices are the ones that stay crisp. Microbial flora should not be altered by a pH 7 dip, since a 3-min dip at this pH does not appreciably affect the pH of the apple slice–only the surface pH is temporarily altered, but the slice as a whole is highly buffered and retains its natural pH.

In our preliminary observations it was noted that both ascorbic acid and low concentrations of SO_2 were effective in protecting color when combined with calcium, but combinations of ascorbic acid with SO_2 did not improve results more than an increase of one ingredient by itself. Likewise, three-way combinations of ascorbic acid, SO_2 and calcium were no more effective than two-way combinations of either ascorbic acid or SO_2 with calcium.

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Ms received 11/16/71; revised 1/17/72; accepted 1/24/72.

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DEHYDRATED CELERY: EFFECTS OF PREDRYING TREATMENTS AND REHYDRATION PROCEDURES ON RECONSTITUTION

INTRODUCTION

DRIED FOODS, instant products, condensed foods, etc., are used in increasing amounts in remanufacturing, institutional outlets and by the housewife. The expanded use of these products in recent years can be attributed in part to the newer and more sophisticated methods of dehydration (VanArsdel and Copley, 1964). Numerous predrying treatments and additives and better packaging also have contributed to the improved rehydration characteristics of many dried products. Methods of rehydration, as would be expected, influence the amounts of water absorbed. Neubert et al. (1966; 1968) reported that merely shaking or swirling rehydration mixtures of dried celery and water can increase significantly the rate and amount of water absorbed.

Even with the development of the newer drying techniques, most vegetables, cut in a range of piece forms and sizes, are still air dried on trays or belts—the most economical and simplest method. The poor rehydration characteristics of many of these air dried vegetables, particularly of the larger pieces, however, continues to stimulate efforts to improve their rehydration qualities.

Loss of differential permeability in the protoplasmic membrane, loss of turgor pressure in the cell, protein denaturation, starch crystallinity, and hydrogen bonding of macromolecules have been suggested as causes of, and at times correlated with, various parameters of rehydration.

Introduction of hydroxyl groups into the cell wall may minimize hydrogen bonding of polysaccharides that compose the cell wall and thereby contribute to minimum cell deformation. Addition of edible polyhydroxyl compounds into fruit and vegetable tissue prior to dehydration improved reconstitution characteristics such as more rapid and complete rehydration, more tender skins, firmer texture, and size and shape more similar to the fresh state (Brandner and Goepp, 1947; Pader and Lauro, 1966; Savage, 1967).

Excessive opening of cotyledons (butterflying) of various legumes during dehydration was reduced by an after-steam immersion in a 10–20% solution of sucrose or other polyalcohols before drying (Meijer, 1966). Bohrer (1967) suggested azeotropic drying for better rehydration qualities; under proper temperature and pressure, combining a solvent such as ethyl alcohol or ethyl acetate with a foodstuff removes the water as an azeotropic mixture with the solvent. A combination of an initial osmotic dehydration of fruit pieces using sucrose followed by dehydration of the sucrose-treated tissue has been suggested (Ponting et al., 1966) for improved quality. Forming hydrophilic polymers within the tissue before dehydration (Schwimmer, 1969) can mechanically improve the appearance and textural qualities of some dried products.

Commercial dried celery is generally unblanched. According to an industry source no distinct improvement in quality nor storage stability accrues with blanching, but instead results in undesirable leaching losses. While Gold (1962) reported some increase in rehydration with celery dice blanched before drying, Neu-

Table 1—Effect of soaking frozen and thawed, unblanched celery, 3/8 in. half-dice, in various sucrose concentrations before air drying on the alcohol soluble solids content in the dried celery and on rehydration characteristics

Predrying treatment		Dried unblanched celery				Rehydrated (cooked) celery[b]				
				Alcohol-soluble solids						
% sucrose solutions[a]	Min	Dehydration ratio	Bulk volume ml/g	% MFB	% increase over control	% of frozen weight	% weight increase over control	Dice form	Texture	Sweetness
None	–	20.4	7.4	66.2	–	27.4	–	Shrunken	Tough	None
15	10	11.0	4.1	80.9	22.2	43.2	58	Variable	Variable	None
30	10	6.6	3.2	88.2	33.2	61.2	123	Full	Crisp, tender to tough	Slight
45	10	4.7	2.6	91.9	38.8	63.1	130	Full	Mostly crisp, tender	Slight
60	10	4.0	2.4	92.6	39.9	65.6	139	Full	Mostly crisp, tender	Slight
None	–	21.3	7.5	74.0	–	26.2	–	Shrunken	Tough	None
15	1	13.1	4.9	77.8	5.1	37.5	43	Shrunken	Tough	None
30	1	9.4	3.8	83.2	12.4	44.9	71	Variable	Variable, tough strings	None
45	1	7.1	3.1	85.7	15.8	52.9	102	Nearly full	Mostly crisp, tender	Very slight
60	1	6.0	2.7	88.6	19.7	56.8	117	Nearly full	Mostly crisp, tender	Very slight

[a]At ambient temperature
[b]All samples had cooked, grayish-yellow-green color.

bert et al. (1966) noted lower rehydration values. Our preliminary studies showed that blanching increased rehydration about 5%.

This study reports the effects of the following predrying treatments on the rehydration characteristics of air-dried celery: (1) immersion in sucrose or other polyhydroxyl compounds; (2) immersion in sulfite, carbonate and/or sucrose solutions; and (3) leaching in water. One series of treatments using hot solutions blanched the celery before drying; all other samples were unblanched. The effect of the first rehydration procedure (by cooking or soaking in cold water) on successive rehydrations, following redrying, is also considered.

EXPERIMENTAL

Celery

Several lots of celery purchased at the local produce market were trimmed by cutting off and discarding the butt end, the upper forked petioles, and the center-heart material. The trimmed, raw celery was either 3/8 in. half-diced or crosscut sliced 1/8 in. thick. Diced celery was tray-frozen, either at −10°F with only moderate air movement or at −25°F in an air-blast cabinet and stored at −10°F. The sliced celery was not frozen.

Predrying and dehydration procedures

Frozen celery (200g per sample) was thawed at room temperature for about 2 hr before any predrying treatment or before drying the untreated controls. The predrying treatment consisted of immersing a thawed sample in 200g of one of the following solutions before air drying: (1) 15, 30, 45, 60% (w/w) sucrose for 1 or 10 min at ambient temperature; (2) hot (185–140°F), 65% solutions of sorbitol, dextrose, glycerol, n-propanol, or sucrose with 0.03M EDTA for 10 min; or (3) various combinations of 1.5% Na_2CO_3 and/or 0.5% $NaHSO_3$ for 30–45 sec, and/or then into 60% sucrose at ambient temperature for 10 min.

Unfrozen, sliced celery was either air dried directly, or treated before drying as follows: 200g of celery was immersed in 400g of 60% sucrose solution at ambient temperature for 10 min; or 300-g samples were each leached with 4–800g aliquots of distilled water at ambient temperature during a 48-hr period.

After draining, treated samples were air dried in a crossflow dryer at 150°F for 1-1/2 hr and then at 117–130°F for 16–19 hr. Untreated samples, dried concurrently, generally dried faster than treated celery. When rehydrated samples were redried, they were dried at 117–120°F for 16–18 hr.

Dehydration ratio was determined from the weight of the fresh or frozen sample before thawing or any predrying treatment divided by the sample weight after dehydration.

Rehydration procedures

Cooked. 1.00g dried untreated celery prepared in the laboratory, or the equivalent amount of dried treated material (based on dehydration ratios), was added to 100–125g of boiling, distilled water in a 250 ml beaker, covered with a watch glass and boiled 15 min. After steeping 5 min, the cooked celery was drained for 1 min and weighed.

Cold water. The same amounts of dried celery used with the cooked procedure were added to 100-g aliquots of distilled water. Mesh screen submerged any floating material. After 24 hr in a refrigerator, the rehydrated celery was drained and weighed.

Commercial air dried unblanched, sulfited celery (1/8 in. crosscut) was rehydrated similarly to laboratory-prepared dried celery except for the following: (1) 25.00g was rehydrated with 1000g of water in a 2000 ml beaker; (2) rehydrated samples were drained for 2 min.

Rehydration percentage, or the percent of the original fresh or frozen weight, was calculated from the (weight of the rehydrated celery × 100) divided by the (weight of the dried sample times the dehydration ratio).

Analytical

Total and alcohol-soluble solids were determined from aliquots of ethanol-celery slurries prepared by blending a known weight of celery with ethanol to give a final alcohol concentration of 80%. Aliquots were removed for total solids assay while the slurry was being stirred; alcohol-soluble solids were determined from aliquots of the clear supernatant liquid. Each aliquot was evaporated to near dryness on a steam table and then dried under vacuum at 140°F for 30 hr.

Bulk volumes were determined by slowly adding celery to a 250-ml graduate while continuously tapping the base of the graduate against a bench top.

Statistical analyses and subjective evaluation

Differences in rehydration percentages due to treatments were tested for significance using Tukey's hsd (honestly significant difference) for multiple comparison (Steel and Torrie, 1960). Subjective evaluation of texture, color, flavor, and over-all appearance was limited to informal comparisons by one to several appraisers.

RESULTS & DISCUSSION

Effect of predrying treatments

Incorporation of sucrose into frozen, diced celery after thawing and before air dehydration improved the rehydration characteristics of the dried celery (Table 1). Dried sucrose-treated celery after cooking weighed more and generally had a better over-all appearance, with pieces that were larger, fuller, more tender and crisp, than untreated celery; color of the treated celery, however, was not improved.

The greater the sucrose concentration and/or the longer the soak period before drying the better the rehydration. The over-all dice size of the best rehydrated samples, however, was still slightly smaller than that of the undried celery. Predrying treatments of 1 min in 15 or 30% sucrose or 10 min in 15% sucrose produced rehydrated celery pieces that were still considerably better than untreated material, even though they can be described as shrunken and small, tough, or having tough strings.

While rehydration percentages of dried sucrose-treated celery increased 43–139% over those of the untreated material, alcohol-soluble solids increased only 5–40%. The dried celery with added sugar, however, although much larger and less shrunken than dried untreated celery, had distinctly smaller bulk volumes.

Soaking unfrozen raw celery, 1/8 in. crosscut, in a sucrose solution before drying also produced a dried product with some improved reconstitution characteristics when compared to the untreated control. Rehydration by cooking or soaking in cold water resulted in treated slices somewhat fuller in size and shape, and generally more tender and crisp; color was slightly more yellow or duller than the untreated samples (Table 2).

This dried sucrose-treated celery, however, rehydrated only about 10 percent-

Table 2—Effect of soaking fresh, unblanched celery slices, 1/8 in. crosscut, in a sucrose solution before air drying on rehydration by cooking or a cold water soak

	Rehydrated celery[a]				Rehydration liquor[a]			
	Cooked		Cold water soak		Cooked		Cold water soak	
Predrying treatment	% of fresh weight	% weight increase over control[b]	% of fresh weight	% weight increase over control[b]	pH	Water-soluble solids, grams[c]	pH	Water-soluble solids, grams[c]
None	36.5 ± 0.5	–	49.8 ± 0.1	–	5.8	0.33	6.1	0.31
	35.0 ± 0.3[d]	–	48.7 ± 0.4[d]	–	5.8	1.59	6.1	1.77
60% Sucrose, 10 min at ambient temp.	46.1 ± 0.3	26.3	60.1 ± 0.4	20.7	5.8	1.34	6.2	1.38
	45.8 ± 0.1	25.5	59.3 ± 0.2	19.1	5.8	1.43	6.2	1.55

[a]Mean of four samples ± standard error
[b]Based on controls with no sucrose added to rehydration water
[c]Calculated from °Brix by refractometer and grams of drained liquid after rehydration
[d]1.74g of sucrose added to rehydration water (equivalent to that in dried sucrose-treated samples)

age units more than the untreated dried celery, a much smaller difference than when frozen celery was the starting material. This smaller difference in rehydration capacity between untreated and treated dried samples prepared from unfrozen 1/8 in. crosscut celery, compared to samples prepared from frozen 3/8 in. half-dice celery, is due in part to distinctly higher rehydration values of the untreated celery prepared from the unfrozen crosscut material, and in part to less sucrose uptake into the unfrozen tissue. Addition of 1.7g of sucrose (equivalent to that in a dried sample of sucrose-treated celery) to the rehydration water for the untreated dried celery did not improve rehydration (Table 2).

When these rehydrated samples were redried and again rehydrated, the sucrose-treated samples were generally no better than the untreated celery (Table 3). Thus, improvement in the rehydration characteristics of treated material apparently is due principally to the physical presence of sucrose which prevents the normal shrinkage of the plant tissue when air-dried and not to any permanent change in water-holding capacity of celery tissue.

Predrying treatments with sodium carbonate, sodium bisulfite, and/or 60% sucrose at ambient temperature, separately or in various combinations, produced distinct differences in rehydration characteristics and appearance (Table 4). Addition of sucrose only made the most significant improvements in rehydration characteristics except for the cooked gray-yellow-green color. Addition of Na_2CO_3 retained the bright green color, softened the texture and also increased rehydration weight. The combination treatment of carbonate and sucrose produced the best rehydrated celery with a rehydration percentage of 71%; the dice were well filled out, the texture was generally tender to firm with some crispness and the color was bright green. Sulfite did not improve rehydration. Celery used for this experiment was frozen relatively slowly at −10°F, and thawed samples lost 45% of their frozen weight. These dried samples, nevertheless, rehydrated as well as other dried sucrose-treated celery prepared from celery frozen rapidly at −25°F and having a weight loss of only 11−20% after thawing.

Blanching in hot solutions of other polyhydroxyl compounds, including sorbitol, dextrose or glycerol also improved rehydration characteristics of dried celery to about the same extent as sucrose (Table 5). A 10-min immersion in n-propanol before drying, however, did not improve rehydration. While the untreated and sucrose-treated celery had a dull yellow-green color after cooking, celery treated with sorbitol, dextrose, glycerol or n-propanol tended to have a more desirable watery gray-green color.

Of the polyhydroxyl compounds tested, sucrose would be preferred because it is a common, low cost additive in many foods, as well as an accepted food of high purity. Flavor of the sucrose-treated celery after rehydration was not particularly sweet. The additional sucrose present could easily be incorporated into such products as soup mixes and canned stewed tomatoes.

Conventional, relatively inexpensive, air-drying equipment probably can be used to dehydrate sucrose-treated celery. Although sucrose-treated celery pieces tend to stick and clump together while drying on trays, this problem should be minimized by using dryers that either continuously mix or periodically turn the product.

Table 3—Influence of water temperature on successive rehydrations (redrying between rehydrations)

Water temperature for rehydration[a]		Rehydrated celery, % of fresh celery weight[b]			
		Control		Sucrose-treated	
First	Second	First	Second	First	Second
Hot	Hot	36.4 ± 0.8	42.2 ± 0.2	45.8 ± 0.2	36.0 ± 1.4
Hot	Cold	35.8 ± 1.5	60.1 ± 0.8	45.8 ± 0.1	58.4 ± 0.6
Cold	Hot	49.3 ± 0.2	23.6 ± 4.0	59.8 ± 0.6	23.6 ± 1.2
Cold	Cold	49.7 ± 0.2	34.5 ± 0.1	59.4 ± 0.6	43.6 ± 7.0

[a]"Hot" represents 15 min cooking; "cold" represents 24 hr soaking in refrigerator.
[b]Mean of two samples ± standard error

Effect of water temperature on rehydrations

It was noted previously that when rehydrated samples were redried and again rehydrated, the samples treated originally with sucrose rehydrated no better than untreated celery (Table 3). The first method of rehydration (cooking or in cold water), however, did affect the degree of reconstitution for the second rehydration of both untreated and su-

Table 4—Effect of soaking frozen and thawed, unblanched celery, 3/8 in. half-dice, in various solutions before drying on rehydration

Predrying treatment		Dried celery		Rehydrated (cooked) celery						
Solution[a]	Min	Dehydration ratio	Bulk volume ml/g	% of frozen weight	% weight increase over control	Dice form	Texture	Color	Sweetness	Cooking liquor pH
None	–	29.8	8.3	22.4	–	Small, shrunken	Tough	Gray-yellow-green	None	5.3
60% Sucrose	10	6.5	2.7	50.5	125	Partially shrunken	Tender to tough	Gray-yellow-green	Very slight	5.6
1.5% Na_2CO_3 − 0.5% $NaHSO_3$ + 60% Sucrose	0.5 } 10 }	6.0	2.5	71.0	217	Full	Crisp, tender	Brightest green	Very slight	8.5
1.5% Na_2CO_3 + 60% Sucrose	0.5 } 10 }	6.0	2.7	71.2	218	Full	Crisp, tender	Bright green	Very slight	8.7
0.5% $NaHSO_3$ + 60% Sucrose	0.5 } 10 }	6.2	2.4	53.8	140	Partially shrunken	Tender to tough	Gray-yellow-green	Very slight	5.8
1.5% Na_2CO_3 − 0.5% $NaHSO_3$	0.5 }	34.5	7.4	38.9	74	Small, shrunken	Soft	Bright green	None	9.2

[a]At ambient temperature

Table 5–Effect of blanching celery in various hot solutions before drying on rehydration

Solution[a]	Dried blanched celery: Dehydration ratio	Dried blanched celery: Bulk volume ml/g	Rehydrated (cooked) celery: % of frozen weight	Rehydrated (cooked) celery: % weight increase over control	Rehydrated (cooked) celery: Dice form	Rehydrated (cooked) celery: Texture	Rehydrated (cooked) celery: Color	Rehydrated (cooked) celery: Sweetness	Cooking liquor pH
None	20.8	6.3	28.1	–	Shrunken	Tough	Yellow-green	None	5.4
65% Sucrose[b]	3.1	2.2	77.7	176	Full	Mostly crisp, tender	Yellow-green	Slight	5.1
65% Sorbitol	2.8	2.0	77.7	176	Full	Mostly crisp, tender	Watery gray-green	Very slight	5.6
65% Dextrose	2.9	2.0	79.3	182	Full	Mostly crisp, tender	Watery gray-green	Very slight	6.0
65% Glycerol	3.1	2.0	77.4	175	Full	Crisp, tender	Pale watery gray-green	Slight	6.0
65% n-Propanol	29.0	7.8	30.7	9	Shrunken	Tough	Watery gray-green	None	5.6

[a]Frozen celery, 3/8 in. half-dice, after thawing, immersed for 10 min at 140–185°F
[b]Contained 0.03M EDTA

crose-treated dried celery. In the first rehydration, all dried samples in cold water for 24 hr rehydrated about 14 percentage units more than cooked celery. In the second rehydration (hot or cold water), all samples that were rehydrated first in cold water had much lower rehydration percentages, while three of four samples originally cooked had distinctly higher rehydration percentages (Table 3).

Fresh celery slices leached in water before drying, when successively rehydrated by the hot or cold water procedures, followed rehydration patterns similar to those of dried unleached celery, except that differences between the original hot and cold water rehydrations were smaller with leached material (Table 6). Dried celery rehydrated a third time (in hot water), after a first rehydration in hot water and a second rehydration in either hot or cold water, continued to increase in weight. Dried celery rehydrated first in cold water and secondly in hot water, when rehydrated a third time in hot water, reversed the trend of poorer rehydrations, doubling the percentage rehydrated the second time and approaching that of the first rehydration value. The dried sample rehydrated the first two times in cold water when rehydrated the third time in hot water, however, continued the trend of lower rehydration percentages.

Commercial air-dried celery, successively rehydrated similarly to dried celery prepared in the laboratory showed the same general trends of changing rehydration weights as influenced by water temperature (Table 7); differences in rehydration weights were all significant at the 1 or 5% level.

Variations in degree of rehydration between the commercial and the laboratory samples may be related to differences in the dried celery:water ratios used for rehydration, dehydration variables, or more likely compositional differences in the starting raw material.

Table 6–Influence of leaching fresh, unblanched celery before dehydration, and of water temperatures used for successive rehydration

Water temperature of successive rehydrations: First	Second	Third	Fourth	Rehydrated celery[a] % of fresh weight: First	Second	Third	Fourth
Unleached celery							
Hot	Hot	Hot	–	36.4	43.2	62.7	–
Hot	Cold	Hot	–	36.8	56.7	59.2	–
Cold	Hot	Hot	–	48.1	21.0	42.4	–
Cold	Cold	Hot	Hot	46.5	31.3	18.6	19.9
Leached in water[b]							
Hot	Hot	Hot	–	39.6	42.5	61.4	–
Hot	Cold	Hot	–	35.3	52.9	58.2	–
Cold	Hot	Hot	–	40.8	18.1	34.5	–
Cold	Cold	Hot	Hot	39.1	14.4	16.3	18.5

[a]Original dried celery weights: unleached, 1.00g; leached, 0.78g (equivalent celery weights based on dehydration ratios)
[b]At ambient temperature for 48 hr

Second rehydration temperatures produced changes, both in the commercial and laboratory samples, that were contrary to the general trend of changing rehydration weights with successive rehydrations. For example, commercial celery rehydrated first in hot water, when rehydrated a second time in hot water, increased only 6.9% but increased 14.4% in cold water; when dried celery was rehydrated first in cold water, a second rehydration in cold water decreased the celery weight only about 7.5% and in hot water a significantly lower 26.6%.

Commercial sliced dried celery after the first rehydration by cooking had a good, acceptable appearance with little or no shrunken pieces, a typical cooked color, and the texture was tender-soft. Additional hot water rehydrations retained the acceptable piece shape and size, and the texture generally remained tender but may have increased in firmness and in some crispness. Cooking following cold water rehydration did not improve piece shape, size, or texture until the second hot water rehydration after the cold treatment, generally correlating with improvements in rehydration weights.

Commercial celery originally rehydrated in cold water had the best appearance with a bright green color, good size, and well-filled out pieces; texture was crisp and tender to slightly tough. Additional cold water rehydrations resulted in progressively poorer appearance and a

Table 7–Effect of water temperature used for successive rehydrations of commercial air dried unblanched celery (1/8 in. crosscut)

Series	Water temperature of successive rehydrations				Weight (grams) of celery after successive rehydrations[a,b]				Level of significance between rehydrations	
	First	Second	Third	Fourth	First	Second	Third	Fourth	1 vs. 2	2 vs. 3
A	Hot	Hot	Hot	–	166.9 ± 0.2	178.5 ± 1.9	227.0 ± 2.1	–	**	**
B	Hot	Cold	Hot	–	167.8 ± 0.7	191.9 ± 2.3	211.0 ± 3.7	–	**	**
C	Cold	Hot	Hot	–	193.9 ± 1.2	142.1 ± 1.3	192.7 ± 3.5	–	**	**
D	Cold	Cold	Hot	Hot	191.6 ± 1.4	179.5 ± 0.1	131.2 ± 2.4	196.1 ± 1.0	**	**
E	Cold	Cold	Cold	–	196.3 ± 0.9	179.4 ± 1.2	119.1 ± 4.5	–	**	**
Level of significance between series[c]					A, B vs. C, D, E**	all interactions** except A vs. B*	all interactions** except D vs. E*			

[a]Original weight per sample of dried celery, 25.00g
[b]Mean of three samples ± standard error, except series C data are means of 6 samples
*5%; **1%

tougher texture, again usually correlating with the lower rehydration weights.

Most of the water-soluble solids in the dried celery was removed during the first rehydration, whether hot or cold; that remaining was removed largely in the second rehydration water (determined by °Brix refractometer and grams of drained liquor). The 12–15g of soluble solids removed from each commercial dried sample during rehydration were 48–60% of the original dried weight. Similar percentages of soluble solids were removed from laboratory dried samples.

Whittenberger and Nutting (1958) related leaching of electrolytes from cell walls to an increase in water-holding capacity. In our studies, the amounts of soluble solids removed from the rehydrated celery with hot and with cold water were similar, and therefore, do not per se appear to influence the gross differences in the amounts of water retained. It was not determined what modifications, physical or chemical, occurred to the remaining celery tissue to produce such distinct differences in rehydration capacity.

It is commonly accepted that excessive heat will destroy the osmotic properties of the cell and the cell turgor, thereby affecting the ability of the tissue to absorb and to retain water. Gane and Wager (1958) noted that elasticity of cell walls is important for good rehydration and that any substantial amount of heat treatment will reduce this elasticity. However, water blanching increased the amorphous content of cell-wall cellulose in dried carrots, and in turn, the higher amorphous content allowed the intact cell walls to more readily absorb water, swell and assume their original shape, resulting in improved reconstitution (Sterling and Shimazu, 1961; Sterling, 1963). Sterling (1955) steamed carrot, potato and apple tissue for as long as 60 min with no disruption of cell walls.

Results of our studies showed that repeated rehydrations in boiling water increased successively the amount of water absorbed while successive cold water treatments reduced the ability of dried celery tissue to reconstitute. It seems evident that certain of the water-insoluble cell wall materials play a significant role in this strongly positive response to rehydration temperature.

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Ms received 5/12/71; revised 2/26/72; accepted 3/4/72.

The author thanks Dr. Joseph R. Wagner for his counsel and many helpful suggestions; Marion C. Long and Henry M. Wright for total- and alcohol-soluble solids analyses; and Dr. Bruce E. Mackey for statistical analyses.

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INFLUENCE OF ALUM ON THE FIRMNESS OF FRESH-PACK DILL PICKLES

INTRODUCTION

ALUM has been used in the manufacture of pickle products for many years, and no one seems to know how the practice originated (Fabian and Krum, 1949). When alum is added during processing (= desalting of brine-stock) or to finished pickle products made from fermented, salt-stock pickles, such as processed dills or sweets, it is reported to play an important role in making the cucumber pickles more crisp and firm. With the development of nonfermented-type products, known as fresh-pack or pasteurized pickles (Etchells, 1938; Etchells and Jones, 1942, 1944), most manufacturers added alum to these products as well. Fresh-pack products, both dill and sweets, have made substantial gains in consumer acceptance, and now, after approximately 35 years from the time of their introduction, they require more than 40% of the national crop of pickling cucumbers (Monroe et al., 1969). In research designed to improve procedures for preparation of pasteurized pickle products, Etchells and Jones (1942, 1944) discussed in detail the methods of manufacturing high-quality products. The addition of alum was not a part of their procedure; nevertheless, they reported that most of the original cucumber crispness or firmness was retained for about 8 months for fresh-pack sweet slices and whole dill pickles; and, for 16 months for fully fermented genuine dills. The basic pasteurization procedure they described was developed under commercial conditions, and was readily adaptable to either hot water or steam as the heating medium. An internal product temperature of at least 160°F, but not over 165°F, maintained for 15 min was called for, followed by prompt cooling of the product to below 90°F. The equilibrated acid content of the various products covered by Etchells and Jones ranged from 0.4–1.7% acetic (= 4 to 17 grains vinegar).

We found no reports in the literature demonstrating the value of alum in the manufacture of fresh-pack pickle products. The research reported here represents a series of experiments on the firmness of fresh-pack dill pickles carried out over a 3-yr period at a pickling plant located in Ohio, with parallel studies being done in our Raleigh laboratory. The experiments were designed to give prime consideration to the influence of alum [$Al_2(SO_4)_3 \cdot 18\ H_2O$], together with different acidity levels of either lactic and/or acetic acids on the firmness of whole cucumbers (unspiced) or whole dill pickles, prepared and pasteurized under both laboratory and commercial conditions.

MATERIALS & METHODS

Laboratory experiments

The four alum salts tested (aluminum potassium sulfate, aluminum ammonium sulfate, aluminum sodium sulfate and aluminum sulfate) were all certified, reagent-grade chemicals. The acidic properties of each alum salt were determined by preparing a series of quantitative aqueous solutions (0.05, 0.10, 0.15, 0.20, 0.25 and 0.30% weight/volume, based upon the actual amount of alum salt rather than the hydrated salt, and titrating 10-ml samples of each with 0.111N sodium hydroxide solution to an endpoint of pH 7.5, measured with a Beckman Zeromatic pH meter. The acidity of each alum salt solution is expressed in milliequivalents (meq) per gram of alum salt. Acetic and lactic acids were of reagent-grade and the concentration of each acid was determined by titrating a 10-ml sample with 0.111N sodium hydroxide to pH 7.5. The Kosher-style, dill pickle cover-brine was obtained from a local pickle manufacturer and represented their regular formula. The sodium chloride content of the samples was determined by a method previously described (Etchells et al., 1964).

Cucumbers, Model variety, size 1-1/8 to 1-3/8 in. diam were packed into 32-oz (1-qt) glass jars so as to maintain close to 60% cucumbers and 40% cover-brine on a weight basis. To obtain this pack-out ratio, each jar contained 12–15 cucumbers weighing 550–560g. The jars were then covered with about 370 ml of the appropriate test brine, leaving headspace of approximately 1/4 in. Next, the jars were closed with 70 mm, 4-lug, "twist-off" caps (White Cap Co., Chicago, Ill.) and pasteurized in a hot-water bath by the method described earlier (Etchells and Jones, 1944). All jars were stored at room temperature (about 78°F).

Experiments at the pickle company

Fresh-pack (pasteurized) dill pickles were made during three growing seasons at the cooperating pickle plant; 350 quart samples of pickles were hand-packed and represented 98 different treatments as to amounts of acetic and/or lactic acids and with or without the addition of alum. The cucumber variety, SMR-15, was used, and the sizes ranged from 1 to 1-3/8 in. diam (commercial sizes 1A and 1B). The packing, capping, pasteurizing, storing and testing of the finished products were essentially the same as that described for the laboratory experiments. One difference was that these were machine-capped and pasteurized in a commercial steam unit to an internal-product temperature of 165°F (held for 15 min) followed by water-spray cooling to about 90°F.

Product evaluation

The procedure used for cucumber pickle evaluation and for brine analyses of each treatment was that used by Monroe et al. (1969). At the time each jar was opened, the following physical and chemical measurements

Table 1—Formulae and acidic properties of commercial alum salts

Chemical name	Trade name	Formulae[a]	Acidic properties of 0.1% aqueous solution[b]	
			pH	Meq as acid/g
Potassium aluminum sulfate	Potassium alum Alum meal Alum flour	$KAl(SO_4)_2 \cdot 12\ H_2O$	3.89	10.5
Ammonium aluminum sulfate	Ammonium alum	$NH_4Al(SO_4)_2 \cdot 12\ H_2O$	3.85	12.9
Sodium aluminum sulfate	Soda alum	$NaAl(SO_4)_2 \cdot 12\ H_2O$	3.75	14.8
Aluminum sulfate	Cake alum Patent alum	$Al_2(SO_4)_3 \cdot 18\ H_2O$	3.74	16.5

[a]Formulae of the first three alum salts listed in the table are sometimes written as double salts. e.g., $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24\ H_2O$.
[b]Alum salt solutions prepared on weight of each salt less water of crystalization

were made: (1) visual signs of spoilage as indicated by turbidity of the brine and gas pressure; (2) product odor; (3) brine acidity as acetic or lactic; (4) brine pH; (5) NaCl content of brine; and (6) pickle firmness in pounds as measured by the USDA Fruit Pressure Tester (Magness and Taylor, 1925). The firmness rating in pounds was the average value for 10 cucumbers each receiving a center punch, using a 5/16 in. diam plunger tip. The firmness rating scale follows: 18 lb and above = very firm; 17–14 = firm; 13–11 = inferior; 10–5 = soft; 4 and below = mushy (Bell et al., 1955).

RESULTS & DISCUSSION

Acidic properties of the different alum salts

There has been little or no information published on the acidic properties of the alum salts or their influence on the pH of pickle products. Fabian and Krum (1949) listed four alum salts used in the manufacture of pickle products. These are shown in Table 1 together with their formulae and acidic properties as determined in the laboratory. By definition, the most acidic alum, aluminum sulfate, is not a "true alum." Chemically, an alum is a combination of a monovalent and trivalent ion salt with 12 or 24 molecules of water of crystallization (Fabian and Krum, 1949). Aluminum sulfate is known as "cake alum" or "patent alum" according to the Merck Index, 8th Edition (1968) and it is accepted and used as alum in the manufacture of pickle products. Over the years, the food industry, and especially the pickle industry, has added aluminum sulfate to their products. For this reason, the experiments reported herein used aluminum sulfate.

To determine the acidic properties of aluminum sulfate, increasing levels of this salt were added to distilled water, and to a commercial Kosher-style dill pickle cover-brine. Increasing concentrations of alum (Table 2) in distilled water decreased the pH very markedly. As mentioned earlier, 0.1% (1g/liter) of alum, which is the amount normally used by the industry for pickle products, gave a pH value of 3.74 and 16.5 meq per gram in distilled water. This is equivalent to 0.0165N acid. To theoretically express 0.1% alum acidity as an equivalent to acetic acid, the following calculations are used:

(1) 1 meq acetic acid = 60 mg
(2) 1g alum = 16.5 meq as acid
(3) to express alum acidity as grams of acetic acid, 1g alum = 16.5 meq as acid x 60 mg of acetic acid = 990 mg = 0.99g of acetic acid
(4) thus, 1g alum has approximately the same acidity as 1g acetic acid.

Table 2 also presents the titratable acidities expressed as acetic and the pH values of a Kosher-style dill pickle brine equilibrated with increasing levels of alum in 1-qt jars with and without cucumbers. The dill pickle brine without cucumbers, but with increasing concentrations of alum, decreased the pH values (3.38 to 3.09) together with an increase in acidity as acetic (0.59 to 0.92%). The acetic acid increased about 0.11% with each 0.1% of alum added; this confirms the alum-acetic acid equivalents calculated above. When cucumbers were covered with the Kosher-style dill pickle brine containing the different alum levels, the cucumbers showed buffering effect, particularly at the lower alum levels (Table 2). This confirms earlier observations (Etchells and Moore, 1971).

Alum-treated, pasteurized cucumbers

The results of laboratory tests on four experimental packs of pasteurized cucumbers, acidified with acetic or lactic acid, each with and without alum are given in Table 3. As previously determined, the concentrations of the two acids needed in the cover-brine to equilibrate at pH 3.8–4.0 were 5.0% acetic and 1.0% lactic. The addition of alum lowered the pH, increased the acidity and reduced pickle firmness. Acetic acid without alum was the only treatment wherein the pickles remained firm after 8 months' storage.

Experiments at a pickle plant on fresh-pack dill pickles

The influence of different levels of lactic and/or acetic acids, with and without alum, on the firmness of fresh-pack dill pickles is given in Table 4. After 10 months' storage at room temperature, the experimental pack was examined. The pH values of the equilibrated cover-brines reflected the ionization behavior for the acids used and the alum-treated lots depressed the pH in all cases. This pH difference was more noticeable at the lower acid concentrations. Cucumber firmness was markedly reduced by the use of alum and was directly related to

Table 2—pH and titratable acidity of alum in water and in a Kosher-style dill pickle brine

Alum added to equilibrate at	Distilled water		Kosher-style pickle brine[a]			
			Without cucumbers		With cucumbers	
				Acid as acetic		Acid as acetic
%	pH	meq/g	pH	%	pH	%
0.00	6.38	0.0	3.38	0.59	3.88	0.67
0.05	3.89	8.9	3.31	0.64	3.75	0.72
0.10	3.74	16.5	3.25	0.71	3.57	0.74
0.15	3.65	26.2	–	–	3.37	0.75
0.20	3.58	35.1	3.14	0.82	3.26	0.77
0.25	3.52	44.6	–	–	3.17	0.78
0.30	3.43	52.1	3.09	0.92	3.12	0.80

[a]The cover-brine contained 1.47% acetic acid, pH 3.25 and 7.0% NaCl. The tests were carried out in 1-qt jars with cucumbers (1-1/8 to 1-3/8 in. diam) or water to replace cucumbers representing 60% of the total weight in each jar. Data shown are averages of duplicate treatments. Equilibration time was 48 hr.

Table 3—Influence of alum on pasteurized cucumbers acidified with acetic or lactic acids after 4 and 8 months' storage periods

Treatment of cucumbers[a] (desired acidity at equilibration)	Brine analysis after storage[b]		Cucumber firmness (pressure test) at storage periods of			
			4 months		8 months	
	pH	Acidity %	lb	rating	lb	rating
Acetic acid						
2.0%, No alum	3.84	2.11	15.8	Firm	14.3	Firm
2.0%, 0.1% alum	3.74	2.18	12.5	Inferior	11.1	Inferior
Lactic acid						
0.4%, No alum	3.95	0.52	15.3	Firm	13.2	Inferior
0.4%, 0.1% alum	3.82	0.61	11.8	Inferior	12.0	Inferior

[a]Model variety cucumbers, 1-1/8 to 1-3/8 in. diam were covered with the test brine containing the acids. 5 ml of alum solution (18.5g/100 ml) was added to each quart of the alum-treated lots.
[b]Brine analyses for pH and acidity (expressed as g/100 ml of each organic acid) are averages of duplicate brine samples at 4 and 8 months' storage periods. NaCl content 2.0%

the quantity of acid in the cover-brine. The striking effect of the influence of alum on the reduction of pickle firmness in lots acidified with different levels of lactic acid is shown in Figure 1. The increase in brine acidity and resultant decrease in brine pH caused by the additive (alum) is also clearly shown. Also, increasing levels of acid resulted in loss of pickle firmness.

The influence of different levels of alum, together with different levels of acetic and lactic acids, on the quality of fresh-pack dill pickles is presented in Table 5. Many of the low acid treatments showed microbial spoilage even though the pasteurization procedure was the same in all cases. However, as the amount of alum increased within each acid concentration, there was a number of jars of pickles that were preserved. For example, in Series I, Experiment 1, Treatments B and C, the control jars spoiled, but those with alum did not. The most noticeable deterioration in pickle firmness occurred with the high concentrations of lactic acid. The acetic acid treatments without alum (Table 5, Series II) were not free of microbial spoilage until the amount of acid added reached 9 ml per quart (0.73% when equilibrated). This is in agreement with Monroe et al. (1969) who recommended a minimum of 0.6% acetic acid for acidification of fresh-pack dill pickles. The addition of alum in every case–where spoilage was not a factor–resulted in a clear-cut loss in pickle firmness.

It should be apparent from the results described here that the continued use of alum in fresh-pack products by pickle manufacturers will continue to produce pickles of inferior quality as to firmness. The industry must remember that the alum in their products represents about 0.10% titratable acidity (calculated as acetic) and exerts a corresponding depression of the brine pH. Thus, with a new, nonalum formula, the proper acidification at equilibration with the cucumbers, should be sufficient to produce a brine pH below 4.0.

Based on findings by Monroe et al. (1969), Etchells and Moore (1971), as well as data of the authors (Table 2) a fresh-pack cover-brine for medium-size, whole dills containing 1.7–1.8% acetic acid, plus 7.2% salt should equilibrate, with the water content of the cucumbers, close to 0.65–0.70% acetic acid, about 2.8% salt and with a brine pH between 3.8 and 4.0. These figures are based on quart jars of pickles, packed so as to maintain 65% pickles and 35% brine. For a slightly looser pack–with 60% pickles–(which may be closer to the conventional machine-packs of industry today) the values cited for equilibrated acidity and salt content would be slightly higher, but the pH values should still fall in the range pH 3.2–4.0, never above pH 4.0.

In addition to the acidity adjustment discussed for a nonalum, fresh-pack formula, the packer should be aware of a similar need resulting from the greater buffering capacity of small-sized cucumbers (7/8 to 1-1/8 in. diam) as compared to the larger-sized fruit (1-1/2 to 2 in. diam). To compensate for this property, the cover-brine formulation for small-sized, fresh-pack pickles such as whole dills will require about 0.15–0.25% more acidity (calculated as acetic acid) than that used for large cucumbers.

In the interest of avoiding spoilage of fresh-pack products, the plant operator should be very careful when making any changes in his basic pasteurization procedure as well as any revisions of product

Table 4–Brine pH and cucumber firmness of fresh-pack dill pickles acidified with lactic and/or acetic acids, with and without alum, examined after 10 months' storage

	Acid treatments		No alum added		Alum added[b]		Cucumber firmness reduced by addition of alum
Code	Added per qt[a] ml	Equilibrated %	Brine pH	Cucumber firmness (PT)[c] lb	Brine pH	Cucumber firmness (PT)[c] lb	lb
Series I, lactic acid							
A	1.5	0.14	4.24	13.5	3.93	12.2	1.3
B	2.0	0.18	4.02	14.2	3.80	12.2	2.0
C	2.5	0.22	3.85	13.8	3.67	12.7	1.1
D	3.0	0.27	3.79	12.5	3.61	11.7	0.8
E	4.0	0.36	3.66	12.0	3.48	11.0	1.0
F	5.0	0.45	3.45	12.2	3.32	11.2	1.0
G	6.0	0.54	3.32	11.5	3.30	9.7	1.8
Series II, acetic acid							
A	1.00	0.08	4.68	13.5	4.24	13.5	0.0
B	1.33	0.11	4.52	13.8	4.18	13.8	0.0
C	1.67	0.14	4.42	15.0	4.15	13.2	1.8
D	2.00	0.16	4.36	13.0	4.14	12.0	1.0
E	2.67	0.22	4.27	13.0	4.07	12.2	0.8
F	3.33	0.27	4.14	14.2	3.94	10.5	3.7
G	4.00	0.32	4.05	13.8	3.88	11.2	2.6
Series III, lactic/acetic mixture							
A	0.75/0.50	0.07/0.04	4.48	13.2	4.12	13.0	0.2
B	1.00/0.67	0.09/0.05	4.30	14.2	4.05	14.0	0.2
C	1.25/0.83	0.12/0.07	4.18	14.2	3.92	13.7	0.5
D	1.50/1.00	0.14/0.08	4.12	13.2	3.89	12.2	1.0
E	2.00/1.33	0.18/0.11	3.89	14.5	3.66	13.5	1.0
F	2.50/1.67	0.23/0.14	3.78	13.8	3.60	13.5	0.3
G	3.00/2.00	0.27/0.16	3.66	14.0	3.49	11.7	2.3

[a]As 85% lactic or 85% acetic acid. Cover-brine was an "Overnight Dill" formula containing 7% salt. Equilibrated salt ranged 2.9–3.0%

[b]Alum as $Al(SO_4)_3 \cdot 18\ H_2O$ added in cover-brine at 0.2% (equiv. 0.75g/qt) and calculated to equilibrate at 0.08%

[c]Pressure test values in pounds; values shown are averages of the center punch for 10 cucumbers, size 1-1/8 to 1-3/8 in. diam. Data shown are averages of duplicate samples within each treatment.

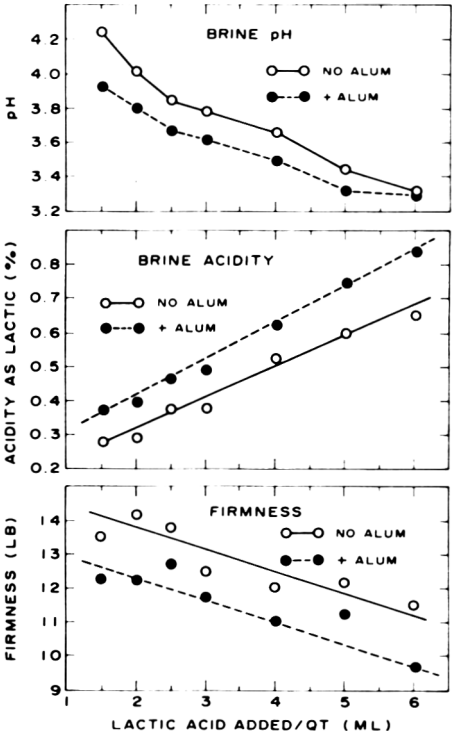

Fig. 1–Influence of alum on the reduction of pickle firmness in lots acidified with different levels of lactic acid.

Table 5—The effect of different levels of alum, acetic and lactic acids on cucumber firmness and microbial spoilage of fresh-pack dill pickles after about 12 months' storage.

Code	Acid treatments: Added per qt[a] ml	Acid treatments: Equilibrated %	Alum added (g per quart jar)[b]: 0.00	0.25	0.40	0.75	1.10	1.67
			Cucumber firmness in pressure test values[c]: lb	lb	lb	lb	lb	lb
Series I, lactic acid								
Experiment 1, low levels								
A	0.5	0.04	11.5(S)	–	11.9(S)	11.7(S)	10.8(S)	–
B	1.0	0.09	10.0(S)	–	12.8	12.0	13.2	–
C	1.5	0.14	12.7(S)	–	13.3	12.4	13.0	–
D	2.5	0.22	12.6	–	13.0	12.8	12.6	–
Experiment 2, high levels								
E	2.25	0.20	4.2(S)	6.4(S)	–	11.4(S)	–	10.6
F	6.75	0.61	11.4	11.2	–	9.1	–	8.8*
G	13.50	1.22	4.5*	4.2*	–	4.7	–	6.2*
Series II, acetic acid								
Experiment 1, low levels								
A	0.33	0.03	11.0(S)	–	11.4(S)	12.0	10.6	–
B	0.67	0.05	12.2(S)	–	11.6(S)	11.6(S)	--[d]	–
C	1.00	0.08	13.2(S)	–	13.0(S)	12.4	12.0	–
D	1.67	0.14	14.2(S)	–	13.8	12.5	13.4	–
Experiment 2, high levels								
E	1.50	0.12	<3.0(S)	5.2(S)	–	6.2(S)	–	7.8(S)
F	4.50	0.36	7.4(S)	7.4(S)	–	9.8	–	11.4
G	9.00	0.73	10.6	11.6	–	9.8	–	10.4

[a]See Footnote a, Table 4.

[b]Alum as $Al_2(SO_4)_3 \cdot 18\,H_2O$ and added in cover-brine, calculated to equalize as follows: 0.25g/qt = 0.027%; 0.40g = 0.043%; 0.75g = 0.08%; 1.10g = 0.119%; and 1.67g = 0.18%.

[c]Pressure test values in pounds with 5/16 in. tip; values shown are averages for the center punch of 10 cucumbers; sizes used 1 to 1-1/8 in. diam for Exp. 1 and 1-1/8 to 1-3/8 in. diam for Exp. 2. Data shown are averages of duplicate sample. (S) indicates observed spoilage by a cloudy brine and gas pressure on the cap for one or both jars. * indicates brine pH of 3.2 and below. Experiment 1 pickles evaluated after 12 months; Experiment 2 pickles after 13 months.

[d]Jars broken

specifications calling for reduced acidification or lower salt content. For example, arbitrarily reducing the acid and salt content in a fresh-pack product, such as whole dill pickles, to achieve some abnormally mild flavor might inadvertently lead to a very serious spoilage problem and a public health hazard as well. There can be no compromise with the proper acidification and pasteurization procedures in the preparation of high-quality, fresh-pack pickle products.

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Ms received 8/17/71; revised 11/22/71; accepted 1/16/72.

Paper no. 3499 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, NC 27607.

This cooperative research was supported in part by a grant from Pickle Packers International, Inc., St. Charles, Illinois.

The authors wish to thank the officials of the H.W. Madison Co., Div. of the J.M. Smucker Co., Medina, Ohio, for their excellent cooperation and for the use of Company facilities; we especially thank William A. Murray, of the Pickle Div. for his support in this investigation and M.L. Lazear Jr. for his able laboratory assistance. We are also indebted to Dale A. Newton, formerly of our laboratory for his fine contribution to the laboratory studies, and to R.E. Kelling of our laboratory for his suggestions and review of the manuscript.

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INFLUENCE OF DIFFERENT ORGANIC ACIDS ON THE FIRMNESS OF FRESH-PACK PICKLES

INTRODUCTION

CRISP TEXTURE of cucumber pickles is an important quality characteristic. This applies to items prepared from brine-stock, or those from green cucumbers, called "fresh-pack" or pasteurized pickles. The role of pectinolytic enzymes as the cause of cucumber softening during brine fermentation has been thoroughly investigated (Bell et al., 1950; Etchells et al., 1955, 1958a) and methods of control have been suggested (Etchells et al., 1955, 1958b; Bell et al., 1955, 1965). However, texture deterioration of pickle products by nonenzymatic reactions, such as by acids, salts and other chemicals, has been given little attention. Lesley and Cruess (1928) and Fabian and Johnson (1938) suggested that the natural organic acids produced in low-salt content fermentations, such as during the manufacture of genuine dill pickles, were in part responsible for reducing the texture of the final product. Texture loss of pickles at elevated storage temperatures (86°F and above) has been reported for processed dills (Pangborn et al., 1959) and for fresh-pack items (Nicholas and Pflug, 1960).

Monroe et al. (1969) reviewed the development of fresh-pack pickles in this country. They reported that the annual volume of these products requires over 40% of the crop of more than 20 million bushels of pickling cucumbers. They also reported on the influence of acetic acid (range 0.20–1.00%) and internal-product pasteurization temperatures (range 120–200°F) on physical, chemical and microbial changes in fresh-pack dill pickles. They concluded that higher temperatures, from 170–200°F, produced bloater (hollow cucumbers) damage; also, faster heating rates decreased pickle firmness, particularly in the top part of the jar. Etchells and Jones (1942) pasteurized fresh-pack sweet slices and fresh-pack dills and noted that they retained most of their original crispness for 8 months. These products, with equilibrated acidities of 0.4–1.7% acetic acid were pasteurized at an internal-product temperature of 165°F for 15 min, followed by prompt cooling to below 90°F. Recently, Etchells et al. (1972) investigated the effect of alum on fresh-pack dill pickles; they found that alum caused a reduction in firmness of the product during storage. This finding was contrary to the widely accepted belief that alum functions as a firming agent in pickle products.

In the manufacture of fresh-pack pickle products, the cover-brine is poured on packed jars of whole or sliced cucumbers just before capping and pasteurizing. The conventional acidulant in pickle manufacture is acetic acid (vinegar), even though other food-grade acids have been suggested (Sausville, 1965). It is added in the cover-brine at a strength calculated to give the desired equilibrated acid concentration. This usually amounts to 35–40% of the cover-brine concentration. Fabian and Wadsworth (1939) found that acetic acid equilibrated more rapidly than lactic in products made with salt-stock. Also, the rate was greatest for both acids during the first 24 hr.

The experiments herein were designed to exert certain physical and chemical stresses on cucumber texture, some of which exceeded those encountered in the pickle industry. Areas of study were: (a) rate of acid-cucumber equilibration with acetic, lactic, citric and oxalic acids; (b) influence of increasing pasteurization (165°F) "holding-times" on the texture of cucumbers packed and equilibrated for 24 hr in water, acetic or lactic acids; (c) firmness of pasteurized cucumbers as influenced by five organic acids; and, (d) lactic acid-softening of cucumbers.

EXPERIMENTAL

CERTIFIED reagent-grade acetic, lactic, citric, malic and oxalic acids were used. The liquid portion of a jar's content is referred to as the "brine" or the "pickle brine;" but, at the time of addition, it is called the "cover-brine." Each acid was incorporated into the cover-brine and determined (w/v) by titrating a 10-ml sample with a 0.111N NaOH solution to pH 7.5 as measured with a Beckman Zeromatic meter. Acid titrations of the cover-brine at time intervals were used to calculate the percent equilibration. The pickle-brine pH was measured with a Beckman Expandomatic meter. The NaCl content of the cover-brines was determined by the method described by Etchells et al. (1964).

Experimental packs for each series were prepared from freshly-harvested cucumbers, carefully graded to size. The pickling varieties used, Model and SMR-58, were obtained from a pickling company in North Carolina. For each series, the cucumbers were thoroughly washed with tap water and regraded; then, 12–15, 1-1/8 to 1-3/8 in. diam cucumbers were weighed (550–560g) and carefully hand-packed into each 32-oz jar. The cover-brine for each jar measured about 370 ml. This gave a ratio of close to 60% solids and 40% brine by weight. The jars were capped with 70 mm, 4-lug, "twist-off" caps (White Cap Co., Chicago, Ill.) and were pasteurized immediately or after a given equilibration period. The laboratory pasteurization was essentially as that described for a hot water, batch operation (Etchells and Jones, 1944). The experimental packs were stored at about 78°F. Cucumber firmness was determined with a USDA Fruit Pressure Tester (Magness and Taylor, 1925) using the procedure of Bell et al. (1955). The firmness, in pounds, was the average of the center punch values for 10 cucumbers, using a 5/16 in. diam plunger tip. The rating scale used was: 18 lb and above = very firm; 14–17 = firm;

Table 1–Rate of acid equilibration between the cover-brine and whole cucumbers[a]

Time (hr)	5% Acetic acid pH	5% Acetic acid Equilibration in %	1% Lactic acid pH	1% Lactic acid Equilibration in %	1% Citric acid pH	1% Citric acid Equilibration in %	4% Oxalic acid pH	4% Oxalic acid Equilibration in %
0.0	2.85	0	2.70	0	2.65	0	2.20	0
1.0	3.05	16	3.00	24	2.85	12	2.40	40
2.5	3.35	40	3.25	38	3.10	28	2.85	65
5.0	3.42	52	3.40	49	3.25	33	3.05	70
10.0	3.65	70	3.60	69	3.50	48	3.45	82
24.0	3.65	85	3.65	79	3.60	64	3.70	88
50.0	3.75	95	3.75	90	3.80	76	3.90	93

[a]The jars contained close to 60% cucumbers and 40% brine on a weight basis.

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Table 2—Influence of increasing pasteurization (165°F) holding-times on the firmness of cucumbers packed in water, acetic or lactic acids

Treatment	Firmness of cucumbers (PT)[a], packed and equilibrated in: Water[c] lb	Rating	Acetic acid[c] lb	Rating	Lactic acid[c] lb	Rating
No heat control	19.3	V. firm	15.8	Firm	17.9	Firm
Time held at 165°F[b]						
0 min	18.0	Firm	14.7	Firm	17.1	Firm
15 min	18.0	Firm	15.5	Firm	17.0	Firm
30 min	17.6	Firm	13.9	Firm	14.8	Firm
60 min	16.8	Firm	12.8	Inferior	13.5	Inferior
120 min	14.5	Firm	12.4	Inferior	11.3	Inferior
180 min	12.6	Inferior	9.5	Soft	9.1	Soft
240 min	13.1	Inferior	8.6	Soft	7.7	Soft

[a]For rating scales of the pressure test values and adjective ratings, see EXPERIMENTAL section.

[b]Come-up time for 1-qt jars of cucumbers, from 90°–165°F was 35 min in the hot-water bath.

[c]The equilibrated pH's of the three cover-liquids were as follows: water, 5.8; acetic acid, 3.7; lactic acid, 3.7.

11–13 = inferior; 5–10 = soft; 4 and below = mushy.

RESULTS & DISCUSSION

Rate of acid-cucumber equilibration

Acetic, lactic, citric and oxalic acids were each added separately in the cover-brines to whole cucumbers (Model variety) in amounts calculated to give an equilibrium pH of about 4.0. The results of the tests are shown in Table 1. Oxalic acid equilibrated at the fastest rate, followed by acetic. Citric acid penetrated at the slowest rate, and after 5 hr was only one-third equilibrated, whereas oxalic acid was more than two-thirds, and acetic and lactic acids about one-half. The rate of acid equilibration is an exponential function; plots of percent acid equilibration against log-time (hours) resulted in fairly straight lines for the four acids. Even so, complete equilibration may take as long as 2 or 3 wk. However, acetic, lactic and oxalic, were about 90% equilibrated in 50 hr. In a commercial operation, the jars of pickles reach the steam pasteurizer in a very short time after the cover-brine is added. Thus, little acid penetration occurs before the heating starts.

Increasing pasteurization (165°F) holding-times with cucumbers packed in water, acetic or lactic acids

The experiments were designed to determine the influence of a fixed internal-product pasteurization temperature (165°F), for 0, 15, 30, 60, 120, 180 and 240 min before cooling on the firmness of cucumbers that have been equilibrated in water, acetic, or lactic acid for 24 hr. Cucumber firmness together with the adjective ratings are shown in Table 2. The highest rating, 19.3 lb was for the no heat, water control treatment. Lactic and acetic acids reduced the firmness by two or three pounds during the equilibration period prior to pasteurization. The water-pack cucumbers retained their firmness during the pasteurization treatments better than those acidified with acetic or lactic acid. Further, water-pack-treated stock was still firm at 120 min, whereas cucumbers from the acetic and lactic acid treatments were down to an inferior rating. Cucumbers from both acid treatments were soft after 180 min.

The effect of acid on cucumber texture could be important, considering the actual degree of equilibration of acid and cucumbers before pasteurization. Apparently, from a texture standpoint, it is better not to have the acid fully equilibrated with the cucumbers at the time of pasteurization.

Firmness of pasteurized cucumbers as influenced by different organic acids

Lactic, citric, malic and oxalic were compared with acetic in cucumber-pasteurization and storage tests (Table 3). All but oxalic acid are commonly used in

Table 3—Acidity and pH of the cover-brines and equilibrated pickle brines, and cucumber firmness from five organic acid treatments

Treatment of cucumbers (desired acidity at equilibration)[a]	Initial cover-brine pH	Initial cover-brine % acid	Examination at: 4 months pH	4 months % acid	4 months lb (PT)[b]	8 months pH	8 months % acid	8 months lb (PT)[b]	Increase in acidity at[c] 4 months %	Increase in acidity at[c] 8 months %
Acetic acid										
2%	2.80	4.94	3.92	2.05	15.8	3.75	2.12	14.3	3	6
4%	2.70	9.89	3.70	4.11	12.5	3.50	4.23	10.4	3	6
Lactic acid										
0.4%	2.80	1.02	4.10	0.47	15.3	3.80	0.56	13.2	18	40
0.8%	2.60	1.97	3.78	0.89	8.6	3.60	1.07	9.5	11	34
Citric acid										
0.4%	2.60	1.00	4.30	0.45	15.7	4.05	0.50	14.2	12	25
0.8%	2.45	2.03	3.75	0.82	9.4	3.55	0.89	7.8	3	11
Malic acid										
0.4%	2.65	1.03	4.15	0.45	15.7	3.90	0.51	13.4	12	27
0.8%	2.50	1.93	3.80	0.83	8.9	3.35	0.83	5.5	4	4
Oxalic acid										
0.16%	2.15	0.40	4.40	0.21	< 3	4.15	0.26	< 3	31	62
0.32%	1.95	0.80	3.65	0.36	< 3	3.50	0.40	< 3	12	25

[a]Model variety cucumbers, 1-1/8 to 1-3/8 in. diam size, packed in 1-qt glass jars. Acid treatments are % (w/v) for each acid and added to equilibrate at the desired acidity.

[b]Pressure test: See EXPERIMENTAL section for details.

[c]Increase in titratable acidity for each treatment is the percent increase from the calculated equilibrated concentration shown under "Treatment of cucumbers."

food products. Oxalic acid was chosen because of its calcium and magnesium sequestering properties. The first lot was made to equilibrate at about pH 4.0. In the second, the acid concentrations were twice those of the first. Samples were analyzed after 4- and 8-months' storage. Firmness values are given in Table 3. The lowest levels of acetic, lactic, citric and malic acids gave pressure test values of 15.3–15.8 lb (= firm) after 4-months' storage. After 8 months, the acetic and citric acid treatments were about 1.5 lb lower, and the lactic and malic acid treatments were more than 2 lb lower than at 4 months. When acid concentrations were doubled, all firmness ratings but one dropped to soft (8.6–9.4 lb). The exception was acetic acid which dropped 3 lb to a rating of inferior to soft. To emphasize the effect on texture, the acetic acid concentrations selected (2.0 and 4.0%) were on the very high-side of that used by industry (range 0.6–2.2%). However, results in Table 3 indicate that acetic acid caused the least loss in firmness, followed by lactic, citric, malic and oxalic acids.

Oxalic acid reduced the cucumbers to mush. The stability constants, log K, for Ca^{++} for the acids are as follows: acetic, 0.53; lactic, 1.07; malic, 1.80; oxalic, 3.0; and citric, 3.50. If one accepts that the primary loss of cucumber texture is caused by the shift in calcium from the pectic substances between the plant cells (Matz, 1962; Hulme, 1970), then an explanation is possible for the texture loss by each of the five acids. Acetic acid is weakly dissociated at pH 4.0 (15%), as compared to lactic acid (61%), malic (80%), citric (88%) and oxalic (95%). This was reflected in the individual acid concentrations required to equilibrate at about pH 4.0 (Table 3). Further evidence of the chelating properties as influenced by pH was suggested by Chaberek and Martell (1959). They reported that citric acid is considerably more efficient in the pH range 7–11, requiring an average of two moles of citric acid to one mole of Ca^{++}, whereas, at pH 5 and below, the ratio is greater than ten to one. This explains in part the rather high degree of cucumber firmness (Table 3) for the citric acid treatment at pH 4.

Table 3 also reveals phenomena not completely understood by the authors. During storage, acidity increased, pH values were depressed and cucumber firmness ratings decreased significantly in all treatments except acetic, which exhibited only minor acidity increases. This is especially puzzling because all treatments were acidified and pasteurized in accord with recommended procedures. Furthermore, no signs of fermentation were apparent. Since the acidity and pH levels of acetic acid treatments remained relatively stable, chemical changes associated with softening process may have been a factor in the increased acidity; because, the greatest changes in acidity were associated with corresponding increased losses in firmness.

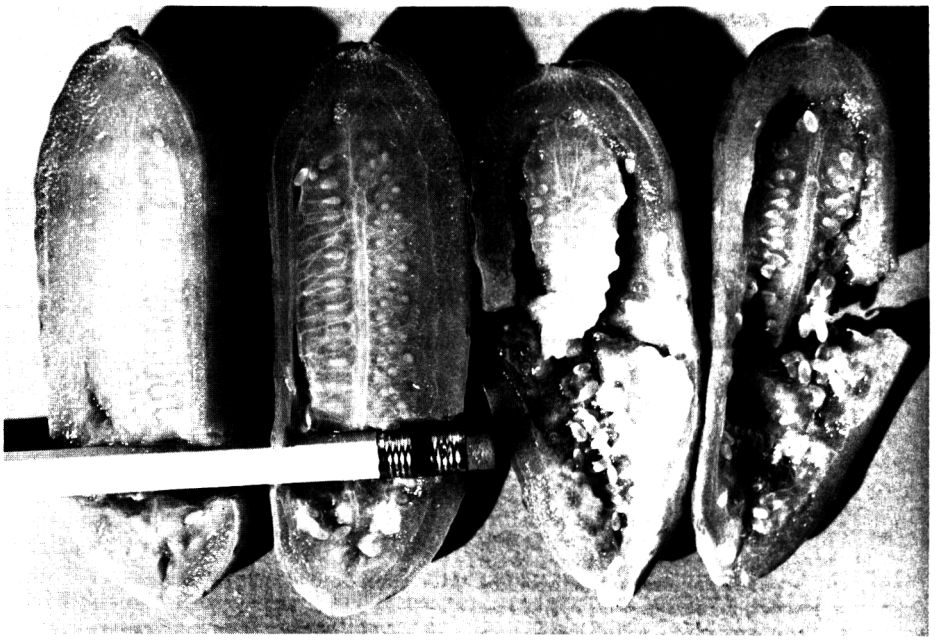

Fig. 1–Cucumber pickles from 1.0% lactic acid experimental fresh-pack after 15 months' storage at room temperature. The soft tissue is illustrated by the ease in which the pencil presses into the cucumber.

Lactic acid-softening of cucumbers

In the previous experiment, 0.8% lactic acid caused cucumber softening after

Table 4–Chemical analyses of pickle brines and firmness of pasteurized cucumbers acidified with lactic acid

Treatment of cucumbers (desired acidity at equilibration)[a]	Chemical analyses of pickle brine at:						Cucumber firmness (PT)[b] at			
	3 months pH	15 months pH	3 months % acid[c]	15 months % acid[c]	3 months % NaCl	15 months % NaCl	3 months lb	3 months Rating	15 months lb	15 months Rating
Lactic acid										
0.2%	3.88	3.75	0.26 (30%)	0.30 (50%)	2.20	2.01	14.8	Firm	9.1	Soft
0.4%	3.55	3.46	0.46 (15%)	0.52 (30%)	2.10	1.96	14.0	Firm	8.4	Soft
0.6%	3.28	3.25	0.69 (15%)	0.76 (27%)	2.05	2.04	11.9	Inferior	4.0	Mushy
0.8%	3.18	3.09	0.90 (12%)	1.00 (25%)	2.10	1.98	9.2	Soft	<3.0	Mushy
1.0%	3.10	3.01	1.09 (9%)	1.21 (21%)	2.30	2.02	6.2	Soft	<3.0	Mushy
Acetic acid (control)										
2.0%	3.45	3.34	1.96	1.99	2.10	2.06	13.1	Inferior	6.7	Soft

[a]SMR-58 variety cucumbers, 1-1/8 to 1-3/8 in. diam and packed in 1-qt glass jars. Cover-brines contained 5g/100 ml NaCl; 0.5, 1.0, 1.5, 2.0 and 2.5g/100 ml lactic acid; and 5.0g/100 ml acetic acid. Pack-out ratio was close to 60% cucumbers and 40% brine by weight.
[b]Pressure test: see EXPERIMENTAL section for details.
[c]Values in parentheses are % increase in titratable acidity for each acid treatment (see Table 3, footnote c).

only 4 months' storage. Lactic acid is one of the important acids in pickling, particularly in the natural fermentation of cucumbers for salt-stock pickles (Etchells et al., 1964) where it is the major acid produced. Consequently, five levels of lactic acid were evaluated as to their influence on firmness of cucumbers (Table 4); a 2.0% acetic acid control lot was included.

Cucumber firmness measurements after 3 months' storage clearly show the softening action of lactic acid. The two low levels, 0.2 and 0.4%, resulted in firm cucumbers, but cucumbers at the two high levels, 0.8 and 1.0%, were soft. This type of softening-spoilage is illustrated in Figure 1. Firmness of cucumbers in the acetic acid control was reduced to inferior at 3 months and soft at 15 months. However, in commercial practice, the equilibrated brine acidities would usually fall in the 0.60–1.5% range, not 2.0%.

Chemical analyses of the brine at 3 and 15 months again showed an increase in acidity of the soft lots accompanied by a drop in brine pH.

The 5% NaCl added in the cover-brine of the five lactic acid treatments equilibrated in the jars containing cucumbers at an average of 2.1 and 2.0% at 3 and 15 months, respectively. Sodium chloride was calculated to equilibrate at 2.0%, hence, variability in the pack-out ratio of cucumbers and cover-brine for each sample was small (Table 4). The addition of NaCl to the test-pack depressed the pH values as compared to the same lactic acid treatments without salt (Table 3). The salt apparently had no effect on the lactic acid softening.

In conclusion, the results reported preclude the use of lactic, citric, malic, or oxalic acids in the manufacture of fresh-pack pickles. Acetic acid (vinegar) (0.6–1.5%) was the acidulant that produced the best-textured fresh-pack pickle products.

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Ms received 9/18/71; revised 1/6/72; accepted 1/16/72.

Paper no. 3498 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, NC 27607.

This cooperative research was supported by a grant from Pickle Packers International, Inc., St. Charles, Ill.

The authors thank the officials of the Mt. Olive Pickle Co., Mount Olive, N.C. and the Perfect Packed Products Co., Henderson, N.C. for their excellent cooperation and assistance throughout this investigation. We are also indebted to Dale A. Newton, formerly of our laboratory, for his fine contribution to the laboratory studies.

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PREPARATION AND EVALUATION OF SOYBEAN CURD WITH REDUCED BEANY FLAVOR

INTRODUCTION

HANG AND JACKSON (1967a, b) prepared soybean cheese using soybeans, skim milk powder, rennet and lactic starter cultures. Although the product possessed a beany flavor it was considered to have some advantages over tofu, the traditional Chinese soybean curd, particularly with regard to keeping quality. Further attempts were made to improve the flavor and texture by the incorporation of larger amounts of skim milk and by ripening the product with various molds (Schroder and Jackson, 1971). Although the ripening brought about desirable changes in the texture, this was offset by the formation of undesirable bitter flavor components, presumably resulting from proteolysis. In order to develop a more acceptable product it was considered necessary to subject the beans to a heat treatment at the start of the process. This would have the effect of (a) destroying native enzymes in the beans thereby preventing further activity during processing; (b) possibly driving off undesirable flavor components; and (c) destroying any microorganisms that may be present. Experiments were undertaken to determine time-temperature treatments of the whole beans and the soybean milk which would achieve the objectives stated above.

In earlier experiments (Schroder and Jackson, 1971) the initial treatment of the beans consisted of a preliminary steaming followed by dehulling. It was considered that the steam treatment activated the lipoxidase enzymes in the beans resulting in the typical beany flavor (Wilkens et al., 1967). Enzyme activation would also occur in the traditional practice of soaking beans in water prior to preparation of soybean milk. In order to overcome this difficulty it was decided that the beans should be blended in hot water without any previous treatment other than rinsing.

MATERIALS & METHODS

THE BASIC PROCESS for the production of soybean curd was as follows: whole soybeans (Grade No. 1, W.G. Thompson & Son Ltd., Blenheim, Ontario, Canada), were quickly rinsed in cold water and placed in a 1-gal Waring Blendor together with boiling water. The ratio of water to beans was 9:1 and the temperature of the water at this stage between 85 and 95°C. The beans and water were then blended at full speed for 3 min. The resulting suspension was held at approximately 100°C for 30 min by injection of live steam. After the steam treatment the suspension was filtered through muslin bags to yield two fractions, namely, a soybean milk and a residue consisting mainly of coarse material derived from the hulls. The soybean milk was maintained at 80°C and the curd precipitated by the addition of 0.2% $CaSO_4$. The curd was allowed to stand for a further 30 min, the temperature being approximately 80°C throughout. The curd was then placed in small cheese hoops and allowed to drain freely for a short time, after which it was pressed overnight at a pressure of approximately 1 psi. A flow sheet for the production of the soybean curd is outlined in Figure 1.

Traditional Chinese curd was prepared in the following manner: whole soybeans were soaked overnight in tap water at 5°C. The soaked soybeans were blended with water and the mixture filtered through a linen cloth. The soybean milk was steamed for 30 min, cooled to 70°C and precipitated with calcium sulphate. When the curd had formed it was cut into cubes with a knife to facilitate the release of whey. The curd was finally placed in small cheese hoops and gently pressed overnight.

Amino acid analysis of the curd was carried out in a Beckman Spinco model 120-B amino acid analyzer (Beckman Instruments Inc., Fullerton, Calif.) according to Spackman et al. (1958). Hydrolysis of the protein was effected in 6N HCl at 110°C for 24 hr and corrections were made for hydrolytic losses by extrapolation back to zero hydrolysis time. All results are reported as percentage of total hydrolyzate; however, no measurement was made of tryptophane.

Protein, crude fiber, moisture, water soluble nitrogen and fat were determined according to the official methods of the AOAC (1965). Crude fat was determined according to the official method for crude fat in soy flour (AACC, 1962).

An attempt was made to utilize the bland soybean curd in the preparation of meat product substitutes. Various meat spices, together with recommendations on their use, were kindly donated by Griffith Laboratories Ltd., Scarborough, Ontario; Fritzsche Brothers of Canada, Toronto, Ontario; and Food Products Ltd., Montreal, Quebec. The soybean curd was ground and formulated in the following proportions:

Soybean curd	468g
$Na_3PO_4 \cdot 12H_2O$	12g
Vegetable protein hydrolyzate	1g
Emulsifier	0.5g
Spices, Smoke flavor, Color	According to manufacturers recommendations

The mixture was either stuffed into sausage casings and steamed for 25 min, or sealed in number 307 × 113 enameled cans (Continental Can Co. of Canada) and autoclaved for 30 min. Problems were encountered during the steaming of the sausage due to bursting of the casings. This was due to the high water-binding capacity of the soybean protein. Loose stuffing of the casings and a higher initial moisture content effectively overcame this problem.

Table 1—Composition of soybean curd

Protein (D.W. basis) (N × 6.25)	55.6%
Fat (D.W. basis)	31.9%
Moisture	76.8%
Water soluble N	0.06%
pH	6.01
% yield	31.7

Table 2—Amino acid composition of soybean curd

Amino acid	% of total crude protein
Lysine	6.18
Histidine	2.37
Arginine	7.25
Aspartic acid	11.62
Threonine	3.60
Serine	4.65
Glutamic acid	19.53
Proline	3.27
Glycine	3.92
Alanine	4.22
½ cystine	2.26
Valine	5.31
Methionine	0.99
Isoleucine	4.95
Leucine	8.02
Tyrosine	3.60
Phenylalanine	5.33

RESULTS

Characteristics of the soybean curd

The pressed curd had virtually no flavor and could best be described as bland. The texture could be modified

Table 3—Composition of residue

Protein (air dry basis)	34.4%
Crude fiber	28.1%
Crude fat	13.6%
Moisture	80.0%

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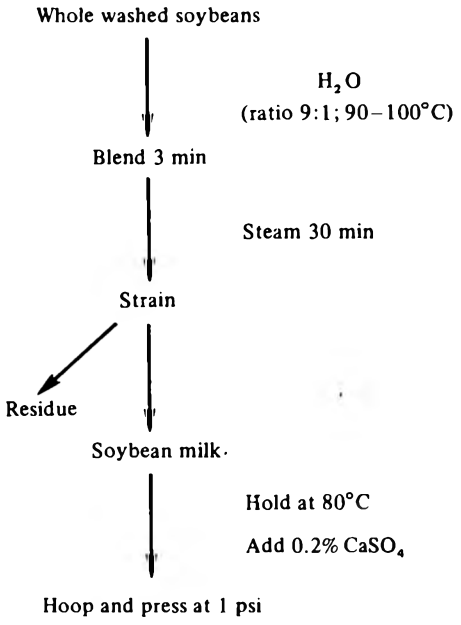

Fig. 1—Flow sheet for the production of soybean curd

according to the amount of pressure applied in the hoops. A pressure of 1 psi resulted in a smooth rubbery texture, whereas lower pressures resulted in paste-like material.

The proximate analysis and amino acid composition of the curd are shown in Tables 1 and 2 respectively. The protein content was very uniform giving a coefficient of variation of 2% from the results of the analysis of eight separate makes.

The amino acid composition compares closely to an analysis of soybean curd produced by a similar method (Hackler and Stillings, 1967).

The low yield of curd is due to the large amount of residue removed during straining. The significance of this will be considered further in the discussion.

Taste panel evaluation of the curd

Fresh curd. A taste panel was used to assess the removal of the beany flavor from the curd. 23 untrained taste panel members were requested to assess the extent of the beany flavor and give some indication of the texture of two curd samples. Beany flavor was recorded as none, very slight, moderate, pronounced, or very pronounced. Texture was recorded as firm, chewy, rubbery, mealy, crumbly, spongy, smooth or pasty. Panel members could check more than one character to describe texture, and if appropriate, could add others. One sample was the test sample and the other was produced by the traditional Chinese method. 18 out of 23 found the test curd to be less beany than the Chinese curd; four found it more beany and one found no difference. To avoid assigning absolute numbers to the extent of the beany flavor, the data were treated as nonparametric. Using both the sign test and Wilcoxon's sign rank test (Steel and Torrie, 1960) the beany flavor of the test curd was found to be highly significantly less than that of the Chinese curd (to the 1% level).

The panel considered the texture of the test curd to be firm, smooth and rubbery, and the texture of the Chinese curd to be smooth and spongy.

Three samples of curd (two test samples and one sample of Chinese curd) were given to panel members and they were requested to rank these in order of preference to determine if they could pick out which sample was different. 19 of 23 ranked the test sample as the best and only four ranked the Chinese sample as the best. It is interesting to note that all panel members of Oriental origin preferred the Chinese curd to the test sample. The product was difficult to assess as it was not a finished product, nor was it one familiar to most of the panel members.

Flavored products. Both the sausage-type meat product and the canned-type meat product were evaluated. The sausage product was evaluated informally and all who tasted the product considered it to be quite acceptable. The canned meat product was evaluated by a taste panel of 30 members. The panel members were allowed to taste the product as prepared or with bread and butter if they so desired. Acceptability was rated on the following scale: very acceptable, moderately acceptable, neither like nor dislike, moderately unacceptable and very unacceptable. The results were as follows:

Very acceptable	15
Moderately acceptable	13
Neither like nor dislike	2

Residue

The crude analysis of the residue is shown in Table 3. The residue after drying could be considered as animal feed.

DISCUSSION & CONCLUSIONS

ONE OF THE major problems limiting the use of soybeans in human nutrition has been the beany flavor. The work reported here indicates that the beany flavor can be significantly decreased by blending the beans with boiling water and steaming the resulting soybean milk. Calcium sulphate precipitation of the soybean milk gives rise to a bland soybean curd that can be varied in texture according to requirements.

The results of the experiments with flavored meat-type products suggest that it is possible to produce acceptable meat product substitutes. The equipment required for this process is very simple and relatively inexpensive.

From the amino acid analysis of the curd and from animal feeding experiments (Schroder, 1969) it is apparent that the nutritional value of the curd is impaired by the low methionine content. Fortunately the cost of methionine is not excessive and the small amount needed to increase the nutritional value to a level similar to that of high quality protein would add little to the overall cost of the product.

The tasteless soybean curd would lend itself to the manufacture of a wide range of products depending upon the pressure applied during processing and the nature of additives. The curd may also be a good starting material for the manufacture of high quality soybean protein hydrolyzates.

The main objective of this work was the production of a bland soybean curd suitable for further processing into meat product substitutes. As a result the overall efficiency of the process was not studied. A more efficient process in terms of yield and energy requirements could almost certainly be achieved by a more critical study of the following items: dehulling before blending; the ratio of beans to water at the time of blending; filtration before steaming; length of the steaming process; and mechanical aids in the filtration process.

The results indicate that nutritious and acceptable meat product substitutes can be prepared from soybean curd prepared in the manner described in this paper.

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Ms received 11/27/71; revised 2/2/72; accepted 2/3/72.

This work was supported in part by the Alberta Agricultural Research Trust. The authors thank Mr. Huan-Mei Mei for his technical assistance and advice.

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WHIPPING PROPERTIES OF SPRAY-DRIED COMPLEXES FROM WHEY PROTEIN AND CARBOXYMETHYLCELLULOSE

INTRODUCTION

PROTEINS present in solutions at low concentrations can be precipitated in high yield as insoluble complexes by selected gums and detergents (Smith et al., 1962). The method is based on the well-known chemical principle by which colloids of opposite charge undergo electrostatic interaction producing a flocculant precipitate (Cornwell and Kruger, 1961).

We have previously described a process for the reclamation of cheese whey proteins with carboxymethylcellulose (CMC), either selectively as fractions (Hidalgo and Hansen, 1971) or as a mixture of the major proteins (Hansen et al., 1971). A pilot plant has been constructed to facilitate the rapid separation of the precipitated complex from the fluid (Hansen and Crauer, 1971). Such processes may be useful for the industrial recovery of special-interest proteins (Smith et al., 1962). For example, the combination of various hydrocolloids with soluble milk proteins may yield new stabilizer products for the food industry, which combine functional properties (Ziemba and Alikonis, 1971) with nutritional value (Karp, 1971). The present study was undertaken to determine the whipping and foaming characteristics of whey protein/CMC complexes.

EXPERIMENTAL

THE WHEY PROTEIN/CMC complexes were prepared by the method of Hansen et al. (1971) from clarified cottage cheese whey using high viscosity-type NaCMC having a designated degree of substitution of 0.65–0.85. Only the fraction recovered at pH 3.2 was used. After neutralization of the complex with NaOH, and prior to drying unless otherwise indicated in the results, H_2O_2 was added at a concentration of 0.02%. The viscous solution (6–7% total solids) was preheated and spray dried at 65°C in a Swenson laboratory spray drier equipped with an external mix air jet (Spraying Systems, Inc.). The powders were then stored in plastic bags at 15°C for about 3 months before use.

The composition of the dried complex was 58–61% protein, determined in the Coleman Nitrogen Analyzer; 8–9% water, determined by drying at 105°C for 3 hr; 4–5% ash, determined by calcination at 650°C; and 25–30% CMC, determined by difference. No H_2O_2 was detected in the powders by a qualitative test (AOAC, 1965).

Whipping trials were carried out using an electric household mixer (Sunbeam Mixmaster). Cold tap water or skim milk was placed in the mixing bowl to which the powdered complex was quickly added. The mixer was run at slow speed until the powder was well wetted (30–60 sec) and then at maximum speed until completion (15–20 min). When desired, homogenization or hydration of the complex was accomplished by thoroughly wetting the powder in a mixing bowl and then homogenizing at room temperature in a small hand-operated laboratory homogenizer. For further hydration, the wetted powders were held at 2–5°C for 24 hr, removed and whipped at full speed.

The specific volume of the foam was determined as an indicator of air uptake during whipping. A sample of the foam was removed from the mixing bowl as the remainder of the foam continued to whip. The sample was placed in a container of known weight and volume and the weight of the foam was determined. The sample, after weighing, was returned to the mixing bowl. It should be noted that specific volume (volume per unit mass) is an indication of air uptake only and not necessarily a measure of foam quality or stability. The standard deviation of the specific volume determination was ± 0.28 ml/g.

Polyacrylamide gel electrophoresis was performed in an E-C vertical, water-cooled apparatus with 7.5% of Cyanogum-41 (American Cyanamid Co.) in Tris-Na_2 EDTA-borate buffer at pH 8.6. Staining was with Amido Black.

Meringues were prepared by adjusting 4% complex in water to pH 9.5 with $Ca(OH)_2$ and

Table 1—Whipping quality of complexes as affected by variations in formulations (whipping time 15 min)

Variable	Specific vol g/ml	Foam stability
Concentration of complex (w/v)		
a) 2.5% in water	3.9	Poor (open texture)
b) 3 % in water	4.5	Poor (open texture)
c) 4 % in water	5.1	Good
d) 5 % in water	6.4	Good (dry)
Dispersing medium–(4% complex)		
a) Water, direct addition	5.1	Good
b) Water, homogenized and aged 24 hr	2.4	Not developed
c) Skim milk, direct addition	5.5	Good
d) Skim milk, homogenized and aged 24 hr	4.6	Good
e) Whole milk, direct addition	2.7	Not developed
Heat treatment–(4% complex in water)		
a) 75°C/5 min	2.6	Not developed
b) 75°C/5 min with 0.02% H_2O_2	2.2	Not developed
c) 120°C/15 min	2.1	Not developed
Temperature of whipping–(4% complex in water)		
a) 6°C	5.1	Good
b) 25°C	5.5	Good
c) 30°C	4.9	Good
pH–(4% complex in water)		
a) 7.5, NaOH adjusted	4.3	Good
b) 7.5, $Ca(OH)_2$ adjusted	6.9	Good
c) 8.5, NaOH adjusted	4.9	Good
d) 8.5, $Ca(OH)_2$ adjusted	7.6	Good
e) 9.5, NaOH adjusted	6.4	Good
f) 9.5, $Ca(OH)_2$ adjusted	8.7	Good, very firm
Sucrose addition–(4% complex in water)		
a) 3%	5.4	Good
b) 5%	4.7	Good
c) 7.5%	4.9	Good
d) 10%	4.5	Poor (sloppy)
Protein addition–(4% complex in water)		
a) 3% sodium caseinate	5.0	Good
b) 3% sodium soyate	4.3	Poor (loose texture)
c) 3% gluten	4.6	Poor (loose texture)

whipping at maximum speed for 15 min. Sugar, equal to twice the weight of the water, was then mixed quickly into the foam with a minimum of agitation. The stiff foam was made into the usual meringue shapes and dried in an oven between 100 and 150°C for 3–4 hr. The unheated, sugar-stabilized foam was suitable for use as cake frosting.

Nonfat dessert topping was prepared by whipping 4% complex in skim milk for 15 min at maximum speed. Flavoring was added to the foam following completion of whipping.

RESULTS

INITIAL TRIALS revealed that spray-dried combinations of whey protein and CMC possessed whipping properties. By whipping dispersions of the powder in water or skim milk, a stiff foam was produced resembling beaten egg whites. The protein, rather than the hydrocolloid, apparently contributed to the whipping properties because no comparable foam was produced by the whipping of solutions containing only CMC.

Effect of variables in formulation

Table 1 summarizes the results obtained when the complex was used under varying conditions for whipping. The best foam was achieved by whipping the 4% complex in water for 15 min. At concentrations below 4%, the volume did not develop to the same extent and the stability was poor as indicated by a more rapid draining of liquid from the foam. At concentrations above 4%, the foam became dry and tended to clog the mixer blades.

Whipping of the complex in different systems revealed that the air uptake was faster in skim milk than in water. The foam did not tolerate fat and unsatisfactory results were obtained in whole milk. The foam did not improve by hydrating the complex in the dispersing media or by homogenizing the mixture; in fact, the results were inferior in the case of the water dispersion. This effect reflects probably an on-going change in the conformation of the protein/hydrocolloid combination after the dry product has been brought into contact with water. The eventual breakdown of the foam after a period of about 1 hr could be a consequence of such change.

Heat treatment of the resolubilized powder proved, under all conditions, detrimental to the whipping quality of the complex and decreased both air uptake and foam stability (Table 1). This effect was unexpected because in preliminary trials the product was shown to withstand a forewarming treatment prior to spray drying of 176°C/30 min without loss of whipping quality. Although heat denaturation of the protein may be contributing to the decrease in foaming ability, we suggest a more likely cause may be an accelerated change in the conformation or structural arrangements of hydrocolloid and protein in solution during heat treatment. This speculation would be consistent with the previous observation that hydration of the complex prior to whipping was undesirable.

The influence of temperature on whipping was determined by equilibrating water and mixing equipment and completing the whipping in temperature-controlled rooms. The temperature rise during the duration of whipping was approxiatemly 10°C. As indicated in Table 1, the best foam volume was attained at 25°C. Whipping at 6°C yielded results similar to our usual procedure of using 12–15°C tap water and whipping at room temperature (25°C).

The pH of the mixture affected the whipping performance of the complex to a considerable extent. With increasing pH up to pH 9.5, foam development increased rapidly. Alkalinities above pH 9.5 were not tested. Improvement in whipping performance was greatest when $Ca(OH)_2$ was used for pH adjustment, emphasizing the importance of divalent cations for the functional properties of hydrocolloids and proteins.

Attempts were made to improve the foam quality by incorporating sucrose or protein into the mixture. Some improvement in quality was achieved at 3% sucrose but not at higher concentration up to 10%. The adverse effect of large quantities of sucrose on foam development applied only to sucrose present in solution during whipping. Sucrose has a definite stabilizing effect on the foams when it is mixed into the foam immediately after whipping (Ziemba and Alikonis, 1971).

Of the proteins tested, only the combination with sodium caseinate yielded acceptable results. Separate trials revealed that 1:1 combinations of sodium caseinate with the complex produced the fastest air uptake over a wide total solids range of 1–10%.

The poor foam structure resulting from the incorporation of gluten could perhaps have been expected since it is known that this protein tends to absorb water from the foam cells (Kumetat, 1955).

Comparison with egg whites

Figure 1 shows that the rate of air uptake of the whipped complex was faster in skim milk than in water. The

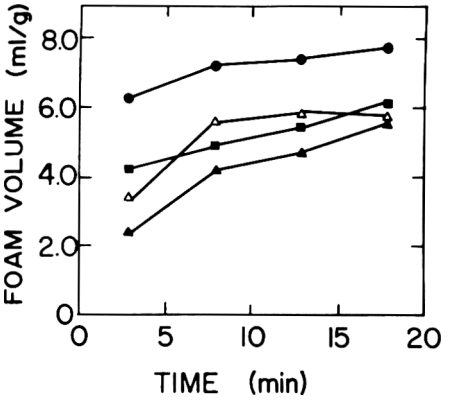

Fig. 1—Foam development of whipped egg white and whey protein/CMC complexes. ● — egg white; ■ — complex in skim milk (4%); ▲ — complex in water (4%); △ — blend (4:4:3) of complex, sodium caseinate and sucrose in water (4.8%). (The pH of egg white was 8.5, the pH of the solutions containing the complex was adjusted to 9.5 with $Ca(OH)_2$.)

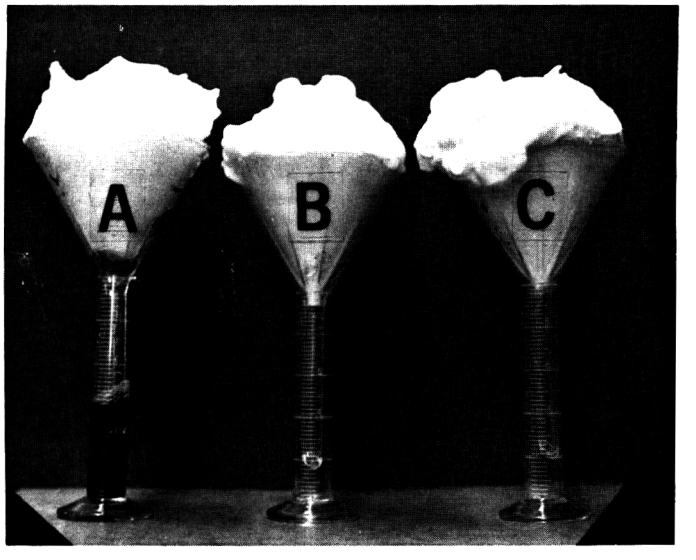

Fig. 2—Foam condition 30 min after whipping of (A) egg white; (B) protein/CMC complex, 4% in water with $Ca(OH)_2$ to pH 9.5; and (C) protein/CMC complex, 4% in water with $Ca(OH)_2$ to pH 9.5 and sugar (67%).

additional protein and carbohydrate in skim milk were most likely the reason for this effect and it was found through a series of trials that a suitable mixture (4:4:3) of the hydrocolloid/protein complex, sodium caseinate and sucrose produced an equally rapid foam development when whipped in water at a concentration of 4.8%. The maximum specific volume attained by the product under these conditions was approximately 80% in comparison with foamed egg whites. The foams produced by whipping the whey protein/CMC complexes resembled beaten egg whites in their physical appearance and velvety smooth texture. In similarity with egg white foams, these exhibited a gradual breakdown which commenced at the moment whipping was stopped and which was manifested by the continuous growth in size of the air bubbles. The foams could, however, be effectively stabilized by the addition of a large amount of sugar, i.e., up to twice the weight of water in the formulation, immediately following whipping. These high density sugar whips remained stable for days and were suitable either for use as cake frostings or for preparation of shaped meringue confections (Wiechers, 1952; Kumetat and Beeby, 1954). Without the addition of high concentrations of sugar, the foams retained their shape for approximately 1 hr and appeared suitable for use as dessert toppings.

Figure 2 shows the condition of these foams 30 min after completion of whipping. In the case of the egg white foam, a considerable amount of liquid had already drained into the cylinder while the foamed complex did not show any drain-off for several hours.

The whey protein/CMC complexes could not be used as a general replacement for egg whites in all products and were, for example, not suitable for the baking of angel food cakes. Kumetat and Beeby (1954) have emphasized that egg substitutes for baking must necessarily be stable against the addition of sugar or sugar and flour. The new product did not meet the latter requirement.

Effects of processing variables

Efforts were made to delineate some of the processing variables during the whey protein reclamation and subsequent spray drying influencing the whipping properties of the dry powder. The experiments involved powders prepared from the same batch of whey protein/CMC complex and processed in the prescribed manner but without addition of H_2O_2 to the whey. The acidic curd was solubilized in water at 6% total solids by adding NaOH to pH 6.5. This viscous mixture was then divided into five portions to which H_2O_2 was added at various concentrations. The preparations were stored at 5°C for 2–3 days until forewarmed to 65°C and spray dried.

The results in Figure 3 demonstrated a general improvement in the whipping properties of the complexes when H_2O_2 was added to the liquid concentrate prior to drying. The best results were achieved after the addition of approximately 0.1% H_2O_2. At the highest concentration (curve e, 0.2%), the foam development had again sharply deteriorated possibly because of the presence of a significant amount of active H_2O_2 in the powder. Tests revealed a positive reaction for H_2O_2 in the product, whereas all other powders were negative. The whipping performance of this powder could be restored by incorporating 0.05M cysteine–HCl in the mixture.

The treatment of the complex with H_2O_2 caused definite changes in the electrophoretic patterns of the proteins, particularly β-lactoglobulin. Figure 4 shows that the extent of alteration was proportional to the concentration of H_2O_2. Fish and Mickelsen (1967) have previously reported that modification occurs in several whey proteins upon exposure to H_2O_2. Therefore, it would appear that these changes play an important role in the whipping properties of complexed powders. Whereas H_2O_2 may be a convenient preservative for whey, the coincidental modification of the whey proteins is a side effect which may be useful in the ultimate application of the products.

The electrophoretic patterns of the

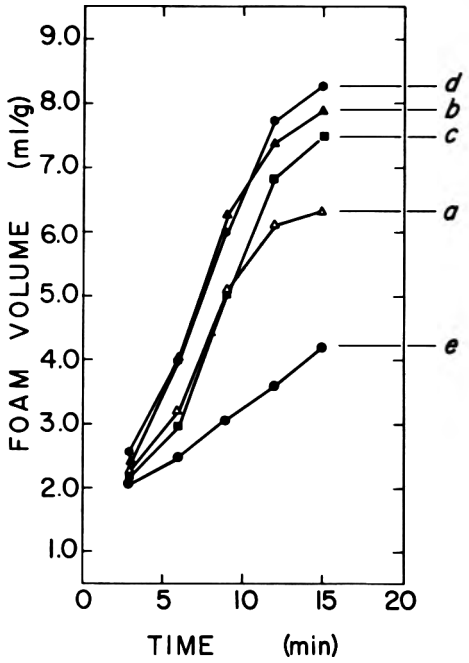

Fig. 3—Effect of H_2O_2 treatment. Curves represent whipping properties of spray-dried complexes from liquid concentrates treated with (a) 0%; (b) 0.025%; (c) 0.050%; (d) 0.10%; and (e) 0.20% H_2O_2. (4% complex in water, pH adjusted to 9.5 with $Ca(OH)_2$.)

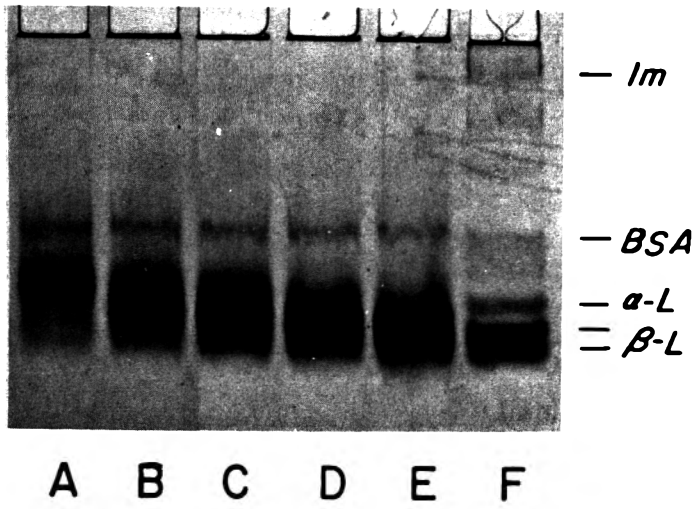

Fig. 4—Electrophoretic patterns showing the effect of H_2O_2 treatment of liquid mixture of whey protein/CMC complexes prior to spray drying. (A–E): Spray-dried complexes from concentrates treated with 0.20%, 0.10%, 0.05%, 0.025% and 0% H_2O_2; (F): Undenatured proteins in whey.

complexes did not show any zones corresponding to undenatured immuno-globulins. The destruction of these proteins may conceivably be an important factor for improving the whipping qualities of whey protein isolates as reported by Wiechers (1952).

Additional whipping trials were conducted on complexes which had been treated with the optimum concentration of 0.1% H_2O_2 to determine the effect of pH and alkali type on their whipping properties. The results in Figure 5 and Table 2 illustrate the definite trend towards larger foam volumes at high pH values and also the potent effect of $Ca(OH)_2$. The significant terms in Table 2 stress the importance of a cooperative effect on the foaming volume at high alkalinity and increased levels of calcium ions.

Table 2—Analysis of variance of specific foam volumes from Figure 4

Source of variance	d.f.	mean square	Level of significance
Total	47		
Whipping time (T)	3	29.007	
pH—effect (P)	2	6.831[b]	P < 0.01
Alkali type (A)	1	38.700[b]	P < 0.01
pH vs. time (P × T)	6	0.532[a]	P < 0.01
Alkali vs. time (A × T)	3	2.277[a]	P < 0.01
pH vs. alkali (P × A)	2	0.201[a]	
Interaction (P × A × T)	6	0.084[a]	
Error (duplicates)	24	0.079	

[a]Tested by error (duplicates)
[b]Tested by corresponding significant interaction term

DISCUSSION

THE PRINCIPLE of complex formation between anionic hydrycolloids and proteins has shown promise for the large scale recovery of proteins from cheese whey (Hansen et al., 1971). Reclamation of proteins by this process leads, however, to products in which the proteins are combined with hydrocolloid in a ratio of approximately 2:1. Although it may be possible to dissociate the complexes by changing either the pH, the ionic strength, or both, a separation of the protein from hydrocolloid is difficult and, at the moment, not practical. For these reasons, it has been important to determine if the combination of protein and hydrocolloids possesses functional properties which may be used to advantage in food manufacture.

The results of this study have shown that whey protein/CMC complexes resemble egg whites with respect to whipping properties. The specific volume and stability of the foams were comparable to egg white foams although the rate of air uptake was somewhat depressed.

Early studies in Australia (Kumetat, 1955, and Kumetat and Beeby, 1954) have demonstrated that milk proteins may be suitably modified to produce excellent substitutes for eggs. Tamsma et al. (1969) have shown that whipping properties can be induced in nonfat dried milk by homogenization. Furthermore, Wiechers (1952) has pointed out that there is much similarity in the functional properties of egg whites (ovalbumin) and whey proteins. He has reported on the use of whey proteins as substitutes for egg whites in preparing meringues and macaroons. We have found that similar products can be prepared successfully from whey protein/CMC complexes. To obtain egg substitutes from whey with the most desirable properties, Wiechers (1952) advocated a reduction in the lactose content and denaturation of the gamma globulin, a protein fraction which he reported had an adverse effect.

The whipping properties of the complex were critically dependent upon a number of variables not only in the formulation of the whipping mixture but also in the manufacturing process for the powder. All of these need to be considered to develop the potentials of the new product. The complexes did not whip well unless the whey proteins had been previously treated with H_2O_2. According to Fish and Mickelsen (1967), substantial changes occur in the whey protein system when milk is exposed to H_2O_2. This reagent alters the zonal electrophoretic patterns and reduces the apparent heat denaturation of the proteins as measured by the Harland-Ashworth method (1947). An improvement in the functional properties of milk proteins after peroxide treatment has been observed in other cases. Buchanan and Bready (1970) have reported that peroxide treatment of liquid skim milk coupled with heat treatment prior to drying can produce powder of excellent bread-baking quality.

The whipping properties of the complex improved considerably when the pH was raised to the alkaline range and the effect was most pronounced when $Ca(OH)_2$ was used for the adjustment. It is noteworthy that when the pH was raised to that of egg whites (pH 8.5–8.9), identical foam volumes were attained by the complex and by egg whites.

So far, the utilization of the complex for whipping purposes seems restricted to applications where the powder is incorporated into the given formula in the dry state. Attempts to prepare reconstituted, sterile liquid products failed in all cases presumably because of conformational changes which occurred in the protein or the CMC when the product was solubilized. We found that hydration of the complex generally had an adverse effect on whipping and the complex did not withstand pasteurization or sterilization.

The manufacture of protein/hydrocolloid complexes is not limited to the use of CMC since we have found (Hidalgo and Hansen, 1969) that other anionic food stabilizers interact in a similar manner. The functional properties of these other types of complexes will, to a large extent, depend upon the chemical characteristics of the hydrocolloid. Their potential uses are currently under investigation.

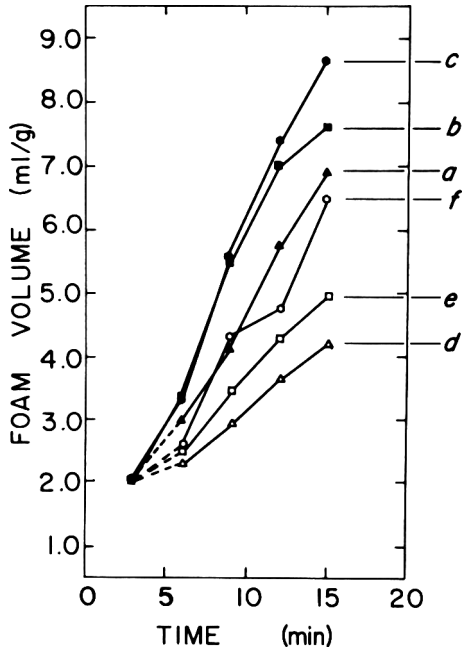

Fig. 5—Effect of pH and alkali type on whipping properties of spray-dried complexes treated with 0.1% H_2O_2. The curves represent complexes with the following adjustments: (a) pH 7.4 with $Ca(OH)_2$; (b) pH 8.5 with $Ca(OH)_2$; (c) pH 9.5 with $Ca(OH)_2$; (d) pH 7.4 with NaOH; (e) pH 8.5 with NaOH; and (f) pH 9.5 with NaOH.

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Ms received 12/17/71; accepted 2/14/72.

Approved as Journal Series Article No. 126-71 of the Ohio Agricultural Research and Development Center, Columbus, Ohio. Investigation supported in part by Public Health Service Research Grant No. FD-00117 from the Office of Research and Training Grants, FDA.

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VACUUM PACKAGING OF LAMB: EFFECTS OF STORAGE, STORAGE TIME AND STORAGE TEMPERATURE

INTRODUCTION

SINCE THE MAJOR areas of lamb consumption are geographically separated from areas of lamb slaughter, some means for reducing product deterioration during transit and handling must be developed. Hoecker (1962) recognized that the obstacles of short retail case-life and discoloration necessitated further research toward the implementation of centralized processing. Jaye et al. (1962) and Ball et al. (1957) reported that meat products stored in gas impermeable packages were more acceptable bacteriologically and organoleptically than those stored in oxygen permeable wraps. Beban et al. (1970) and Ordal (1962) reported that gas impermeable packages controlled the growth of psychrotrophic organisms associated with fresh meat spoilage. Warnecke et al. (1966) reported that vacuum packaging provided a selective media for bacterial growth and Beban et al. (1970) reported lower numbers of spoilage bacteria on vacuum-packed cuts. However, Reagan et al. (1971) reported that lamb loins stored under vacuum had higher psychrotrophic counts and that bacteria multiplied more rapidly on chops from loins which had previously been stored under vacuum.

Rikert et al. (1957) reported that the presence of vacuum in the package may be necessary to maintain normal meat color, while Beban et al. (1970) reported that normal muscle color could be maintained for as long as 30 days under vacuum. However, Fredholm (1963) reported that meats stored under vacuum for 14 days or longer at 0–1°C did not always regain a bright red color when exposed to air and that the surfaces of vacuum packaged cuts were often greyish-brown.

Numerous reports have indicated that meat cuts stored at lower temperatures exhibit longer retail case-life and lower bacterial counts (Birmingham et al., 1966; Butler et al., 1953; Gardner, 1965; Rey et al., 1970; Roth, 1967). Mallmann and Churchill (1946) reported that foods with relatively high microbial populations could be held for a considerable period of time, without loss of quality, if held at relatively low temperatures. A number of workers have isolated bacteria which grow below 0°C (Ayres, 1960; Ingram, 1951; and Straka and Stokes, 1960). However, Berry and Morgan (1934) re-

Table 1—Experimental design for comparisons of the effects of vacuum packaging, storage time and storage temperature on the acceptability of lamb cuts

Exp. no.	Cut	Pairs of samples	Treatment comparison	Storage temperature (°C)	Storage intervals (days)	Display intervals (days)	Evaluations during or after retail display
1	Sirloins	13	Vacuum packaged storage vs. fresh	0	14	4	Color daily and odor after 4 days of retail display
1	Racks	28	Vacuum packaged storage vs. fresh	0	14	4	Color daily and odor after 4 days of retail display
2	Legs	35	Vacuum packaged storage vs. fresh	0	11	11	Color at 2 day intervals during retail display
3	Legs	25	Vacuum packaged storage vs. fresh	0	14	2	Color, odor and weight loss after 2 days of retail display
4	Loins	148	Length of storage and storage temperature	0, 7	8, 12, 16, 24, 32, 40	9	Psychrotrophic plate counts initially and following retail display Weight loss following 5 and 9 days of retail display Color, odor and palatability following retail display Weight loss during thawing and cooking
5	Legs	120	Length of storage	0	7, 14, 21, 28, 35, 42	2	Weight loss during storage and display Color, odor and palatability following retail display
6	Shoulders	12	Length of storage	0	6, 12	2	Weight loss during storage and display Color and odor following retail display
7	Sirloins	13	Storage temperature	0, −18	14, 72	4	Color daily during retail display Odor following retail display

Table 2—Comparison of color and odor scores for fresh chops and chops from sirloins and racks stored 14 days in vacuum packages (Exp. 1)

Cut	Storage condition[f]	Color score[a] Day of retail display[c] 1	2	3	4	Odor score[b] 4
Sirloin	Fresh	4.1[d]	6.3[d]	7.3[d]	7.3[d]	1.5[d]
	Stored	4.4[d]	6.1[d]	7.1[d]	8.0[e]	2.9[e]
Rib	Fresh	6.0[d]	7.2[d]	7.4[d]	7.4[d]	1.1[d]
	Stored	5.8[d]	7.5[d]	7.1[d]	7.3[d]	3.0[e]

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[c]Means with a common underline in the same horizontal row do not differ significantly ($P > 0.05$).
[d,e]Means in the same vertical column within cuts bearing different superscripts differ significantly ($P < 0.05$).
[f]Interactions between storage conditions and length of retail display period (days) were not significant ($P > 0.05$).

Table 4—Comparison of color scores, odor scores and weight losses for fresh leg roasts and leg roasts which were vacuum packaged and stored for 14 days prior to retail display (Exp. 3)

Treatment	Color score[a]	Odor score[b]	Weight loss during storage and display (%)
Fresh	3.3[c]	1.1[c]	0.2[c]
Stored	5.0[d]	2.5[d]	0.6[d]

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[c,d]Means in the same vertical column bearing different superscripts differ significantly ($P < 0.05$).

ported that the minimum temperature for psychrotrophic growth was approximately $-10°C$.

The present study was designed to determine the feasibility of using the vacuum packaging concept as a means for extending the storage life of lamb cuts and to determine the effects of vacuum packaged storage, storage time and storage temperature on the subsequent retail case-life and palatability of lamb cuts.

MATERIALS & METHODS

A TOTAL OF 202 lamb cuts including 13 pairs of sirloins, 28 pairs of racks and 60 pairs of leg roasts were utilized to determine the effects of vacuum packaged storage on the subsequent retail case-life and palatability of the retail cuts. An additional 148 pairs of loins, 120 pairs of leg roasts, 12 pairs of shoulders and 13 pairs of sirloins were utilized to determine the effects of storage time and temperature on the subsequent retail case-life and palatability of vacuum packaged lamb cuts.

The lamb carcasses were fabricated into retail or wholesale cuts on the 8th day postmortem. Retail cuts, designated for fresh comparisons, from one side of the carcass were wrapped in oxygen permeable film and immediately displayed under retail conditions. Wholesale cuts from the other side of the carcass were vacuum packaged and stored for various time intervals, according to the experimental design in Table 1, and subsequently fabricated into retail cuts.

The retail cuts were displayed in a self-service retail display case maintained at 0°C, under 82 footcandles of incandescent light. Color and odor were evaluated using a 9-point

Table 3—Comparison of color scores for fresh leg roasts and leg roasts which were vacuum packaged and stored for 11 days prior to retail display (Exp. 2)

Storage condition[e]	Color score[a] Day of retail display[b] 1	3	5	7	9	11
Fresh	2.6[c]	2.8[c]	3.3[c]	4.1[c]	5.3[c]	6.4[c]
Stored	4.9[d]	5.5[d]	6.6[d]	7.3[d]	7.6[d]	9.0[d]

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means with a common underline in the same horizontal row do not differ significantly ($P > 0.05$).
[c,d]Means in the same vertical column bearing different superscripts differ significantly ($P < 0.05$).
[e]Interactions between storage conditions and length of retail display period (days) were not significant ($P > 0.05$).

Table 5—Comparison of color and odor scores during subsequent retail display for chops from fresh loins and loins stored for varying periods under vacuum (Exp. 4)

Period of storage[h] (days)	Day of retail display[a] 1	3	5	7	9
	Color score[b]				
0 (Fresh)	4.5[de]	6.0[d]	6.0[d]	7.0[d]	7.9[de]
8	3.6[d]	7.6[e]	7.6[e]	7.6[de]	7.4[d]
16	5.8[fg]	7.9[e]	7.7[e]	7.9[de]	8.3[e]
24	6.6[g]	7.5[e]	7.5[e]	7.7[de]	8.1[e]
32	5.4[ef]	7.7[e]	7.6[e]	7.6[de]	8.4[e]
40	4.5[de]	6.8[de]	7.7[e]	8.1[e]	7.9[de]
	Odor score[c]				
0 (Fresh)	1.0[d]	1.2[d]	1.0[d]	2.4[d]	2.5[d]
8	1.7[e]	2.9[g]	2.5[e]	2.8[de]	3.0[e]
16	2.0[f]	2.5[ef]	3.0[g]	3.0[e]	3.0[e]
24	1.9[ef]	2.8[fg]	2.9[fg]	3.0[e]	3.0[e]
32	2.6[g]	2.8[fg]	3.0[g]	3.0[e]	3.0[e]
40	2.9[g]	2.6[efg]	2.6[ef]	3.0[e]	3.0[e]

[a]Means with a common underline in the same horizontal row do not differ significantly ($P > 0.05$).
[b]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[c]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[d,e,f,g]Means with different superscripts in the same vertical column differ significantly ($P < 0.05$).
[h]For each storage period one half of the loins were stored at 0°C and the other half at 7°C. Interactions between length of storage period (days), storage temperature and length of retail display period (days) were not significant ($P > 0.05$).

scale where 1 = very bright color and 9 = greyish or greenish color and a 3-point scale where 1 = no detectable off-odor and 3 = definite objectionable odor. Weight losses were determined to the nearest gram during the various storage and display periods. Psychrotrophic plate counts were determined by swabbing one cm^2 of the surface of the longissimus muscle, plating on standard methods agar and incubating plates at 3°C for 7 days (Reagan et al., 1971).

Samples for palatability determinations were frozen upon removal from retail display and stored at −18°C for periods not exceeding 30 days. Samples were cooked to an internal temperature of 75°C in a 177°C oven and evaluated by a trained three-member taste panel using a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely). Thawing and cooking losses were determined by weighing the frozen, thawed and cooked cuts to the nearest gram.

Data were analyzed using analysis of variance (Snedecor and Cochran, 1967) and the mean separation technique of Duncan (1955).

RESULTS & DISCUSSION

THE PATTERN of color deterioration for chops from fresh and vacuum packaged sirloins and racks is presented in Table 2. Significant color deterioration ($P < 0.05$) was noted between the first and second and second and third days of retail display for chops from fresh and vacuum packaged sirloins and between the first and second days for chops from fresh and stored racks. Thus, chops from vacuum packaged (stored) cuts did not generally deteriorate in color at a faster rate than their fresh counterparts. Color scores for sirloin and rib chops from fresh vs. vacuum packaged cuts did not differ on the first day of retail display. This suggests that normal color was maintained during the 14 day storage period under vacuum. The latter finding is in agreement with Rikert et al. (1957) and Beban et al. (1970). Sirloin and rib chops from vacuum packaged cuts had significantly ($P < 0.05$) less desirable odor than their fresh counterparts. If a mean odor score of 2.5 is assumed to be the point of undesirability (Jeremiah et al., 1971), chops from both sirloins and racks which had been stored under vacuum were unacceptable at the end of the 4-day retail display period. However, these data do not identify the degree of odor acceptability at 1, 2 or 3 days of retail display.

Fresh and vacuum packaged leg roasts (Table 3) displayed similar patterns of color deterioration during retail display; but the color of fresh leg roasts was significantly more desirable ($P < 0.05$) than that of their stored counterparts at every interval of display. In contrast to the data for sirloins and racks, leg roasts sustained significant color deterioration during refrigerated storage under vacuum. A subsequent comparison (Table 4) revealed that fresh leg roasts had significant advantages ($P < 0.05$) over their stored counterparts in desirability of color and odor and in reduced shrinkage during storage and display.

Data in Table 5 compare color and odor scores for chops from fresh loins and loins which were vacuum packaged and stored at 0°C or 7°C for various lengths of time. One-half of the loins were stored at 0°C and the other half at 7°C in each storage period. After three days of retail display all of the chops from loins which had been vacuum packaged and stored were unsaleable due to discoloration (based on mean color scores of 6.5 or greater, Jeremiah et al., 1971), while fresh chops did not become unsaleable until the 7th day of retail display. With the exception of chops stored for 24 days, chops from both fresh and stored loins sustained significant discoloration

Table 6—Expected retail case-life of fresh chops and chops from loins stored for varying periods under vacuum (Exp. 4)

Period of storage (days)	Expected retail case-life (days)		
	Color[a]	Odor[b]	Microbial count[c]
0 (Fresh)	3.5	8.4	3.6
8	3.3	6.5	3.1
12	1.9	4.5	2.3
16	1.8	6.3	2.6
24	2.4	4.8	2.1
32	5.2	6.5	3.3
40	3.0	4.3	1.4

[a]Loin chops with color scores of 6.5 or higher were considered unsaleable.
[b]Loin chops with odor scores of 2.5 or higher were considered unsaleable.
[c]Loin chops with $\log_{10}$ microbial counts greater than 4.6 were considered unacceptable.

Table 7—Comparison of palatability ratings and weight losses for chops from fresh loins and loins stored in vacuum packages for varying periods (Exp. 4)

Day of retail display	Period of storage (days) 0 (Fresh)	8	16	24	32	40
			Weight loss during display (%)			
5	4.9[b]	3.1[b]	3.3[b]	7.3[b]	4.4[b]	3.5[b]
9	6.1[b]	4.6[b]	4.3[b]	5.7[b]	5.8[b]	4.6[b]
			Weight loss during thawing and cooking (%)			
1	20.1[d]	19.9[d]	18.6[c]	22.9[e]	22.1[e]	15.5[b]
3	18.8[cd]	19.5[d]	18.2[c]	19.6[d]	23.3[e]	15.4[b]
5	18.2[c]	19.8[d]	17.0[b]	18.0[bc]	18.1[c]	18.4[c]
7	19.0[cd]	16.9[b]	19.3[d]	16.8[b]	18.2[c]	18.8[cd]
9	20.8[d]	17.6[c]	18.3[c]	15.2[b]	17.8[c]	16.0[b]
			Flavor rating[a]			
1	5.9[b]	5.8[b]	5.9[b]	4.3[c]	3.5[d]	4.1[cd]
3	6.1[b]	5.5[c]	4.6[d]	3.8[de]	3.2[e]	3.7[e]
5	5.8[b]	5.1[c]	3.9[d]	3.3[de]	2.6[e]	3.2[de]
7	5.6[b]	4.3[c]	2.8[d]	2.2[de]	1.6[e]	2.4[de]
9	5.2[b]	3.6[c]	2.4[c]	1.6[c]	2.1[c]	1.9[c]
			Juiciness rating[a]			
1	6.3[bc]	6.5[b]	6.4[b]	6.4[bc]	6.0[c]	6.1[bc]
3	6.5[b]	6.5[b]	6.1[b]	6.2[b]	6.2[b]	6.4[b]
5	6.3[b]	5.8[c]	6.4[b]	6.2[b]	6.0[bc]	6.2[b]
7	6.4[b]	6.1[bc]	6.1[bc]	5.9[c]	6.1[bc]	5.7[c]
9	6.3[b]	5.8[c]	6.0[bc]	6.1[bc]	6.0[bc]	6.2[bc]
			Tenderness rating[a]			
1	6.3[b]	7.0[c]	6.5[bc]	6.7[bc]	6.8[bc]	7.1[c]
3	6.4[b]	6.5[b]	6.5[b]	6.6[b]	6.8[b]	7.0[b]
5	6.5[bc]	5.8[c]	6.3[bc]	7.0[b]	6.6[bc]	6.7[b]
7	6.4[b]	6.4[b]	6.3[b]	6.5[b]	6.8[b]	6.5[b]
9	6.6[bcd]	6.1[d]	6.4[cd]	6.7[bc]	6.6[bcd]	7.2[b]
			Overall satisfaction rating[a]			
1	5.9[b]	6.1[b]	5.9[b]	4.5[c]	3.4[d]	4.7[c]
3	5.9[b]	5.7[b]	4.7[c]	4.0[cd]	3.3[d]	4.2[c]
5	5.8[b]	4.9[c]	4.0[cd]	3.6[de]	2.8[e]	3.5[de]
7	5.7[b]	4.3[c]	2.9[d]	2.2[de]	1.7[e]	2.5[d]
9	5.3[b]	3.7[c]	2.4[d]	1.7[d]	2.2[d]	2.4[d]

[a]Means based on a 9-point scale (9 = like extremely; 1 = dislike extremely)
[b,c,d,e]Means in the same horizontal row bearing different superscripts differ significantly ($P < 0.05$).

Table 8–Comparison of palatability ratings, color and odor scores and weight losses during storage and display for fresh leg roasts and leg roasts stored in vacuum packages for varying periods (Exp. 5)

Characteristic	Period of storage (days)						
	0 (Fresh)	7	14	21	28	35	42
Weight loss during storage and display (%)	0.3^{a}	0.3^{a}	0.5^{b}	0.5^{b}	0.7^{b}	0.6^{b}	1.2^{c}
Odor score following storage[e]	–	1.5^{a}	1.4^{a}	2.5^{b}	2.8^{c}	2.2^{b}	2.3^{b}
Odor score following display[e]	1.1^{a}	1.4^{a}	1.2^{a}	2.0^{c}	2.0^{c}	1.7^{b}	1.6^{b}
Color score following display[f]	3.5^{a}	3.3^{a}	3.8^{ab}	4.5^{b}	5.2^{c}	5.8^{c}	4.3^{b}
Flavor rating[g]	5.8^{ab}	6.0^{a}	5.5^{c}	5.6^{bc}	5.4^{c}	5.0^{d}	5.6^{bc}
Juiciness rating[g]	6.1^{ab}	6.3^{a}	5.8^{d}	6.1^{ab}	5.9^{bcd}	5.9^{bcd}	6.1^{abc}
Tenderness rating[g]	5.9^{bc}	6.4^{a}	5.8^{bc}	6.2^{ab}	6.0^{bc}	5.7^{b}	6.1^{a}
Overall satisfaction rating[g]	5.7^{ab}	6.0^{a}	5.4^{b}	5.5^{b}	5.4^{b}	4.9^{c}	5.6^{b}
Shear force value (kg) Semimembranosus	4.0^{a}	3.8^{a}	3.5^{b}	3.5^{b}	3.4^{b}	4.1^{a}	–
Shear force value (kg) Biceps femoris	3.6^{a}	3.0^{c}	3.2^{b}	2.9^{d}	2.9^{d}	3.2^{b}	3.7^{a}

[a,b,c,d]Means in the same horizontal row bearing different superscripts differ significantly ($P < 0.05$).
[e]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[f]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[g]Means based on a 9-point scale (9 = like extremely; 1 = dislike extremely)

between the first and third days of retail display. After the third day of retail display the rate of discoloration was much slower. However, after the third day of display the majority of the chops from vacuum packaged loins were already of questionable acceptability due to discoloration.

Odor scores for fresh chops and chops from loins stored under vacuum (Table 5) indicate that odor desirability was significantly reduced by vacuum packaging for each period of storage. Chops from loins which had been stored under vacuum for 32 and 40 days were unacceptable from the odor standpoint (based on mean odor scores of 2.5 or greater, Jeremiah et al., 1971) after one day of retail display. All of the chops from stored loins were unacceptable in odor after three days of retail display; whereas, fresh chops did not become unacceptable in odor until the ninth day of retail display.

Previous results (Jeremiah et al., 1971) have suggested that a $\log_{10}$ psychrotrophic count of 4.6 is a useful index of incipient microbial spoilage in lamb cuts. Log_{10} psychrotrophic counts greater than 4.6, mean color scores of 6.5 or higher and mean odor scores of 2.5 or higher were used to indicate unacceptability in retail cuts. Table 6 shows the expected retail case-life of fresh chops and chops from loins which had been stored under vacuum for various time periods. Storage in vacuum packages for any period of time shortened the effective retail case-life of loin chops, especially from the standpoint of odor. The relatively long retail case-life expectancy for chops from loins stored for 32 days does not fit into the logical trend, and may have resulted from sampling errors or larger proportions of the initial microflora being mesotrophs, rather than typical spoilage-type psychrotrophs. The color stability of these chops was previously noted in Table 5. Based on the microbial data, 8 days may be the maximum period for storage of lamb loins which are vacuum packaged on the eighth day postmortem (in agreement with Reagan et al., 1971).

Mean palatability ratings and weight losses for chops from fresh loins and loins stored in vacuum packages are presented in Table 7. Weight losses during retail display did not differ significantly ($P > 0.05$) between storage periods after five and nine days of retail display. Since chops from fresh and stored loins sustained similar weight losses during retail display, there is apparently no compensatory loss in weight upon removal from vacuum packages. Weight losses during thawing and cooking were not linearly related to increases in storage period or time in the retail case. On the first day of retail display fresh chops were significantly ($P < 0.05$) more desirable in flavor than chops from loins which had been stored under vacuum for periods of 24 days or more. Chops from loins which had been stored for eight days or more under vacuum were significantly less desirable ($P < 0.05$) in flavor, if they had been displayed for three days or more under retail conditions.

Differences in juiciness ratings were

Table 9–Comparison of color scores, odor scores and weight losses for shoulder roasts stored under vacuum or unprotected for six or twelve days (Exp. 6)

Trait	Period of storage (days)			
	Unprotected		Vacuum packaged	
	6	12	6	12
Color score[a]	4.2^{c}	3.8^{c}	4.2^{c}	8.3^{d}
Odor score[b]	1.0^{c}	1.3^{c}	1.0^{c}	2.8^{d}
Weight loss during storage, from facial trimming, and during retail display (%)	8.5^{c}	15.2^{d}	1.3^{e}	1.9^{e}

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[c,d,e]Means in the same horizontal row bearing different superscripts differ significantly ($P < 0.05$).

Table 10–Comparison of psychrotrophic counts, color scores and odor scores for loin chops following storage at different temperatures in vacuum packages (Exp. 4)

Storage temperature (°C)	Day of retail display							
	0	1	2	3	4	5	7	9
	Psychrotrophic count ($\log_{10}$)							
0	4.5^{a}	4.2^{a}	5.1^{a}	5.7^{a}	6.3^{a}	7.4^{a}	–	8.3^{a}
7	5.2^{b}	4.6^{b}	5.3^{a}	6.1^{b}	6.5^{a}	7.4^{a}	–	7.5^{b}
	Color score[c]							
0	–	4.8^{a}	6.1^{a}	7.2^{a}	7.4^{a}	7.5^{a}	7.8^{a}	8.2^{a}
7	–	5.2^{a}	7.3^{b}	7.3^{a}	7.9^{a}	7.7^{a}	7.7^{a}	7.9^{b}
	Odor score[d]							
0	–	1.9^{a}	2.2^{a}	2.6^{a}	2.8^{a}	2.9^{a}	2.9^{a}	3.0^{a}
7	–	2.2^{b}	2.5^{b}	2.7^{a}	3.0^{a}	2.7^{b}	3.0^{a}	3.0^{a}

[a,b]Means in the same vertical column within the same comparison bearing different superscripts differ significantly ($P < 0.05$).
[c]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[d]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)

not associated with differences in vacuum packaging, length of storage under vacuum or length of display in the retail case. The higher numerical tenderness ratings, for chops from loins which had been stored under vacuum, suggest that some autolysis of muscle proteins occurs during storage under vacuum packaged conditions. However, since the tenderness ratings generally did not differ significantly between fresh chops and chops from vacuum packaged loins, it is doubtful that the tenderness differential is great enough to warrant vacuum packaging for that purpose alone. Overall satisfaction ratings closely paralleled ratings for flavor and generally decreased as time in vacuum packaged storage increased. Chops from loins stored 24 days or longer in vacuum packages were significantly less desirable in palatability than fresh chops after 1 day of retail display. By the fifth day in the retail case chops from loins stored 8, 16, 24, 32 or 40 days under vacuum were less desirable ($P < 0.05$) in palatability than fresh chops. The data in Table 8 reveal that fresh leg roasts and those stored for seven days under vacuum had significantly more desirable ($P < 0.05$) color scores following display than those from roasts stored for periods of 21 days or longer. Undesirable odors were significantly ($P < 0.05$) more prevalent in leg roasts stored for 21 days or longer when compared to leg roasts stored for 14 days or less. Fresh leg roasts and those which were stored for seven days in vacuum packages lost significantly ($P < 0.05$) less weight during storage and display than leg roasts stored for 14 days or longer. Leg roasts stored seven days had significantly ($P < 0.05$) more desirable flavor and overall satisfaction ratings than those stored for longer periods in vacuum packages (Table 8). However, flavor ratings for roasts stored 21 or 42 days did not differ significantly ($P > 0.05$) from those for fresh roasts. Increased periods of storage in vacuum packages were not significantly related to either tenderness or juiciness ratings, but roasts stored seven days under vacuum were rated significantly higher ($P < 0.05$) in tenderness than fresh leg roasts. Shear force values for the biceps femoris indicated that storage for 7 to 35 days under vacuum significantly ($P < 0.05$) enhanced tenderness.

Data in Table 9 compare shoulder roasts stored 6 or 12 days in vacuum packages with those which were unprotected during storage. These data indicate that storage in vacuum packages for periods as long as 6 days did not result in significant decreases in either color or odor scores. However, roasts stored for 12 days under vacuum were significantly ($P < 0.05$) less desirable in both color and odor than those stored without protection. Significantly larger ($P < 0.05$) weight losses were sustained by shoulder roasts stored without protection as compared to those stored in vacuum packages, primarily as a result of the losses associated with the facial trimming necessary to regain surfaces with acceptable lean color.

The data in Table 10 indicate that the storage of vacuum packaged loins at 0°C vs. 7°C did not significantly affect the subsequent color of loin chops and that chops from loins stored at 7°C did not discolor more rapidly than those stored at 0°C. The latter finding disagrees with the reports of Allen and Foster (1960), Birmingham et al. (1966) and Butler et al. (1953). Storage of loins at 7°C did result in higher psychrotrophic counts and less desirable odor scores ($P < 0.05$) for loin chops until the second and third days of retail display, respectively. The fact that storage at 0°C resulted in lower psychrotrophic counts is in agreement with Reagan et al. (1971). Data not presented in tabular form indicated that vacuum packaged storage at 0°C vs. 7°C had no significant ($P > 0.05$) effect on subsequent weight loss during display.

A comparison of mean color and odor scores for chops from sirloins stored at different temperatures under vacuum is presented in Table 11. No significant advantage ($P > 0.05$) in color was observed for storage at −18°C as compared to storage at 0°C during the first three days of display. However, chops stored at the lower temperature display significant advantages ($P < 0.05$) in color and odor after four days of retail display.

A comparison of weight losses and palatability ratings for chops from vacuum packaged loins stored at different temperatures is presented in Table 12. Chops from loins stored at 0°C displayed significant advantages in flavor and overall satisfaction during the first seven days of retail display, compared to their counterparts stored at 7°C. Higher storage temperatures had no significant effect on tenderness; thus accelerated rates of aging at elevated temperatures were not evident

Table 11—Comparison of color and odor scores for chops from vacuum packaged sirloins following storage at different temperatures (Exp. 7)

Storage temperature (°C)	Color score[c] Day of retail display 1	2	3	4	Odor score[d] 4
0	4.5[a]	5.9[a]	7.6[a]	8.6[a]	2.9[b]
−18	4.9[a]	6.1[a]	7.1[a]	7.6[b]	2.0[a]

[a,b]Means in the same vertical column bearing different superscripts differ significantly ($P < 0.05$).
[c]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[d]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)

Table 12—Comparison of weight losses and palatability ratings for chops from vacuum packaged loins following storage at different temperatures (Exp. 4)

Trait	Storage temperature (°C)	Day of retail display 1	3	5	7	9
Flavor rating[c]	0	5.2[a]	4.6[a]	4.1[a]	3.0[a]	2.2[a]
	7	4.4[b]	3.7[b]	3.1[b]	2.3[b]	2.5[a]
Tenderness rating[c]	0	6.8[a]	6.6[a]	6.4[a]	6.5[a]	6.6[a]
	7	6.7[a]	6.7[a]	6.5[a]	6.5[a]	6.6[a]
Juiciness rating[c]	0	6.3[a]	6.3[a]	6.2[a]	5.9[a]	6.0[a]
	7	6.2[a]	6.3[a]	6.1[a]	6.0[a]	6.1[a]
Overall satisfaction rating[c]	0	5.5[a]	4.8[a]	4.2[a]	3.1[a]	2.3[a]
	7	4.6[b]	3.9[b]	3.3[b]	2.3[b]	2.6[a]
Weight loss during thawing and cooking (%)	0	20.2[a]	17.5[a]	19.8[a]	18.9[a]	17.9[a]
	7	18.8[a]	21.0[b]	16.7[b]	17.0[a]	16.0[a]

[a,b]Means in the same vertical column within the same comparison bearing different superscripts differ significantly ($P < 0.05$).
[c]Means based on a 9-point scale (9 = like extremely; 1 = dislike extremely)

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Ms received 7/2/71; revised 12/29/71; accepted 1/24/72.

T.A. 9273, Texas Agricultural Experiment Station.

This study was partially supported by the American Sheep Producer's Council, Inc., Denver.

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VACUUM PACKAGING OF LAMB: EFFECT OF CERTAIN FACTORS ON RETAIL CASE-LIFE AND PALATABILITY

INTRODUCTION

CENTRALIZED processing of carcasses offers various economic advantages (Long, 1969; Mottin, 1962; Volz and Marsden, 1963; Hoecker, 1962). Boning and trimming prior to vacuum packaging would allow case-ready cuts to be prepared at a central location with less labor and overhead costs. Haines (1933) reported that fat offers an inferior growth media for normal spoilage type bacteria. Jeremiah et al. (1971) found that the retail case-life of lamb cuts was primarily reduced by discoloration but also by microbial proteolysis and lipolysis. Gould (1963) reported that pretrimming significantly reduced microbial contamination on the cut surfaces of meat. However, since Horwood and Minch (1951) found large numbers of bacteria on food handlers hands, boning and trimming operations may actually contaminate cuts.

Various researchers have observed that meat discolors more rapidly when stored or displayed in lighted areas (Lane and Bratzler, 1962; Marriott, 1965; Roth, 1967; Townsend and Bratzler, 1958). Other workers have found that lighting has little influence on the acceptability of meat (Clauss et al., 1957; Gould, 1963; Rikert et al., 1957a; Voegeli et al., 1953). Goeser (1962) and Gould (1963) reported that the incident heat from display lighting increased the surface temperature of meat. Controlled sanitation is the primary means for extending the case-life of the fresh product (Briskey et al., 1960; Butler et al., 1953; Volz and Marsden, 1963). However, Roth (1967) found that temperature and lighting conditions during display affected case-life more than sanitation conditions during cutting and packaging.

Various workers have reported that gas-impermeable films maintain meat cuts in an acceptable condition for longer periods than permeable films (Ball et al., 1957; Jaye et al., 1962; Kraft and Ayres, 1952). Rikert et al. (1957b) reported that a vacuum was necessary for meats to retain their desirability in a gas-impermeable film; but Fredholm (1963) found vacuum packaged cuts to be less desirable than unpackaged cuts. Ingram (1962) reported that the effect of a packaging material on microorganisms was dependent on its permeability to oxygen, carbon dioxide and water. Various workers have reported lower microbial growth in gas-impermeable materials (Halleck et al., 1958; Ordal, 1962; Warnecke et al., 1966).

Reagan et al. (1971) and Jeremiah et al. (1972) have questioned the feasibility of vacuum packaging cuts for subsequent refrigerated storage. They found that, in general, vacuum packaging is associated with reduced desirability of odor and flavor and increased psychrotrophic counts. The present study was initiated to determine the effects of boning and trimming, subcutaneous fat thickness, lighting, packaging materials and sanitation level on the acceptability of vacuum

Table 1—Experimental design for comparison of the effects of various processing and storage conditions on the acceptability of lamb cuts

Expt. no.	Cut	Number of samples	Treatment comparison	Storage temp (°C)	Storage interval (days)	Display interval (days)	Evaluations during or after retail display
1	Loins	148	Subcutaneous fat thickness levels	0, 7	8, 12, 16, 24, 32, 40	9	Psychrotrophic plate counts initially and following retail display. Color, odor and palatability following retail display
2	Shoulders	42	Boning prior to vacuum packaged storage	0, −18	14, 72	2	Color and odor following retail display
3	Legs	34	Boning prior to vacuum packaged storage	0	14	2	Color and odor following retail display
4	Racks	140	Trimming prior to vacuum packaged storage and the presence of lighting during storage	0	14	4	Color daily and odor following retail display
5	Legs	120	The presence of lighting during storage	0	14	2	Color, odor and palatability following retail display.
6	Legs	70	Oxygen permeable wrap vs. vacuum packaging and fresh vs. stored	0	0, 11	11	Color at 1 or 2 day intervals, and odor after 11 and 22 days
7	Racks	56	Sanitation levels and fresh vs. stored.	0	14	4	Color daily and odor following retail display
8	Legs	50	Chilling intervals postmortem	0	14	2	Color daily and odor following retail display
9	Racks	140	Chilling intervals postmortem	0	14	4	Color daily and odor following retail display

packaged lamb cuts and to identify the optimum time postmortem to vacuum package lamb cuts for subsequent refrigerated storage.

EXPERIMENTAL

A TOTAL OF 610 wholesale lamb cuts were assigned to treatment comparisons according to the experimental design outlined in Table 1 and were utilized to evaluate the effects of various processing and storage conditions on the subsequent acceptability of retail cuts, fabricated from vacuum packaged lamb cuts.

The carcasses in experiments 1 through 7 were fabricated into wholesale or retail cuts and stored or displayed on the 8th day postmortem. The carcasses used to determine the optimum time postmortem for vacuum packaging (experiments 8 and 9) were fabricated and stored at daily intervals postmortem. Loins from 100 Choice carcasses and 48 Good carcasses were assigned to groups based on five levels of subcutaneous fat thickness within the Choice grade (2.0 mm or less, 2.1–4.0 mm, 4.1–6.0 mm, 6.1–8.0 mm and 8.1 mm or more) and two fat thickness levels among Good grade carcasses (2.0 mm or less and 2.1–4.0 mm).

Retail cuts were displayed in a standard display case or on stainless steel tables at 0°C under 82 footcandles of incandescent light. Normal sanitation levels consisted of thoroughly washing, scrubbing and rinsing tables and equipment with pressurized, hot (82°C) water. Improved sanitation consisted of thoroughly washing the tables, equipment and cutting tools (knives, saws, etc.) with a solution of Septisol and rinsing with a bactericidal solution (Vestal 1-Stroke Environ). Bactericidal solution was provided during the fabrication procedure for continuous cleansing of workmen's hands and equipment.

Color and odor were evaluated using 9-point (9 = greyish or greenish; 1 = very bright) and 3-point scales (3 = definite objectionable odor; 1 = no detectable off-odor), respectively. Palatability traits were evaluated by a trained three-member panel using a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely), after the samples were oven broiled in a 177°C electric oven to an internal temperature of 75°C.

Data were analyzed using paired comparisons and analysis of variance (Snedecor and Cochran, 1967). Mean separation was performed using the procedure outlined by Duncan (1955).

Table 2—Percentages of unacceptable loin chops from vacuum packaged loins (Exp. 1)

		USDA quality grade and subcutaneous fat thickness (mm)						
		Choice					Good	
Basis of unacceptability	Day of retail display	2.0 or less	2.1 to 4.0	4.1 to 6.0	6.1 to 8.0	8.1 or more	2.0 or less	2.1 to 4.0
Microbial[a]	0	13.6	0.0	9.1	0.0	4.5	6.2	4.5
	1	30.0	20.0	30.0	30.0	20.0	40.0	40.0
	2	44.4	66.7	50.0	50.0	41.7	66.7	41.7
	3	58.3	75.0	75.0	66.7	83.3	66.7	83.3
Color[b]	1	27.3	18.2	9.1	27.3	18.2	25.0	22.7
	2	50.0	75.0	50.0	66.7	66.7	33.3	50.0
	3	72.7	81.8	63.6	72.7	81.8	81.2	63.6
Odor[c]	1	22.7	13.6	22.7	18.2	27.3	25.0	36.4
	2	33.3	41.7	33.3	58.3	50.0	33.3	41.7
	3	68.2	63.6	54.5	63.6	68.2	75.0	59.1
Flavor[d]	1	27.3	13.6	22.7	18.2	22.7	31.2	18.2
	3	60.0	50.0	60.0	40.0	70.0	60.0	60.0

[a]Based on a mean $\log_{10}$ psychrotrophic count of 5.4 or greater
[b]Based on a mean color score of 6.5 or greater
[c]Based on a mean odor score of 2.5 or greater
[d]Based on a mean flavor score of 4.5 or less

Table 3—Comparison of color and odor scores for rib chops from trimmed vs. untrimmed racks stored in vacuum packages at 0° C for 14 days (Exp. 4)

	Storage condition	
Trait	Trimmed	Intact
Color score[a], day 1 of retail display	5.67[c]	5.61[c]
Color score[a], day 2 of retail display	6.73[d]	6.74[d]
Color score[a], day 3 of retail display	7.60[e]	7.42[e]
Color score[a], day 4 of retail display	7.86[e]	7.64[e]
Odor score[b], day 4 of retail display	2.97[c]	2.95[c]

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[c,d,e]Means in the same horizontal row and means in the same vertical columns with different superscripts differ significantly ($P < 0.05$).

RESULTS & DISCUSSION

A COMPARISON of the percent unacceptable chops from vacuum packaged loins of various fatness levels following retail display is presented in Table 2. Jeremiah et al. (1971) observed that a $\log_{10}$ psychrotrophic count of 5.4 was indicative of readily detectable spoilage in lamb cuts. On this basis, relatively large percentages of the chops from Choice loins with 2.0 mm of subcutaneous fat or less were microbiologically unacceptable upon removal from vacuum packaged storage, and by the 2nd day of retail display 40% or more of the chops in all fatness groups were microbiologically unacceptable. A comparison of the percent unacceptable chops based on discoloration (color scores of 6.5 or greater) reveals that one-third or more of the chops from loins of all fatness levels were unacceptable by the end of the 2nd day of retail display.

Data in Table 2 also indicate that one-third or more of the chops from loins of all fatness levels possessed objectionable odors and thus would be unacceptable (odor scores of 2.5 or greater) by the end of the 2nd day of retail display. It was also noted that odor development, rather than being confined to any particular fatness level, progressed at approximately the same rate in chops from vacuum packaged loins in all fatness groups. Objectionable flavors (due to spoilage) were observed in 13% or more of the chops from loins of all fatness levels and were thus unacceptable after 1 day of retail display. By the end of the 3rd day of retail display 40% or more of all the chops were unacceptable in flavor, but no definite trend in the development of off-flavor was noted among fatness levels.

A comparison of color and odor scores for rib chops from racks which were trimmed vs. those which were not trimmed prior to vacuum packaged storage at 0°C for 14 days is presented in Table 3. These data reveal no significant differences in the color or odor acceptability of chops from trimmed vs. untrimmed racks. However, it can be noted that the color of rib chops from both treatments deteriorated significantly ($P < 0.05$) during the 2nd and 3rd days of retail display.

A comparison of color scores, odor scores and weight losses for boneless vs. bone-in shoulder and leg roasts which

Table 4—Comparison of color scores, odor scores and weight losses during storage for boneless vs. bone-in shoulders and leg roasts stored 14 days at 0°C in vacuum packages (Exp. 2 and 3)

Trait	Storage condition			
	Shoulders		Leg roasts	
	Boneless	Intact	Boneless	Intact
Color score[a], after retail display	3.10[c]	6.80[d]	4.70[c]	5.82[d]
Odor score[b], after vacuum storage	2.00[c]	1.60[d]		
Odor score[b], after retail display	1.00[c]	1.40[d]	2.00[c]	2.29[c]
Weight loss, during vacuum storage (%)	0.60[c]	0.06[d]	0.71[c]	0.51[d]
Weight loss, during retail display (%)	0.50[c]	0.36[c]	1.35[c]	0.33[d]
Weight loss, total (%)	1.10[c]	0.42[d]	2.06[c]	0.85[d]

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[c,d]Means in the same horizontal row, within the same comparison, with different superscripts differ significantly ($P < 0.05$).

were vacuum packaged and stored at 0°C for 14 days is presented in Table 4. These data reveal that during storage in vacuum packages, boneless shoulders developed odors which were significantly ($P < 0.05$) less desirable than those from shoulders which were intact during vacuum packed storage. However, after 2 days of retail display both the color and odor of the boneless shoulders were significantly more desirable ($P < 0.05$) than those of their intact counterparts. These results suggest that the objectionable odors which accumulate during vacuum packed storage dissipate more rapidly and/or to a greater extent from boneless than from intact cuts. Based upon previous research findings (Jeremiah et al., 1971), the point at which lamb color becomes commercially unacceptable is described by the color score 6.5. Using this criterion, the color of the intact shoulders was unacceptable after 2 days of retail display. Boneless shoulders sustained significantly ($P < 0.05$) greater weight losses than their intact counterparts, which was particularly apparent during the storage period under vacuum conditions. Removal of the bones from shoulder roasts resulted in greater surface areas of lean exposed and the severing of numerous muscle fibers, which enhanced the loss of moisture via purging of loosely-held water from the muscle mass. Boneless leg roasts were significantly ($P < 0.05$) brighter and more acceptable in color following 2 days of retail display than their intact counterparts. However, no significant difference was observed in odor acceptability between roasts from the two treatments. Boneless leg roasts sustained significantly ($P < 0.05$) greater weight losses during storage in vacuum packages and during retail display than their intact counterparts. The significant ($P < 0.05$) advantages in moisture retention during storage and display indicate that intact leg roasts are superior to boneless cuts for subsequent storage under vacuum from the shrinkage standpoint.

A comparison of color scores, odor scores and weight losses from boneless vs. bone-in shoulders after storage under vacuum at −18°C is presented in Table 5. While color scores for the thawed cuts did not differ significantly, the intact shoulders were significantly ($P < 0.05$) brighter in color after 2 days of retail display than their boneless counterparts. However, it is doubtful that this latter difference is sufficient to be of economical significance. Intact shoulders also displayed more desirable odor scores ($P < 0.05$) than their boneless counterparts after freezing, but this difference was not apparent after 2 days of retail display. Further evidence is thus provided for the postulation that the objectionable odors which develop during storage under vacuum dissipate more rapidly and/or to a greater extent in boneless than from intact cuts. While the objectionable odors formed during vacuum packed storage of boneless cuts fully dissipate during retail display in oxygen permeable film, it appears that consumers would reject the product unless sufficient time is allowed to elapse between removing the cuts from vacuum and displaying them for sale. Boneless cuts lost significantly ($P < 0.05$) more weight during storage and retail display than their intact counterparts. Vacuum packaging of intact cuts for subsequent storage would appear to have

Table 5—Comparison of color scores, odor scores and percent weight losses for boneless vs. bone-in shoulders stored in vacuum packages for 72 days at −18°C (Exp. 2)

Trait	Storage condition	
	Boneless	Intact
Color score[a], after thawing	4.36[c]	4.18[c]
Color score[a], after retail display	3.91[c]	3.45[d]
Odor score[b], after thawing	2.00[c]	1.54[d]
Odor score[b], after retail display	1.09[c]	1.00[c]
Weight loss, during thawing (%)	3.11[c]	0.39[d]
Weight loss, during display (%)	1.55[c]	0.65[d]
Weight loss, total (%)	4.61[c]	1.04[d]

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[c,d]Means in the same horizontal row with different superscripts differ significantly ($P < 0.05$).

Table 6—A comparison of case-life and palatability characteristics for leg roasts which were vacuum packaged and stored under retail lighting or in the dark (Exp. 5)

Trait	Storage conditions	
	Light	Dark
Odor score[a], after vacuum storage	1.9[d]	2.2[e]
Odor score[a], after retail display	1.5[d]	1.7[d]
Color score[b], after retail display	4.8[d]	4.2[e]
Weight loss, during storage and retail display (%)	0.6[d]	0.6[d]
Flavor rating[c]	5.6[d]	5.5[d]
Juiciness rating[c]	6.0[d]	6.1[d]
Tenderness rating[c]	6.0[d]	6.0[d]
Overall satisfaction rating[c]	5.5[d]	5.5[d]
Semimembranosus, shear force (kg)	3.7[d]	3.5[d]
Biceps femoris, shear force (kg)	3.3[d]	3.0[e]

[a]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[b]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[c]Means based on a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely)
[d,e]Means in the same horizontal row bearing different superscripts differ significantly ($P < 0.05$).

Table 7–Comparison of color and odor scores for rib chops from racks which were vacuum packaged and stored under retail lighting or in the dark (Exp. 4)

Trait[c]	Storage conditions[g]	
	Light	Dark
Color score[a], day 1 of retail display	5.2[d]	6.1[d]
Color score[a], day 2 of retail display	6.4[e]	7.0[e]
Color score[a], day 3 of retail display	7.3[f]	7.8[f]
Color score[a], day 4 of retail display	7.6[f]	7.9[f]
Odor score[b], day 4 of retail display	3.0	3.0

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[c]Means in the same horizontal row underscored by a common line do not differ significantly.
[d,e,f]Means in the same vertical column bearing different superscripts differ significantly ($P < 0.05$).
[g]Interactions between storage conditions and length of retail display period (days) were not significant ($P > 0.05$).

significant economic advantage in lowering weight losses, but the cuts would probably have lowered product acceptability in the retail case.

Leg roasts that were stored in the dark exhibited significantly ($P < 0.05$) lower odor scores immediately after removal from vacuum packages than those stored under incandescent light (Table 6). However, odor scores for roasts from the two treatments were not different after 2 days of retail display in oxygen permeable film. These data suggest that the objectionable odors which accumulated during vacuum packaged storage in both treatments dissipated during the 2-day retail display period. Significantly ($P < 0.05$) brighter lean color was observed for leg roasts that were stored in the dark and subsequently displayed in the retail case; but the magnitude of this difference makes its practical significance questionable. The presence or absence of light during storage had no significant effect on weight loss during storage and display nor upon any of the palatability ratings of the leg steaks. The latter finding is in agreement with the report of Clauss et al. (1957). Shear force values for the biceps femoris were lower for roasts stored in the dark, but those for the semimembranosus did not differ between treatments.

Data in Table 7 compare color and odor scores for chops from racks which were vacuum packaged and stored under incandescent light or in darkness for 14 days at 0°C. These data reveal significant ($P < 0.05$) advantages in color for chops from racks stored in the light at every interval throughout the retail display period. The color desirability of chops from both treatments decreased significantly ($P < 0.05$) during the 2nd and 3rd days of retail display. The presence of light during storage had no significant effect on the odor of chops after 4 days of retail display, as has been previously noted for leg roasts.

A comparison of color scores for leg roasts subjected to three sets of retail display conditions, involving the wrapping material in which roasts were stored and/or displayed is presented in Table 8. These data indicate that fresh leg roasts wrapped in oxygen permeable film displayed significantly ($P < 0.05$) more desirable lean color throughout the retail display period than either fresh leg roasts which were vacuum packaged or leg roasts displayed in oxygen permeable film after an 11-day storage period (0°C) in vacuum packages. Color scores for leg roasts deteriorated rapidly and were significantly ($P < 0.05$) lower for all treatments on the 5th day of retail display. The lean color of vacuum packaged leg roasts deteriorated significantly ($P < 0.05$) between the 3rd and 5th and 9th and 11th days of display. Lean color scores for leg roasts wrapped in oxygen permeable film decreased significantly ($P < 0.05$) between the 3rd and 5th, 5th and 7th, 7th and 9th, and 9th and 11th days of retail display. The color of leg roasts which were vacuum packaged and subsequently wrapped in oxygen permeable film deteriorated significantly ($P < 0.05$) during each interval except that time period between the 7th and 9th days of retail display. These results suggest that fresh leg roasts were significantly ($P < 0.05$) more desirable in color than leg roasts which were stored in vacuum packages and subsequently wrapped in oxygen permeable film. Furthermore, leg roasts which were exposed to oxygen retained desirability of color for a longer period of time than roasts which had been previously vacuum packaged. These findings are in agreement with the reports of Fredholm (1963) and Landrock and Wallace (1955), but do not substantiate the reports of Ball et al. (1957), Jaye et al. (1962), Kraft and Ayres (1952) or Rikert et al. (1957b).

Table 8–Comparison of color scores for leg roasts wrapped in vacuum packages and/or in oxygen permeable film (Exp. 6)

Trait[b]	Display conditions[h]		
	Oxygen permeable film	Vacuum packaged	Vacuum packaged for 11 days, followed by oxygen permeable film
Color score[a], day 1 of retail display	2.6[c]	4.8[c]	4.9[c]
Color score[a], day 3 of retail display	2.7[c]	4.8[c]	5.5[d]
Color score[a], day 5 of retail display	3.3[d]	5.8[d]	6.6[e]
Color score[a], day 7 of retail display	4.2[e]	5.9[d]	7.5[f]
Color score[a], day 9 of retail display	5.3[f]	6.0[d]	7.6[f]
Color score[a], day 11 of retail display	6.3[g]	6.7[e]	8.1[g]

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means in the same horizontal row underscored by a common line do not differ significantly.
[c,d,e,f,g]Means in the same vertical column bearing different superscripts differ significantly ($P < 0.05$).
[h]Interactions between display conditions and length of retail display period (days) were not significant ($P > 0.05$).

Color and odor scores for rib chops from wholesale racks which were fabricated under conditions of normal vs. improved sanitation are presented in Table 9. These data reveal significant advantages ($P < 0.05$) in color desirability after 1 day of retail display for chops from racks which were fabricated under conditions of improved sanitation. However, the color of the chops cut under normal sanitation conditions appeared to deteriorate at approximately the same rate as chops cut under conditions of improved sanitation. At the end of the retail display period, no significant differences in color or odor were noted between chops that were fabricated under different conditions of sanitation. Odor scores for chops from fresh racks were significantly more

Table 9–Comparison of color and odor scores for rib chops from wholesale racks fabricated under conditions of normal vs. improved sanitation (Exp. 7)

Trait	Fresh		Stored	
	Normal sanitation	Improved sanitation	Normal sanitation	Improved sanitation
Color score[a], day 1 of retail display	6.9[d]	5.1[c]	6.2[f]	5.3[e]
Color score[a], day 2 of retail display	7.4[d]	7.0[c]	7.4[e]	7.6[e]
Color score[a], day 3 of retail display	7.9[d]	6.9[c]	6.9[e]	7.4[f]
Color score[a], day 4 of retail display	7.3[c]	7.4[c]	7.0[e]	6.7[e]
Odor score[b], day 4 of retail display	1.0[c]	1.1[c]	3.0[e]	3.0[e]

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[c,d,e,f]Means in the same horizontal row, within the same comparison, bearing different superscripts differ significantly ($P < 0.05$).

Table 10–Mean separation data for color[a] and odor[b] scores of rib chops stratified according to chilling time (days) postmortem prior to vacuum packaging and days of subsequent retail display (Exp. 9)

Chilling time postmortem (day)[g]	Color scores[c] Retail display time (day)				Odor scores[b]
	1	2	3	4	4
1	6.7[d]	7.7[d]	7.5[d]	7.7[de]	3.0[d]
2	6.8[d]	7.3[d]	7.9[d]	8.0[d]	3.0[d]
3	4.5[f]	5.8[e]	7.3[d]	7.5[de]	3.0[d]
4	5.4[ef]	5.8[e]	7.4[d]	7.9[de]	2.9[d]
5	5.5[ef]	7.3[d]	7.3[d]	7.3[e]	3.0[d]
6	6.3[de]	7.8[d]	7.9[d]	8.0[d]	3.0[d]
7	4.5[f]	5.7[e]	7.4[d]	8.0[d]	2.9[d]

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[c]Means in the same horizontal row underscored by a common line do not differ significantly.
[d,e,f]Means in the same column bearing different superscripts differ significantly ($P < 0.05$).
[g]Interactions between chilling time postmortem (days) and length of retail display period (days) were not significant ($P > 0.05$).

desirable ($P < 0.05$) than those from stored racks, regardless of the sanitation level.

Mean separation data for color and odor scores of rib chops stratified according to day of retail display and chilling time (day) postmortem prior to vacuum packaging are presented in Table 10. Chops from racks which were vacuum packaged 3, 4, 5 or 7 days postmortem were brighter ($P < 0.05$) in color after 1 day of display than those from racks which were vacuum packaged at 1 or 2 days postmortem. Chops from racks which were vacuum packaged 3, 4 or 7 days postmortem did not decrease significantly in color during the 2nd day of retail display. If a mean color score of 6.5 or higher is accepted as the point at which chops are no longer saleable (Jeremiah et al., 1971), only those chops from racks which were vacuum packaged after the 2nd day postmortem were saleable after 1 day of retail display. Similarly, only those chops from racks which were vacuum packaged 3, 4 or 7 days postmortem were saleable after 2 days of retail display (Table 10). None of the chops were bright enough in color after 3 days of display to be considered acceptable.

Mean odor scores for rib chops from racks which were vacuum packaged at various postmortem intervals did not differ significantly at the end of the 4-day retail display period. If a mean odor score of 2.5 or higher is accepted as the point at which chops are no longer saleable (Jeremiah et al., 1971), all of the chops would be unacceptable from an odor standpoint after 4 days of retail display.

Table 11 presents the mean color and odor scores for leg roasts which were vacuum packaged at various postmortem intervals, stored under refrigeration for 14 days and displayed under retail conditions for 2 days. Vacuum packaging significantly ($P < 0.05$) reduced the desirability of odor for those leg roasts which were packaged 1, 2 and 5 days postmortem. These data indicate that leg roasts which were vacuum packaged the 4th or 5th days postmortem had significantly ($P < 0.05$) higher color scores, while those vacuum packaged on either the 3rd or 4th days postmortem were significantly ($P < 0.05$) more desirable in odor. Using the values 2.5 or higher and 6.5 or higher as unacceptability levels for odor and color scores, respectively, leg roasts which were vacuum packaged at intervals less than 3 or more than 4 days postmortem are unacceptable in odor after 2 days of retail display. Since the color scores for leg roasts which were vacuum packaged the 3rd day postmortem are very near the point of unacceptability, it appears that the optimum time to vacuum package leg roasts for subsequent storage from both color and odor standpoints would be 4 days postmortem. Vacuum packaging of leg roasts on the 3rd day postmortem resulted in significantly greater weight losses than those incurred by vacuum packaging at 1, 2 or 5 days postmortem. Therefore, it appears the optimum time to vacuum package lamb cuts which will ultimately be displayed as chops or roasts, to minimize deterioration, is the 4th day postmortem.

Table 11–Comparison of color scores, odor scores and weight losses for fresh and vacuum packaged leg roasts grouped according to chilling time (days) postmortem prior to vacuum packaging (Exp. 8)

Characteristic	Storage treatment	Chilling time postmortem (day) 1	2	3	4	5
Color score[a]	Fresh	3.0[c]	3.4[d]	4.2[e]	3.0[c]	2.8[e]
	Vacuum packaged	5.0[d]	5.2[d]	6.4[e]	4.2[c]	4.4[c]
Odor score[b]	Fresh	1.0[c]	1.0[c]	1.2[c]	1.0[c]	1.2[e]
	Vacuum packaged	3.0[d]	3.0[d]	1.4[c]	1.2[c]	2.8[d]
Weight loss during storage and display (%)	Vacuum packaged	0.3[c]	0.2[c]	0.6[d]	0.4[cd]	0.3[c]

[a]Means based on a 9-point scale (9 = greyish or greenish; 1 = very bright)
[b]Means based on a 3-point scale (3 = definite objectionable odor; 1 = no detectable off-odor)
[c,d,e]Means in the same horizontal row bearing different superscripts differ significantly ($P < 0.05$).

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Ms received 7/9/71; revised 12/29/71; accepted 1/24/72.

T.A. 9274, Texas Agricultural Experiment Station.

This study was partially supported by the American Sheep Producer's Council, Inc., Denver.

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COMPARISON OF PRE-COOKED IRRADIATED CHICKEN AND LAMB WITH AND WITHOUT PARTIAL DEHYDRATION

INTRODUCTION

MERITS OF ionizing radiation for extension of shelf life of flesh foods have been well recognized. For prolonged storage stability at ambient temperature, radiation sterilization processes for various types of meats (Heiligman, 1965; Wierbicki et al., 1970) and fish patties (Sinnhuber et al., 1968) have been reported. These processes involve irradiation of these products at high doses (2.3–5.7 Mrad) and often at cryogenic temperatures. Limited extension in shelf life of fish at refrigeration temperature by low dose irradiation (0.05–1.0 Mrad) has been reported (Dassaw and Yiyauchi, 1965).

Since sub-sterilization doses of irradiation do not eliminate all microorganisms, attempts have been made to enhance the microbicidal effectiveness of radiation by the combined use of complementary agents such as chemical additives (Lee et al., 1965a, b), antibiotics (Awad and Sinnhuber, 1965) and heat (Kempe, 1955). The net result of a combined treatment either physical and/or chemical, is to obtain shelf-stable foods with a reduced irradiation dose.

The potential of such combined treatment for stabilizing shrimps at ambient temperature for a period of 4 months has been reported (Kumta and Sreenivasan, 1970; Gore et al., 1970). This process of preservation, referred to as 'dehydro-irradiation', involves the incorporation of sodium chloride and a suitable antifungal agent in the fish followed by partial dehydration and irradiation at the threshold dose at room temperature. Agarwal (1970) reported the stability of dehydro-irradiated white pomfret for more than 5 months without refrigeration.

In India and other developing countries, where an adequate network of refrigeration for the handling of flesh foods has yet to be established, there is a need for preserving such foods without refrigeration. This study was undertaken with a view to examine the usefulness of the dehydro-irradiation process for the preservation of lamb and chicken. Possi-

Table 1—Proximate analysis of precooked chicken and lamb

Proximate composition %	Chicken		Lamb	
	Non-dehydrated	Partially dehydrated	Non-dehydrated	Partially dehydrated
Water	64.21	47.22	58.89	45.72
Protein	22.56	35.26	25.08	35.00
Fat	9.58	13.22	12.59	15.40
Salt	0.49	0.93	0.33	0.83
Ash	2.20	3.60	1.76	2.25

Table 2—Sensory characteristics of irradiated chicken[a]

Sensory characteristics	Storage time (days)	Irradiation dose, Mrads				
		4.5	1.0	2.0	1.0[b]	2.0[b]
Preference	0	5.1 ± 0.4[c]	6.1 ± 0.4	5.4 ± 0.3	5.5 ± 0.4	5.1 ± 0.3
	18	5.4 ± 0.3	6.6 ± 0.3	5.4 ± 0.4	6.1 ± 0.6	6.0 ± 0.7
	40	5.4 ± 0.4	6.1 ± 0.4	5.6 ± 0.5	5.4 ± 0.6	5.6 ± 0.5
	69	5.3 ± 0.4	6.4 ± 0.4	5.1 ± 0.5	5.8 ± 0.6	5.1 ± 0.6
	94	4.7 ± 0.4	5.9 ± 0.4	5.5 ± 0.5	5.2 ± 0.6	5.6 ± 0.4
Off-odor	0	2.2 ± 0.3	1.6 ± 0.4	2.4 ± 0.3	1.8 ± 0.2	2.1 ± 0.3
	18	2.0 ± 0.2	1.6 ± 0.3	2.3 ± 0.4	2.0 ± 0.5	2.1 ± 0.5
	40	2.1 ± 0.2	1.5 ± 0.3	1.8 ± 0.4	1.9 ± 0.3	1.9 ± 0.2
	69	2.2 ± 0.4	1.3 ± 0.2	2.1 ± 0.3	1.6 ± 0.3	2.6 ± 0.5
	94	2.6 ± 0.3	2.4 ± 0.5	2.8 ± 0.5	2.4 ± 0.5	2.5 ± 0.5
Off-flavor	0	1.4 ± 0.2	1.5 ± 0.2	1.5 ± 0.1	2.4 ± 0.4	2.0 ± 0.4
	18	1.9 ± 0.3	1.5 ± 0.3	2.6 ± 0.3	2.2 ± 0.6	2.0 ± 0.6
	40	2.5 ± 0.3	2.0 ± 0.5	2.5 ± 0.5	2.9 ± 0.7	2.5 ± 0.4
	69	2.5 ± 0.4	1.7 ± 0.3	1.7 ± 0.4	2.3 ± 0.4	2.0 ± 0.3
	94	2.6 ± 0.3	2.2 ± 0.3	2.8 ± 0.4	2.2 ± 0.2	2.6 ± 0.2
Irradiation flavor	0	3.4 ± 0.5	1.6 ± 0.4	2.2 ± 0.3	1.4 ± 0.2	2.4 ± 0.5
	18	2.8 ± 0.3	1.4 ± 0.2	2.4 ± 0.3	1.8 ± 0.2	1.8 ± 0.2
	40	1.8 ± 0.3	1.6 ± 0.3	2.5 ± 0.4	1.9 ± 0.2	1.8 ± 0.2
	69	1.9 ± 0.3	1.3 ± 0.2	2.9 ± 0.8	1.1 ± 0.1	2.8 ± 0.6
	94	3.3 ± 0.6	2.8 ± 0.5	2.4 ± 0.5	2.5 ± 0.6	1.9 ± 0.4
Discoloration	0	1.4 ± 0.2	1.0 ± 0.1	1.2 ± 0.1	1.0 ± 0.0	1.7 ± 0.4
	18	2.3 ± 0.3	2.1 ± 0.2	2.5 ± 0.3	1.6 ± 0.2	2.2 ± 0.1
	40	2.1 ± 0.3	1.8 ± 0.3	2.2 ± 0.6	1.6 ± 0.3	2.4 ± 0.3
	69	2.6 ± 0.4	2.3 ± 0.6	2.1 ± 0.4	1.9 ± 0.3	1.4 ± 0.2
	94	1.8 ± 0.2	1.8 ± 0.3	1.8 ± 0.2	1.9 ± 0.3	2.2 ± 0.4
Friability	0	2.4 ± 0.3	1.6 ± 0.4	1.9 ± 0.4	1.9 ± 0.4	2.2 ± 0.4
	18	2.4 ± 0.4	1.8 ± 0.4	2.1 ± 0.4	1.8 ± 0.4	1.8 ± 0.4
	40	2.9 ± 0.3	2.6 ± 0.6	2.4 ± 0.3	2.5 ± 0.5	2.5 ± 0.3
	69	2.6 ± 0.4	2.7 ± 0.5	3.0 ± 0.5	3.0 ± 0.6	3.1 ± 0.4
	94	2.6 ± 0.3	2.8 ± 0.4	2.8 ± 0.4	2.9 ± 0.4	3.2 ± 0.4
Mushiness	0	2.1 ± 0.3	1.6 ± 0.3	2.0 ± 0.3	1.6 ± 0.3	1.5 ± 0.3
	18	2.3 ± 0.3	1.6 ± 0.3	2.0 ± 0.2	1.9 ± 0.4	1.8 ± 0.2
	40	2.4 ± 0.2	2.0 ± 0.4	2.0 ± 0.3	2.1 ± 0.2	2.0 ± 0.2
	69	2.1 ± 0.2	1.9 ± 0.3	1.9 ± 0.3	2.0 ± 0.6	1.9 ± 0.3
	94	2.0 ± 0.2	1.9 ± 0.3	2.1 ± 0.3	2.2 ± 0.3	2.2 ± 0.3

[a] Average of 8 panelists
[b] 0.07% parabens added
[c] ± sign indicates standard error

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ble interaction of individual, as well as combined, treatments of parabens and partial dehydration on the organoleptic characteristics of the meats were studied. Sterilized samples of the meats given 4.5 Mrad at −30°C were used as standard references for comparison.

EXPERIMENTAL

Preparation of samples

Fresh, boneless leg of lamb and boneless chicken breasts (skin on) were used for this study. Both types of meat were formed into rolls by pressing in molds under controlled conditions. The process consisted of adding 1.25% sodium chloride (salt), 1.0% sodium tripolyphosphate (TPP) and, when desired, a mixture of 0.04% propyl-p-hydroxybenzoate (propyl paraben) and 0.03% methyl-p-hydroxybenzoate (methyl paraben) to the meats prior to forming into rolls. [Parabens are on the GRAS list of the U.S. Food and Drug Administration (FDA) and are allowed in certain food products up to a maximum concentration of 1000 ppm. The USDA Meat Inspection Regulations prohibit the direct addition of parabens to meats. These restrictions are not applicable in India.] Salt and TPP were used to reduce the loss of natural juices during cooking for enzyme inactivation and to improve the texture of the rolls so that they could be sliced without crumbing or falling apart.

Meat rolls were prepared by processing 3.5 kg of meat (with additives mixed with meat) in stainless steel cylinders (10.4 cm diam × 4.6 cm length) under pressure (5 kg/cm²) and holding at room temperature for 2 hr. The meats were enzyme inactivated by autoclaving the cylinders at 105°C for about 85 min until the meat reached an internal temperature of 80°C. The cylinders were cooled to 4–6°C. The meat was removed from the cylinders and cut into 1 cm thick (ca. 120g) slices.

Some of the slices of each type of meat were air dehydrated to approximately 45% moisture (cross flow dryer operating at 50°C, 240m/min air velocity, 4 hr). Nondehydrated, as well as partially dehydrated, meat slices of each type of meat were individually packed under vacuum (6 cm of Hg) in laminated pouches (14 × 16 cm) made of high density polyethylene (0.076 mm), aluminum foil (0.099 mm) and polyethylene-terephthalate (0.127 mm). The meat was in contact with the polyethylene, which is approved by the FDA as a food contactant in gamma-irradiated foods (FDA, 1968).

Table 3—Sensory characteristics of partially dehydrated irradiated chicken[a]

Sensory characteristics	Storage time (days)	Irradiation dose, Mrads			
		0.5	1.0	0.5[b]	1.0[b]
Preference	0	6.0 ± 0.1[c]	6.1 ± 0.5	6.1 ± 0.3	6.0 ± 0.4
	18	6.0 ± 0.5	6.4 ± 0.5	6.1 ± 0.5	6.0 ± 0.4
	40	4.5 ± 0.5	5.0 ± 0.5	4.5 ± 0.5	3.7 ± 0.6
	69	4.4 ± 0.5	5.2 ± 0.5	4.5 ± 0.6	3.5 ± 0.6
	94	4.4 ± 0.6	4.2 ± 0.4	4.5 ± 0.4	3.9 ± 0.4
Off-odor	0	1.6 ± 0.2	1.6 ± 0.3	1.6 ± 0.3	1.9 ± 0.3
	18	1.5 ± 0.3	1.4 ± 0.2	1.5 ± 0.3	1.8 ± 0.4
	40	1.5 ± 0.3	1.6 ± 0.4	2.5 ± 0.5	3.1 ± 0.7
	69	1.9 ± 0.5	1.8 ± 0.5	2.8 ± 0.6	2.5 ± 0.6
	94	2.6 ± 0.5	2.4 ± 0.5	2.0 ± 0.3	2.2 ± 0.4
Off-flavor	0	1.5 ± 0.3	1.9 ± 0.3	1.5 ± 0.2	1.8 ± 0.2
	18	1.2 ± 0.4	1.8 ± 0.4	1.8 ± 0.4	1.4 ± 0.3
	40	2.2 ± 0.6	2.2 ± 0.4	2.9 ± 0.6	3.5 ± 0.8
	69	2.6 ± 0.6	1.9 ± 0.5	3.2 ± 0.7	3.8 ± 0.8
	94	2.5 ± 0.5	2.8 ± 0.4	2.2 ± 0.4	3.1 ± 0.4
Irradiation flavor	0	1.0 ± 0.0	1.4 ± 0.3	1.0 ± 0.0	1.4 ± 0.3
	18	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.4 ± 0.3
	40	1.2 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.4 ± 0.3
	69	2.0 ± 0.8	1.1 ± 0.1	1.5 ± 0.3	1.5 ± 0.3
	94	2.0 ± 0.3	1.5 ± 0.4	2.0 ± 0.6	2.9 ± 0.8
Discoloration	0	2.2 ± 0.6	2.2 ± 0.4	1.9 ± 0.4	2.2 ± 0.4
	18	3.0 ± 0.4	3.2 ± 0.4	2.9 ± 0.6	3.6 ± 0.6
	40	2.6 ± 0.6	3.3 ± 0.8	2.7 ± 0.8	3.6 ± 0.8
	69	3.5 ± 0.8	2.8 ± 0.3	3.4 ± 0.5	3.3 ± 0.6
	94	3.5 ± 0.6	3.1 ± 0.5	2.9 ± 0.6	2.8 ± 0.6
Friability	0	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
	18	1.0 ± 0.0	1.4 ± 0.3	1.1 ± 0.1	1.1 ± 0.1
	40	1.1 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.0
	69	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.2 ± 0.2
	94	1.6 ± 0.6	1.7 ± 0.6	1.5 ± 0.5	1.5 ± 0.5
Mushiness	0	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.5 ± 0.2
	18	2.0 ± 0.4	1.5 ± 0.2	1.4 ± 0.2	1.5 ± 0.2
	40	2.1 ± 0.4	1.9 ± 0.6	2.1 ± 0.7	2.1 ± 0.7
	69	1.5 ± 0.5	1.4 ± 0.4	1.4 ± 0.3	1.5 ± 0.5
	94	2.4 ± 0.9	2.2 ± 0.8	2.1 ± 0.6	2.1 ± 0.8

[a]Average of 8 panelists
[b]0.07% parabens added
[c]± sign indicates standard error

Irradiation

The packaged samples were gamma irradiated using the U.S. Army Natick Labs. Cobalt 60 facility (dose rate of 0.5 × 10⁴ rads/min). In both types of meat, irradiation sterilized (4.5–5.2 Mrad at −30 ± 10°C) samples were used for reference. This dose is the established 12D dose for chicken and the predicted 12D dose for lamb. Products given substerilization doses were at 4–5°C at the start of the irradiation and the irradiation was done without refrigeration (from here on referred to as ambient temperature irradiation). Doses, as stated, are minimum doses and may range up to a maximum dose of 120% of the minimum dose. Samples of nondehydrated chicken, both with or without parabens added, were given 1.0 or 2.0 Mrad. Corresponding lamb samples were given only one irradiation dose, 1.0 Mrad. Partially dehydrated chicken with and without parabens were irradiated at 0.5 and 1.0 Mrad; whereas, corresponding lamb samples were only irradiated at 0.5 Mrad.

Following irradiation, all the samples were stored at 21°C, with a relative humidity of 50%, until subjected for analysis and sensory evaluation.

Evaluations

Chemical, microbial and organoleptic evaluations were done on chicken shortly after irradiation and after 18, 40, 69 and 94 days storage. The lamb was evaluated shortly after irradiation and after 20, 46 and 70 days storage.

Chemical analyses. Moisture, fat, protein, ash, salt and TPP were determined by the standard AOAC (1970) methods. Free fatty acids (FFA) were determined by methods described by Pearson (1971). pH was read directly using a Beckman Zeromatic pH meter. Nonprotein-nitrogen (NPN) was estimated by the method described by Jacobs (1951).

Headspace gases were analyzed with a Fisher Gas Partitioner, Model 25. It has Fisher column number 11-134-45 which is a pair of matched columns. Column one is a 2m HMPA column (30% hexamethyl-phospheramide of 60–80 mesh chromosorb P) and column two is a 1.6m column of 60–80 mesh chromosorb P followed by 2.3m of 60–60 mesh activated molecular sieve 13×. Helium was the carrier gas with a flow rate of 110 cc/min.

Microbial and toxin analysis. All irradiated samples used in sensory evaluations were tested to ascertain the absence of preformed toxin of *Cl. botulinum*, types A and B, using a modification of the method recommended by the U.S. Dept. of Health, Education and Welfare (1969). These same samples were also evaluated for aerobic and anaerobic bacteria, yeast and molds using standardized microbiological techniques (Powers, 1971, personal communication).

Organoleptic evaluation. Following microbial evaluation, the samples were evaluated organoleptically by an eight-member trained technical panel. The samples were evaluated for preference using the 9-point hedonic scale

(Peryam and Pilgrim, 1957) and for discoloration, off-odor, irradiation flavor, off-flavor, mushiness and friability using the 9-point intensity scale with a rating of one indicating "none" and 9 indicating "extreme."

The partially dehydrated samples were rehydrated by submerging in tap water (approximately 20°C) for 3 hr. All samples were held for 15 min at 170° prior to serving to the panelists.

Statistical analysis. The organoleptic scores were statistically analyzed by computer using the technique of analysis of variance for factorial design. The program of the computer was written at the 95% confidence level. The organoleptic data for chicken or lamb were analyzed separately for each withdrawal and for the combined withdrawals.

RESULTS & DISCUSSION

Chemical indices

Proximate analyses for the precooked chicken and lamb are shown in Table 1. The high levels of ash are due to the addition of salt and TPP.

There were no significant differences in the NPN values for either the various chicken samples (range 0.91–1.37%) or lamb samples (range 0.69–1.03%) prior to storage or after storage. These data indicate that the pre-irradiation heat treatments used for inactivating the autolytic enzymes were adequate. There were also no significant differences in the FFA values of the chicken samples (range 1.41–3.07 expressed as % oleic acid of the extracted fat) or the lamb samples (range 2.37–3.46) which suggests the absence of hydrolytic changes in the fat during storage. Changes in pH (chicken, range 6.20–6.60; lamb, range 6.26–6.60) were also statistically insignificant.

Headspace gas composition

Besides N_2 and O_2, all irradiated samples were found to contain CO_2 and H_2 as major components and CH_4 and CO as minor components. With few exceptions, which were probably due to variations in the extent of evacuation of air during vacuum packaging, H_2 and CO_2 increased significantly (95% confidence level) with increased storage time. Because of these increases, there was a decrease in the percent N_2. Since the NPN in the samples remained constant during storage and there was no microbial activity, it would seem that the CO_2 was evolved due to chemical reactions within the food. These types of changes in the composition of headgas in irradiated foods have been reported by other investigators. Pratt et al. (1967) working with irradiated beef, ham and other foods, and Mendlesohn and Brooke (1968) working with irradiated clam meat, have noted significant increases in the volatile constituents, including CO_2, in the headspace due to storage.

Microbiological and toxin evaluations

All samples tested were free of *Cl. botulinum* toxin. Aerobic and anaerobic plate counts and yeast and mold counts were all less than 10 per gram (the lowest dilution tested). These results indicate that a 0.5 Mrad dose for partially dehydrated and a 1.0 Mrad dose for nondehydrated meats of chicken and lamb may be adequate to produce shelf stable items. No positive conclusion can be drawn concerning the effects of the partial dehydration or addition of parabens on the microflora since no microorganisms were detected in any of the samples tested.

Organoleptic evaluations

The evaluation scores for the various samples are shown in Tables 2, 3 and 4. It is noteworthy that the samples were only warmed, without any additional culinary treatment for serving to the panelists. This resulted in lower preference scores than would have been obtained if the meats had been prepared using common culinary practices. This procedure was used because the main objective of the sensory evaluation was to determine the influences of the experimental treatments on the sensory attributes and not on acceptability.

Comparative evaluation

Organoleptic characteristics of the nondehydrated samples of chicken and lamb given sterilizing doses of 4.5 Mrad at −30 ± 10°C were compared statistically (95% confidence level) with the samples given low doses. The chicken samples, either with or without parabens and given 1.0 Mrad, had less off-odor, less irradiation flavor and were less mushy than the radiation sterilized samples, although the preference scores for both samples were statistically the same.

Chicken samples that were given 2.0 Mrad, irrespective of the parabens treatment, were scored statistically similarly to the irradiation sterilized chicken samples (4.5 Mrad at −30 ± 10°C) in all the sensory parameters tested. The chicken samples given 1.0 Mrad, however, were preferred to the samples given 2.0 Mrad. This was due to less off-odor and less irradiation flavor in the 1.0 Mrad samples. Low dose irradiated (1.0 Mrad) lamb samples, with or without parabens, were preferred to the irradiation sterilized (4.5 Mrad at −30 ± 10°C) samples. This was due to less discoloration and less mushiness. The addition of parabens did not influence the organoleptic attributes or

Table 4–Sensory characteristics of irradiated lamb[a]

Sensory characteristics	Storage time (days)	Nondehydrated Irradiation dose, Mrads 4.5	Nondehydrated Irradiation dose, Mrads 1.0	Nondehydrated Irradiation dose, Mrads 1.0[b]	Partially dehydrated Irradiation dose, Mrads 0.5	Partially dehydrated Irradiation dose, Mrads 0.5[b]
Preference	0	4.8 ± 0.4[c]	5.8 ± 0.2	6.5 ± 0.4	5.2 ± 0.4	5.8 ± 0.4
	20	4.4 ± 0.6	6.0 ± 0.5	5.4 ± 0.6	4.9 ± 0.6	4.3 ± 0.5
	47	4.5 ± 0.6	6.6 ± 0.5	5.6 ± 0.6	4.3 ± 0.6	4.4 ± 0.5
	70	5.2 ± 0.4	6.3 ± 0.4	6.0 ± 0.6	6.1 ± 0.1	5.4 ± 0.6
Off-odor	0	2.2 ± 0.4	1.8 ± 0.2	1.4 ± 0.3	2.1 ± 0.4	1.8 ± 0.3
	20	2.0 ± 0.4	1.6 ± 0.4	1.6 ± 0.3	1.6 ± 0.4	1.7 ± 0.3
	47	2.1 ± 0.5	1.9 ± 0.6	2.4 ± 0.6	3.1 ± 0.7	2.9 ± 0.8
	70	1.2 ± 0.2	1.6 ± 0.3	1.4 ± 0.3	1.4 ± 0.1	1.5 ± 0.2
Off-flavor	0	1.1 ± 0.1	1.9 ± 0.4	1.4 ± 0.1	2.1 ± 0.4	1.8 ± 0.4
	20	2.4 ± 0.5	1.7 ± 0.4	2.0 ± 0.5	2.1 ± 0.7	2.4 ± 0.7
	47	2.4 ± 0.5	2.0 ± 0.5	2.4 ± 0.6	2.7 ± 0.7	2.7 ± 0.8
	70	1.5 ± 0.3	1.5 ± 0.3	2.4 ± 0.4	1.8 ± 0.6	1.7 ± 0.4
Irradiation flavor	0	2.1 ± 0.5	2.0 ± 0.4	1.5 ± 0.2	2.2 ± 0.4	1.5 ± 0.3
	20	2.9 ± 0.6	1.4 ± 0.2	1.1 ± 0.1	2.1 ± 0.5	1.3 ± 0.3
	47	2.4 ± 0.5	2.0 ± 0.7	2.3 ± 0.6	2.7 ± 0.9	2.9 ± 0.8
	70	2.2 ± 0.7	1.8 ± 0.5	1.4 ± 0.2	1.2 ± 0.1	1.1 ± 0.1
Discoloration	0	2.6 ± 0.8	1.8 ± 0.2	1.6 ± 0.4	2.6 ± 0.5	1.9 ± 0.2
	20	2.3 ± 0.5	1.6 ± 0.3	1.6 ± 0.3	2.6 ± 0.6	2.9 ± 0.5
	47	2.4 ± 0.5	2.1 ± 0.6	2.3 ± 0.7	3.9 ± 0.7	3.9 ± 0.7
	70	2.2 ± 0.5	1.2 ± 0.2	2.1 ± 0.3	3.1 ± 0.7	2.2 ± 0.7
Friability	0	3.5 ± 0.5	2.5 ± 0.7	2.9 ± 0.7	2.9 ± 0.7	2.8 ± 0.7
	20	3.1 ± 0.5	2.4 ± 0.6	2.4 ± 0.5	1.5 ± 0.3	2.0 ± 0.6
	47	2.9 ± 0.4	2.1 ± 0.5	2.3 ± 0.4	2.1 ± 0.4	1.8 ± 0.3
	70	2.5 ± 0.3	2.0 ± 0.5	1.9 ± 0.3	2.4 ± 0.6	2.4 ± 0.8
Mushiness	0	2.4 ± 0.4	1.2 ± 0.1	1.3 ± 0.3	1.2 ± 0.1	1.2 ± 0.1
	20	3.4 ± 0.5	2.1 ± 0.5	2.0 ± 0.4	1.0 ± 0.0	1.2 ± 0.2
	47	2.3 ± 0.5	1.4 ± 0.2	1.9 ± 0.3	1.1 ± 0.1	1.1 ± 0.1
	70	2.4 ± 0.5	1.4 ± 0.3	1.5 ± 0.2	1.4 ± 0.3	1.4 ± 0.2

[a] Average of 8 panelists
[b] 0.07% parabens added
[c] ± sign indicates standard error

acceptability of nondehydrated samples of chicken (1.0 and 2.0 Mrad) and lamb (1.0 Mrad). Similarly this treatment did not alter the sensory characteristics of partially dehydrated samples of chicken and lamb given 0.5 Mrad. Increase in irradiation dose from 0.5–1.0 Mrads in the partially dehydrated chicken with parabens caused a significant lowering in preference and this decrease was probably caused by an increase in off-odor and off-flavor.

Partial dehydration of chicken had no effect on odor and flavor attributes but adversely affected the preference of the samples. This decrease in preference seemed to be due to changes in texture and color as the dehydrated samples were tough and had a yellow discoloration. A combination treatment of parabens and partial dehydration seemed to cause an additional increase in off-odor and off-flavor scores. This decrease in overall acceptability could be due to "browning" reaction as it has been noted that "browning" as well as a protein denaturation occurs during dehydration of flesh products (Connell, 1957; Sawant and Mager, 1961; Pearson et al., 1966).

CONCLUSIONS

THE OVERALL results of these studies indicate that irradiation of enzyme inactivated meats at doses as low as 1.0 Mrad can produce shelf stable, acceptable meat items. This dose requirement can probably be reduced to 0.5 Mrad by partial dehydration of the meats prior to the irradiation treatment. Additional work, using inoculated pack studies, is needed to ascertain the freedom from spoilage and toxigenic microorganisms of meats treated as described in these studies.

Although partial dehydration caused slight discoloration and toughening of texture of the chicken and lamb, the combined process did produce meat items that were acceptable, free from microbial spoilage and *Cl. botulinum* toxin during storage without refrigeration for 2–3 months, the longest storage time investigated.

No conclusions can be drawn concerning the effects of the addition of parabens on microbial stability since none of the samples, regardless of treatment, showed any evidence of microbial spoilage.

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Ms received 11/14/71; revised 1/27/72; accepted 2/3/72.

Presented at the 30th Annual Meeting of the Institute of Food Technologists in San Francisco.

This paper reports research undertaken at the U.S. Army Natick (Mass.) Laboratories and has been assigned No. TP 1116 in the series of papers approved for publication.

This research was conducted under a fellowship awarded by the International Atomic Energy Agency of the United Nations. This fellowship was administered by the National Academy of Sciences—National Research Council, Washington, D.C.

The authors acknowledge the assistance of Dr. E. Wierbicki, Irradiated Food Products Div., Food Lab., and Mrs. Mary Ann Wall, Data Analysis Office, of the U.S. Army Natick Labs., for their contributions in this investigation.

The findings in this report are not to be construed as an official Dept. of the Army position.

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EMULSION STABILITY AND PROTEIN EXTRACTABILITY OF OVINE MUSCLE AS A FUNCTION OF TIME POSTMORTEM

INTRODUCTION

WHEREAS PRERIGOR meat is a more efficient emulsifier than postrigor meat (Trautman, 1964; Acton and Saffle, 1969), its usefulness is limited by the short period of time for which it retains this desirable property. For the manufacturing of emulsion-type sausages using prerigor meat, it is important to know what changes occur when muscle goes into rigor, and how long prerigor meat retains its desirable properties. An understanding of the reasons for the superior emulsifying properties of prerigor meat might lead to a way of retaining them, or to restore them in postrigor meat.

Hamm (1966a) investigated the water-holding properties of meat at various times postmortem. He found that the higher water-holding capacity of prerigor compared to postrigor meat could be largely attributed to its higher pH and consequent enhanced hydration, plus the presence of ATP, a natural polyphosphate. Hamm (1966b) also described how the higher water-holding capacity of prerigor meat could be preserved by prerigor freezing or prerigor mincing followed by salting. Acton and Saffle (1969) have demonstrated that prerigor meat has a higher emulsifying capacity than postrigor meat and that this higher emulsifying capacity could be preserved by preblending meat with salt and ice. Biochemical changes in meat going into rigor have been described by Bendall (1960) and Newbold (1966). Postmortem anaerobic glycolysis results in the formation of lactic acid with a consequent pH drop. At the onset of rigor crossbridges are formed between the thin and thick filaments of the myofibrils.

The aim of this study was to obtain information on the changes occurring in muscle as related to emulsifying efficiency, when muscle goes into rigor. Thus data were collected on the time required for the development of rigor in a number of ovine muscles. Information was also collected on the emulsifying properties as well as changes in pH, water-holding capacity and protein extractability of two muscles at various times postmortem.

EXPERIMENTAL

Meat source

The meat used throughout the experiments was obtained from 3-yr-old wethers.

Duration of time before rigor development

The muscles were excised within 30 min postmortem and kept in polyethylene bags. It was assumed that the muscle was in rigor when its pH remained constant.

pH measurements

5g of meat was homogenized in 45 ml of a 3% NaCl solution with an Ultra Turrax Mixer Emulsifier Type Tp 18/2 (Janke & Kunkel K. G. Staufen i. Br., W. Germany). The pH of the homogenate was measured with a Radiometer pH meter Model 22 using a glass electrode.

Water-holding capacity

The water-holding capacity was determined on a 10% meat homogenate in 3% saline by centrifugation for 15 min at 10,000 G, 1 hr after homogenization, and weighing the resulting sediment. During the 1-hr interval the homogenate was neither stirred nor agitated. Water-holding capacity was defined as

$$\frac{\text{Wt of sediment}}{\text{Wt of original meat}} \times 100\%$$

Emulsifying properties

Emulsions were made of 2½% meat homogenate in 3% saline (50 ml) and an oil blend of 40% castor oil and 60% paraffin oil (50 ml). The 'required' hydrophilic lipophilic balance (HLB) (Griffin, 1949; Van Eerd, 1971) of the oil blend was 13.0. After emulsification with an Ultra Turrax Mixer Emulsifier for 15 sec, the emulsions were poured into 100-ml measuring cylinders. The aqueous phase separation, measured 1 hr after emulsification, was taken as an index of emulsifying efficiency. The HLB of myosin and actomyosin were determined by the method of Griffin (1949), as modified by Van Eerd (1971). The HLB range was obtained by making blends of paraffin oil ('required' HLB = 10.6) and castor oil ('required' HLB = 14.6).

Protein extractability of meat

The protein concentration in the superna-

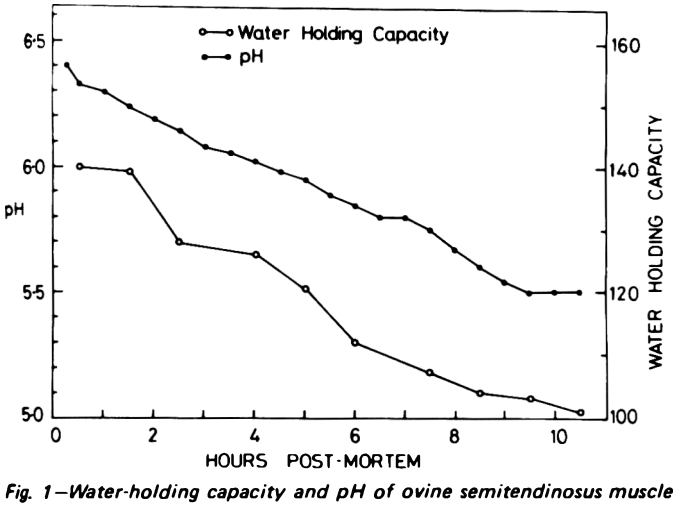

Fig. 1—Water-holding capacity and pH of ovine semitendinosus muscle as a function of the time postmortem. (See Methods for definition of water-holding capacity.)

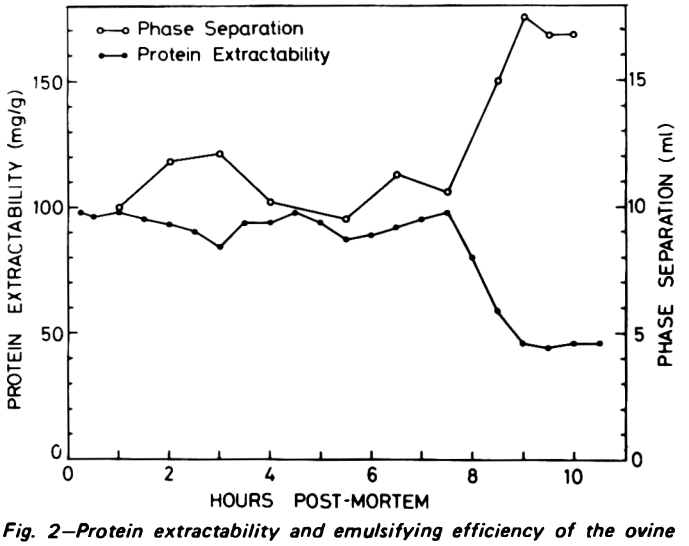

Fig. 2—Protein extractability and emulsifying efficiency of the ovine semitendinosus muscle of Figure 1 as a function of the time postmortem.

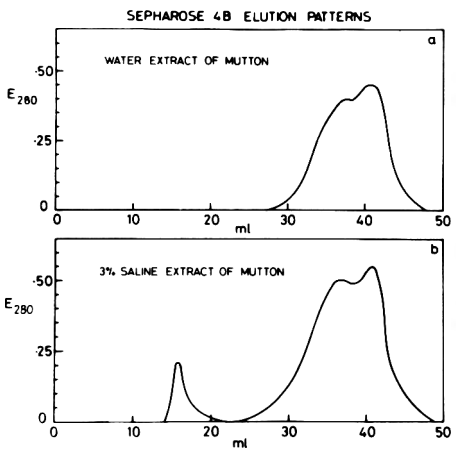

Fig. 3—Elution patterns of a water and 3% saline extract of ovine semitendinosus after chromatography on Sepharose 4B.

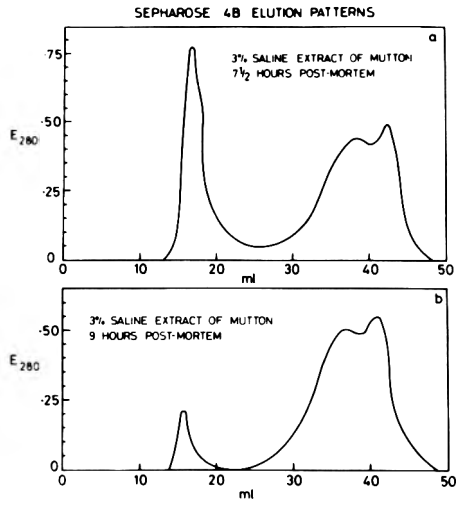

Fig. 4—Sepharose 4B elution patterns of ovine semitendinosus extracts extracted with 3% saline, immediately before and after the development of rigor.

tants of the homogenates after water-holding capacity measurements was determined and the protein extractability expressed as mg protein/g meat.

Column chromatography

0.5-ml samples (3–6 mg protein) of meat protein extracts were applied to a 30 × 1.5 cm column, packed with Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). The proteins were eluted with a buffer of pH 6.5 consisting of 0.5M NaCl, 0.025M phosphate and 0.01% sodium azide. The elution was followed by measuring the absorption of the eluate at 280 nm.

Protein determination

Protein concentrations were determined using the biuret method of Gornall et al. (1949). Protein concentrations of <1 mg/ml were determined using the method of Lowry et al. (1951). The myofibrillar proteins content in muscle extracts was determined by measuring the ratio of the myofibrillar and sarcoplasmic proteins content in the eluate after Sepharose 4B chromatography.

Myofibrillar protein preparation

Myosin and actomyosin were prepared from semitendinosus muscle by the method of Szent-Györgyi (1951). Extraction of these proteins was commenced within 30 min after slaughter.

All of the above procedures were carried out at room temperature (approximately 23°C).

Table 1—Duration of time before the onset of rigor mortis in ovine muscles

Muscle	Hours[a]
Semitendinosus	8.2 ± 0.8
Psoas major	8.2 ± 0.6
Pectoralis	10.7 ± 0.9
Biceps femoris	11.9 ± 1.3
Longissimus dorsi	13.5 ± 0.7
Semimembranosus	14.2 ± 1.1

[a]The data are the averages and standard deviations from five experiments.

RESULTS & DISCUSSION

DURATION OF TIME before rigor development for six muscles taken from five sheep is shown in Table 1. It can be seen that there is a large intermuscular variation, the semimembranosus muscle taking about twice as long to go into rigor as the semitendinosus muscle. Time before rigor development is influenced by the storage temperature of the muscle, higher temperatures accelerating rigor development and lower temperatures retarding it (Cassens and Newbold, 1967).

Changing properties of muscle going into rigor

In Figures 1 and 2, pH, water-holding capacity, protein extractability and emulsion separation are plotted against time postmortem for a semitendinosus muscle. The protein extractability remained constant for 7.5 hr, after which it decreased by about 45% within 1 hr and then remained constant. The phase separation of emulsions, as an index of emulsifying efficiency also was constant for 7.5 hr, and then increased rapidly to a final constant value. The sudden drop in protein extractability coincided with the increase in phase separation and thus decreased emulsion efficiency.

The water-holding capacity and the pH dropped steadily as a function of time postmortem until soon after the completion of the decrease in protein extractability. Measurements of pH, water-holding capacity, emulsifying efficiency and protein extractability were repeated with four semitendinosus muscles and two longissimus dorsi muscles. The sudden drop in protein extractability was less for the longissimus dorsi than for the semitendinosus: their averages being 33% and 45% respectively. This agrees with the findings of Acton and Saffle (1969) that the protein extractability of postrigor beef was 34% less than that of prerigor beef.

Chromatography of proteins extracted from semitendinosus muscle with 3% saline

Sepharose 4B was used for the separation of the high molecular weight myofibrillar proteins from the comparatively low molecular weight sarcoplasmic pro-

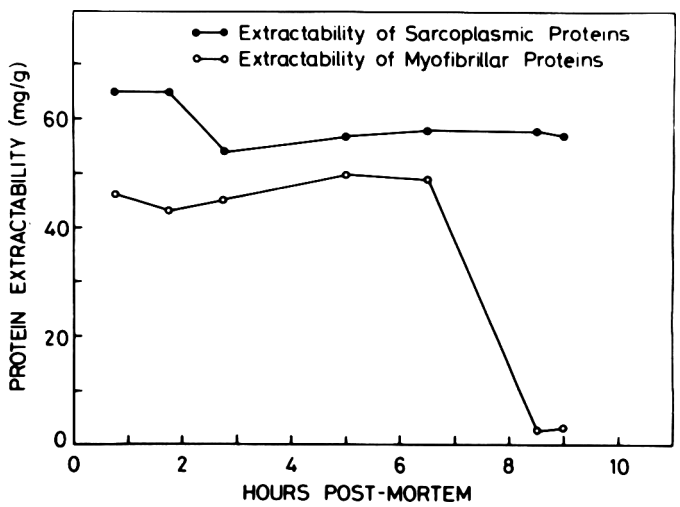

Fig. 5—Extractability of myofibrillar proteins and sarcoplasmic proteins of ovine semitendinosus muscle as a function of time postmortem.

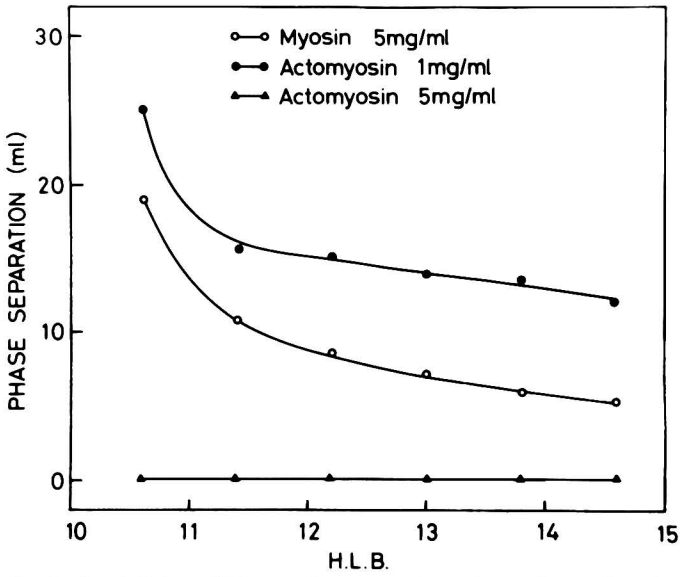

Fig. 6—Emulsifying efficiency of actomyosin and myosin as a function of HLB.

teins. Proteins extracted from postrigor muscle with water gave a double peak on Sepharose 4B (Fig. 3a), while a salt extract showed an additional peak of very high molecular weight protein (Fig. 3b). Figure 4 shows the elution patterns of saline extracts of semitendinosus muscle just before, and just after the sharp decrease of protein extractability. When the protein extractability dropped, the peak representing high molecular weight protein was greatly reduced.

The myofibrillar protein extractability of another semitendinosus muscle going into rigor is shown in Figure 5. It can be seen that this extractability dropped from 45 mg/g in the prerigor state to 3 mg/g in the postrigor state. Figure 5 also shows extractability of the 'low' molecular weight proteins as a function of time postmortem. There was not much variation in these values and therefore it is concluded that the drop in protein extractability was due almost entirely to a decrease in the extractability of the myofibrillar proteins. The decrease in protein extractability coincided with a decrease in emulsion efficiency, so it was assumed that this decrease in emulsifying efficiency was caused by decreased extractability of myofibrillar proteins, which was probably caused by the formation of crossbridges between the thin and thick filaments.

On extraction of prerigor meat with saline solution the principal myofibrillar protein extracted is myosin. Myofibrillar proteins extracted from postrigor meat are predominantly actomyosin. Therefore myosin and actomyosin were isolated to study their emulsifying properties. At a concentration of 5 mg/ml myosin showed good emulsifying properties with an HLB $>$ 14.6 (Fig. 6). Actomyosin at this concentration was a very efficient emulsifier: none of the prepared emulsions showed any phase separation. Dilution of the actomyosin to 1 mg/ml still resulted in good emulsifying properties with an HLB $>$ 14.6 (see Fig. 6). Hegarty et al. (1963) also reported very good emulsifying properties of myosin and actomyosin but did not find a substantial difference in efficiency between them. If the extractability of actomyosin of postrigor meat could be enhanced, meat emulsifying efficiency would be improved considerably.

The protein extractability and the emulsifying efficiency stay high until 1–2 hr before the muscle is in rigor, as defined by reaching an ultimate pH. However, this does not necessarily mean that the manufacturing properties of prerigor meat are optimal until then, as the pH of prerigor meat drops with a consequent decrease in the water-holding capacity (Fig. 1).

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Ms received 10/31/71; revised 1/10/72; accepted 1/16/72.

This work was supported in part by funds from the Australian Meat Research Committee.

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BEEF COLOR AS RELATED TO CONSUMER ACCEPTANCE AND PALATABILITY

INTRODUCTION

CONSUMER STUDIES have shown that physical appearance of a retail cut in the display case is the most important factor determining retail selection of meat products (Danner, 1959; Dunsing, 1959a, b). Consumers select meat cuts primarily for leanness and then for appearance and freshness, with judgments for the latter two attributes based primarily on brightness of color (ASPC, 1964; Rhodes et al., 1955; Seltzer, 1955). The importance of attractive lean color was further emphasized by Shaw (cited by Nelson, 1964), who reported that 36.7% of the meat purchases from self-service counters were unplanned and that these impulse purchases were made primarily because of attractive appearance.

The descriptions of beef color included in USDA (1965) quality grading standards are subjective and, as such, are subject to wide variations in interpretation. Allen (1968) described "ideal" beef color as "cherry red." However, Billmeyer and Saltzman (1966) concluded that since people differ in their individual response to visual colors, they will also differ in their interpretations of subjectively defined terms for color. Barton (1968a) concluded that an objective method for measuring color was necessary before comparisons of color characteristics and/or preferences could be made between laboratories or locations. Inconsistencies were also noted in subjective scores for color from one observation period to the next, if there was a substantial time lapse between successive evaluations (Barton, 1968b). Pirko and Ayres (1950), Dean and Ball (1960), Snyder (1965) and Stewart et al. (1965) have successfully measured fresh meat pigments spectrophotometrically while Ockerman and Cahill (1969) related machine readings to visual color scores for beef. The present study was initiated to identify subjective visual color standards which could be utilized at various locations (packer, retailer, etc.), to define these color standards in terms of hue, value and chroma, and to test representative steak samples for consumer acceptability.

Table 1—Description of standard scale for beef muscle color

Color score	Description
1	Very pale pink
2	Pale pink
3	Pink
4	Slightly pale red
5	Cherry red
6	Slightly dark red
7	Moderately dark red
8	Dark red
9	Very dark red

EXPERIMENTAL

62 WHOLESALE BEEF loins were selected on the basis of a visual muscle color scale ranging from very pale pink to very dark red (Table 1). Loins included in the present study ranged in color from pale pink to moderately dark red. Selections were also confined to loins from youthful carcasses (A maturity) as evaluated by the USDA beef carcass grading standards (USDA, 1965).

The loins were fabricated by removing the bone and cutting four steaks (4 cm thick) from each sample. The subcutaneous fat was trimmed to a uniform thickness of 5 mm on one steak from each loin. These steaks were then permitted to oxygenate (bloom) for a period of 75 min under refrigeration and in the dark. Following this period of oxygenation, the steaks were subjectively scored by a trained three-member panel for color using a nine-point scale (Table 1), for marbling using USDA marbling scores (small + = 15; modest − = 16; modest = 17, etc.), and for texture by use of a nine-point scale (9 = very fine; 1 = very coarse). Two other steaks from each loin were wrapped and frozen for palatability and chemical studies.

Following visual color evaluation by the trained panel, these steaks were placed in transparent plastic backing trays, wrapped in oxygen permeable film (Goodyear—"Choice Wrap"), and randomly grouped in sets of five steaks per group for display at three different food stores in the local trade area. These stores were selected so that their clientele would be representative of various ethnic and socio-economic groups.

A technician was present in each store during the display period to interview consumers concerning their preferences regarding muscle color and to explain the questionnaire

Table 2—Values for Munsell notation and physical and chemical characteristics for steaks grouped according to visual color score

Visual color score	Statistic[f]	Hue	Value	Chroma	pH	Total pigment mg/g	Marbling score	Texture score
2	Mean	11.30[a]	3.44[a]	5.90[a]	5.52[a]	4.11[a]	20.00[a]	7.20[a]
	SD	0.79	0.18	0.35	0.04	0.84	6.12	1.09
	CV	6.99	5.37	5.99	0.77	20.50	30.62	15.21
3	Mean	10.69[a]	3.11[b]	5.51[a]	5.51[a]	4.34[a]	18.64[a]	5.43[b]
	SD	0.84	0.26	0.34	0.06	0.81	4.24	1.28
	CV	7.85	8.51	6.15	1.04	18.64	22.72	23.65
4	Mean	10.55[a]	2.77[c]	5.48[a]	5.52[a]	5.29[ab]	17.71[a]	4.88[bc]
	SD	0.89	0.28	0.47	0.06	0.95	3.67	1.27
	CV	8.47	9.97	8.61	1.06	17.94	20.73	25.99
5	Mean	11.10[a]	2.61[cd]	4.87[b]	5.54[a]	5.53[b]	16.00[a]	4.47[c]
	SD	0.48	0.23	0.57	0.05	1.22	3.93	0.51
	CV	4.28	8.71	11.70	0.85	22.09	23.44	11.56
6	Mean	10.95[a]	2.38[de]	4.43[b]	5.59[a]	5.41[b]	18.33[a]	3.00[d]
	SD	0.73	0.38	1.25	0.08	1.52	4.23	1.41
	CV	6.70	15.82	28.34	1.34	28.14	23.06	47.14
7	Mean	11.16[a]	2.07[e]	3.26[c]	5.87[b]	4.96[ab]	18.80[a]	4.00[d]
	SD	2.15	0.18	0.23	0.27	1.02	8.29	1.00
	CV	19.31	8.89	7.06	4.60	20.57	44.09	25.00
Overall	Mean	10.86	2.77	5.09	5.56	5.02	17.84	4.84
	SD	0.93	0.43	0.87	0.13	1.14	4.56	1.45
	CV	8.60	15.60	17.17	2.33	22.67	25.66	29.98

[a,b,c,d,e]Means in the same column with different superscripts differ significantly ($P < 0.05$).
[f]CV = coefficient of variation; SD = standard deviation of the mean.

Table 3—Percent consumer acceptance of steaks in different color score groups

Visual color score[a]	Desirability score[b] (%)							Occurrence %	
	1	2	3	4	5	6	7	Desirable[c]	Undesirable[d]
2	5.2	5.2	15.6	18.2	23.4	16.2	16.4	56.0	26.0
3	0.4	1.3	4.4	11.4	28.8	27.1	28.9	82.8	6.1
4	0.5	1.3	7.1	14.9	30.9	28.0	17.5	76.5	8.9
5	1.7	3.6	10.7	18.4	28.1	20.8	16.9	65.8	15.9
6	3.0	2.0	17.5	20.0	22.0	20.0	23.5	65.5	22.5
7	26.7	15.7	28.7	15.0	5.3	3.7	4.3	13.3	71.0

[a]Color scores coded as follows: 2 = pale pink; 7 = moderately dark red.
[b]Desirability scores: 1 = extremely undesirable; 4 = neither desirable nor undesirable; 7 = extremely desirable.
[c]Combined percentages of steaks rated 5, 6 and 7
[d]Combined percentages of steaks rated 1, 2 and 3

regarding the acceptability of muscle color and the overall appearance of the individual steaks. Each consumer scored the samples using a seven-point hedonic scale (7 = extremely desirable; 1 = extremely undesirable). A total of 50 consumers evaluated each sample at each location.

The second steak (2 cm thick) was cut from each boneless loin strip at the time of fabrication, trimmed of all subcutaneous fat, cut to fit into a glass petri dish, and allowed to oxygenate (bloom) for 75 min. Following oxygenation, the color of each sample was measured by use of a Gardner Automatic Color Difference Meter (Model AC-1) by placing the uncovered surface of the steak over the instrument port. Readings for Rd, a and b were obtained with the instrument calibrated against a standard plate (5.9 Rd, 23.2 a and 6.6 b). Each sample was then measured with a Photovolt-610 Reflectance Meter standardized against a porcelain plate with a reflectance value of 75% T. Three individual readings were taken per sample using three different tristimulus color filters. A reflectance attachment to the Bausch and Lomb Spectronic-20 Spectrophotometer was then used to measure the color of each sample at 30 nm intervals from 415–685 nm. The Macbeth-Munsell Disk Colorimeter was used to obtain a visual color match for each sample, using Munsell standard disks for red, yellow, white and black, and values for hue, value and chroma were calculated. Results of all measurements were converted to C.I.E. values (X, Y and Z) for correlation analyses. The Photovolt Reflectance Meter and Gardner Color Difference Meter are tristimulus colorimeters and are described by Francis and Clydesdale (1969a). The Macbeth-Munsell system is a widely used system of color description which has been described by Munsell (1967) and Nickerson (1958). Several other reports have described the use of the reflectance attachments for spectrophotometers to measure color (Judd, 1952; Mackinney and Little, 1962; Billmeyer and Saltzman, 1966; and Francis and Clydesdale, 1969b).

Another steak (2.5 cm in thickness) from each loin was cooked to an internal temperature of 75°C in a 177°C oven and evaluated for tenderness, juiciness and flavor by a five-member trained taste panel by use of a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely). Samples were presented to the panel as 2.5 cm cubes and in groups of five samples per day. Three core samples (1.3 cm diam) from each steak were also evaluated for tenderness with the Warner-Bratzler shear. The cores were removed from the steak in a manner such that the muscle fibers were parallel to the coring device. Shear values were obtained after the core samples had cooled to 21°C.

The remaining steak from each loin was utilized for the chemical determination of pigment concentration. Total pigment concentration was determined according to the procedure of Rickansrud and Henrickson (1967).

Means, standard deviations and coefficients of variation were calculated for each trait within each color score group. Multiple regression analyses were computed according to the procedures outlined by Snedecor and Cochran (1967).

Table 4—Mean values for the palatability attributes of steaks from each color score group

Visual color score	Statistic	Flavor	Juiciness	Tenderness
2	Mean	6.92[a]	6.00[a]	6.84[a]
	SD[b]	0.36	0.84	0.59
	CV[b]	5.25	13.94	8.62
3	Mean	6.56[a]	6.40[a]	6.94[a]
	SD	0.53	0.92	0.69
	CV	8.11	14.32	10.00
4	Mean	6.57[a]	6.33[a]	6.88[a]
	SD	0.68	0.79	0.78
	CV	10.49	12.52	11.28
5	Mean	6.51[a]	6.15[a]	6.40[a]
	SD	0.48	0.66	1.21
	CV	7.50	10.76	18.38
6	Mean	6.35[a]	6.48[a]	6.67[a]
	SD	0.49	0.70	2.37
	CV	7.70	10.79	35.58
7	Mean	6.20[a]	6.64[a]	7.32[a]
	SD	0.69	0.77	2.11
	CV	11.17	11.54	28.82
Overall	Mean	6.53	6.32	6.79
	SD	0.57	0.77	1.20
	CV	8.72	12.23	17.67

[a]Means do not differ significantly ($P < 0.05$) from other means in the same column.
[b]SD = standard deviation; CV = coefficient of variation.

RESULTS & DISCUSSION

DATA in Table 2 provide a description of the samples within each visual color score group in terms of Munsell notation and physical and chemical characteristics. These data indicate that both value (lightness or darkness) and chroma (intensity of color) decreased as the subjective visual color score increased. A comparison of means indicates no significant difference in hue among visual color score groups, but the darkest steaks (those with the highest visual color scores) exhibited greater variability in hue than did those in lighter color score groups. Significant differences ($P < 0.05$) in value were noted between samples scored 2 vs. 3, 3 vs. 4, 4 vs. 6 and 5 vs. 7 and in chroma for 4 vs. 5 and 6 vs. 7. Therefore, visual color appears to become darker due to increased saturation of black and a reduction of the natural red hue. Data in Table 2 also indicate that samples with visual color scores of 2 or 3 had significantly less ($P < 0.05$) total pigment than those scored 5 or 6. The darker colored steaks had coarser texture than those which were light in color. The significantly higher pH for steaks with a visual color score of seven probably resulted from the inclusion of some "dark cutters" in this group.

A comparison of consumer acceptability of the steaks from each visual color score group is presented in Table 3. Steaks with visual color scores of seven were rated extremely undesirable by nearly 27% of the consumers interviewed,

Table 5–Simple correlation coefficients of physical characteristics and objective color measurements with color scores and total pigment concentration

Measurement	Visual color	Total pigment
Hue	0.09	−0.28*
Value	−0.81**	−0.32*
Chroma	−0.73**	−0.00
Gardner		
Rd	−0.79**	−0.38**
a	−0.67**	0.09
b	−0.78**	−0.12
X	−0.80**	−0.31*
Y	−0.79**	−0.38**
Z	−0.71**	−0.46**
Photovolt		
Blue	−0.68**	−0.53**
Green	−0.70**	−0.41**
Amber	−0.72**	−0.30*
X	−0.61**	−0.18
Y	−0.70**	−0.41**
Z	−0.68**	−0.54**
Spectronic-20 Reflectance		
415 nm	−0.26	−0.40**
445 nm	−0.42**	−0.41**
475 nm	−0.28*	−0.28*
505 nm	−0.64**	−0.27
535 nm	−0.57**	0.39**
565 nm	−0.56**	−0.41**
595 nm	−0.75**	−0.40**
625 nm	−0.77**	−0.16
655 nm	−0.76**	−0.15
685 nm	−0.74**	−0.25
X	−0.81**	−0.31*
Y	−0.80**	−0.38**
Z	0.08	−0.03
Macbeth-Munsell		
Red	−0.79**	−0.17
Yellow	−0.73**	−0.39**
White	−0.62**	−0.50**
Black	0.81**	0.25
X	−0.82**	−0.32**
Y	−0.81**	−0.37**
Z	−0.76**	−0.41**
pH	0.55**	0.01
Marbling	−0.11	−0.11
Texture	−0.60**	−0.30**

*($P < 0.05$)
**($P < 0.01$)

Table 6–Comparison of means and standard deviations for the parameters of beef color acceptable to consumers

		Visual color scores			
		2		6	
Objective measure	Standard	Mean	SD	Mean	SD
Total pigment, mg/g	Chemical	4.34	0.81	5.41	1.52
Macbeth-Munsell					
Disk Colorimeter	Red	29.14	3.07	15.35	7.83
	Yellow	5.58	1.39	2.05	0.93
	White	2.04	0.38	0.45	0.16
Gardner Color Difference					
Meter	Rd	10.66	1.17	7.13	1.00
	a	29.06	3.14	23.25	5.23
	b	11.70	1.08	8.93	1.57
Photovolt-610					
Reflectance Meter	Blue	7.30	1.04	4.83	0.93
	Green	10.50	1.87	7.17	1.54
	Amber	20.10	3.85	11.75	4.60
Bausch and Lomb					
Spectronic-20					
Spectrophotometer	415 nm	5.00	0.35	3.83	0.61
	445 nm	8.00	1.90	6.17	1.69
	475 nm	12.60	0.82	9.83	3.01
	505 nm	13.90	2.92	9.08	1.84
	535 nm	8.60	1.52	6.00	0.71
	565 nm	11.60	3.78	7.42	2.18
	595 nm	17.80	2.84	9.83	2.32
	625 nm	37.00	3.92	21.25	7.03
	655 nm	43.00	4.34	25.17	8.22
	685 nm	43.80	3.01	27.08	7.57

while approximately 29% of the consumers rated steaks which were assigned a color score of three as extremely desirable. Approximately 83% of the consumers rated the steaks with a visual score of three as desirable and 77% of the consumers rated steaks with a score of four as desirable. However, only 56% and 13% of the consumers assigned desirable ratings to steaks which were scored two and seven in visual color, respectively. These data indicate that consumers prefer steaks which are neither extremely dark nor extremely pale. Only 6% of the consumers rated steaks with a color score of three as undesirable and 9% of the consumers assigned undesirable ratings to steaks assigned a color score of four. Therefore, in this study the consumers preferred steaks that were more pale than the "cherry red" previously proposed as "ideal" (Allen, 1968).

Palatability ratings for steaks assigned to the various color score groups are presented in Table 4. Mean scores for flavor, juiciness and tenderness did not differ among steaks in different color score groups. These data suggest that muscle color is not an accurate indicator of beef palatability. When pH, visual color score, marbling score and texture score were included as independent variables in multiple regression equations, only 10% of the variation in flavor and juiciness and 6% of the variation in tenderness could be explained.

The simple correlation coefficients presented in Table 5 indicate that both value and chroma were significantly related ($P < 0.01$) to visual color scores. However, the relationship between hue and visual color score was low and nonsignificant. All objective measures of color with the Gardner, Photovolt, and Macbeth-Munsell instruments were significantly related to the visual color scores ($P < 0.01$). All readings on the Bausch and Lomb Spectronic-20 were significantly related to subjective color scores except the reading at 415 nm and the calculated value for Z. Both hue and value were significantly ($P < 0.05$) related to the total pigment concentration. The Rd measurement from the Gardner was significantly related to total pigment concentration as were the X, Y and Z notations calculated from the Gardner readings. The measurements with the Photovolt were significantly ($P < 0.01$) related to pigment concentration. Both the Y and Z notations from the Photovolt were significantly related ($P < 0.01$) to total pigment concentration, but the X notation was not. The simple correlation coefficients derived for comparison of measurements obtained by use of the Macbeth-Munsell system and the Bausch and Lomb Spectrophotometer with total pigment concentration were quite variable and generally low in magnitude (Table 5). Both muscle pH and texture were significantly related to the visual color scores ($P < 0.01$); muscle texture was also related to total pigment concentration ($P < 0.05$). Simple correlation coefficients were computed for all of the measurements in the first column of Table 5 with flavor, juiciness and tenderness ratings. With the exception of hue, all of the measurements (including X, Y and Z) had low and nonsignificant relationships to the palatability attributes. Hue was correlated −0.30, −0.31 and −0.36 (all $P < 0.05$) with ratings for

Table 7—Comparative accuracy of certain combinations of color measurements in predicting visual color scores for beef steaks

Method	Reference standard or measurement	Visual color scores R^2	b'
Macbeth-Munsell			
Disk Colorimeter	Red	0.68	–0.30
	Yellow		0.07
	White		–0.19
	Black		0.34
Gardner Color Difference			
Meter	Rd	0.68	–0.21
	a		0.62
	b		–1.20
Photovolt-610			
Reflectance Meter	Blue	0.55	–0.11
	Green		–0.24
	Amber		–0.42
Bausch and Lomb			
Spectronic-20			
Spectrophotometer	415 nm	0.68	–0.03
	445 nm		0.06
	475 nm		0.05
	505 nm		–0.11
	535 nm		0.08
	565 nm		–0.25
	595 nm		–0.20
	625 nm		–0.37
	655 nm		–0.29
	685 nm		–0.17
Total pigment and pH	pH	0.40	0.55
	Total pigment		0.31

flavor, juiciness and tenderness, respectively.

Data in Table 6 include the means for objective color measurements for steaks included in visual color groups 2 and 6. It is suggested that steaks with colorimetric readings above (for color group 2) or below (for color group 6) these limits would be unacceptable to a majority of the consumers in the geographical area sampled in the present study. Based upon this assumption, it may be practical and helpful to utilize these limits as criteria for objectively identifying cuts which are likely to be considered unacceptable by the consumer.

Standard partial regression coefficients and coefficients of determination for objective measures of muscle color are presented in Table 7. These data indicate that visual muscle color score can be predicted with almost equal accuracy by using the Macbeth-Munsell, Gardner or Bausch and Lomb systems, while the Photovolt system accounted for 13% less of the variation in visual muscle color scores than the other systems. The objective measures of muscle color accounted for considerably more of the variation in visual muscle color scores than did total pigment concentrations and pH. The standard partial regression coefficients included in Table 7 indicate that the red and black Munsell readings were relatively more important than the white and yellow readings in accounting for differences in subjective muscle scores and that the yellow reading was of least importance. Similar comparisons for the Gardner apparatus indicated that the reading for "b" was twice as important as the reading for "a" and six times as important as the reading for Rd in accounting for variations in visual color scores. Readings at wavelengths of 625 and 655 on the Bausch and Lomb Spectronic-20 were most closely related to visual color scores. Values indicate that pH was nearly twice as important as total pigment concentration in accounting for the variation in visual color scores.

Data from the present study indicate that pigment concentration does not serve as a reliable guide for monitoring visual color of beef cuts. It is concluded that a variety of instruments may be used for estimating meat color. The description of acceptable limits for beef color using objective measures could provide a basis for more meaningful comparisons in future research studies and would be useful to industry.

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Ms received 11/15/71; revised 1/31/72; accepted 2/3/72.

T.A. 9531, Texas Agricultural Experiment Station. This study was partially supported by a grant from the American Meat Institute Foundation.

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DEVELOPMENT OF A PROTOTYPE SAUSAGE EMULSION PREPARATION SYSTEM

INTRODUCTION

THE SCIENCE of meat emulsions has been investigated extensively in recent years, but it remains a fertile field for future research. Much of the knowledge on the properties of sausage emulsions has been obtained with commercial production-type equipment. When commercial equipment is used, large quantities of meat and other ingredients are required, which increases the cost of experimentation. Also, commercial conditions, and even pilot-size operations, are difficult to control closely. These problems, therefore, indicated the need for an efficient and a highly controllable laboratory model system for preparing sausage emulsions in order to evaluate a variety of emulsion parameters, involving a minimum of materials, time and labor.

A widely accepted practice for the preparation of sausage emulsions has been to utilize three stages: (a) grinding, (b) chopping and mixing in a cutter and/or (c) comminution in an emulsion mill or emulsitator. Presently, grinders, mixers, cutters, colloid mills and emulsitators are the principal types of machines utilized by the sausage industry. In general, the equipment increases the degree of fineness, amount of air incorporated, temperature of the product and surface area.

Model systems have been developed for studying dilute protein extracts and selected variables (Swift et al., 1961; Hegarty et al., 1963; Carpenter and Saffle, 1964; Trautman, 1964; Webb et al., 1970; Tsai et al., 1970).

Recently, laboratory model systems for preparing sausage emulsions have been developed by Ockerman and Cahill (1970) and Morrison et al. (1971). Ockerman and Cahill (1970) developed micro-chopper, micro-stuffer and micro-smokehouse units, but two persons were required for the satisfactory operation of the units. Morrison et al. (1971) developed a three-stage system for preparing sausage-type emulsions on a laboratory basis. These systems were found to be of significant value in preparing laboratory sausage products; however, higher efficiency, more control and greater precision were needed to prepare emulsions under closely controlled conditions.

The purpose of this investigation was to develop and construct an efficient and controllable laboratory model system for preparing sausage emulsions in order to evaluate a variety of emulsion parameters.

MATERIALS & METHODS

Sampling procedure

Muscle tissue samples were taken from beef top rounds, trimmed free of surface fat and connective tissue, ground through a 3/8 in. plate and then mixed thoroughly. The sample was divided in lots of 250g each, placed in Whirl-Pak bags and stored at −27°C. The frozen samples were thawed at 2°C for 48 hr, reground three times through a 3/16 in. plate and thoroughly mixed with the excess drip loss. The beef samples were then used for analytical purposes and emulsion preparation. Total moisture and fat were determined on the muscle tissue samples by the procedures outlined in AOAC (1965).

Formula composition

Sausage emulsions were prepared with beef muscle tissue, soybean oil, water and salt. Formula calculations were made as described by Morrison et al. (1971). Table 1 shows sample formulae for the two meat:water ratios and three total lipid levels used. The procedure of Morrison et al. (1971) was modified to facilitate the preparation of emulsions with total lipids higher than 65%. A base emulsion containing 50% total lipids was first prepared and a calculated amount of the emulsion was replaced with soybean oil to arrive at the desired lipid level while maintaining the total volume. The meat:water ratio was defined as the ratio of the lean muscle tissue to the total added water. The evaluation of performance of all models tested was accomplished by using a total lipid level of 50% and a meat:water ratio of 1.5:1.0. In addition, selected model systems were evaluated for performance at 15% and 65% total lipid levels at a meat:water ratio of 2.0:1.0. Salt level was kept constant at 3% of the combined weight of lean muscle tissue plus total added water.

Chopping time

The total chopping time in each stage of emulsion preparation was recorded with a stop watch.

Cook stability

The method of Townsend et al. (1968), with slight modification, was used to measure the cook stability (CS) of the emulsions. 30g of uncooked emulsion were placed in a centrifuge tube (20 mm × 108 mm) and cooked for 30 min in a 70°C water bath. The cook stability was reported as a percentage by calculating the ratio of the weight of the cooked, unreleased emulsion to that of the uncooked emulsion, multiplied by 100.

Physical properties

The panel rating system, developed by Morrison et al. (1971), for scoring the physical properties (PP) of cooked emulsions by sensory evaluation was used with slight modification (Table 2).

A physical properties score (PP score) of 21 or higher was considered as a satisfactory emulsion, provided that no individual property had a score less than three on the four-point scale or four on the five-point scale.

RESULTS & DISCUSSION

THE EQUIPMENT, points of adding ingredients and the various stages of emulsion preparation and evaluation for the

Table 1—Composition of formulae used for the preparation of sausage-type emulsions[a]

	Meat:water ratio (1.5:1.0)	Meat:water ratio (2.0:1.0)	
	% total lipids	% total lipids	
Ingredients	50 (g)	15 (g)	65 (g)
Beef muscle tissue	51.5	103.6	37.6
Soybean oil	79.4	21.8	97.0
Water	29.1	34.6	15.4
Salt	2.4	4.1	1.6

[a]The amounts of added oil and added water were adjusted in specific formulae to compensate for the lipid and water present in the meat tissue to obtain the specified total water and lipid levels by the procedures of Morrison et al. (1971).

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Table 2—Panel rating system for scoring physical properties of cooked sausage emulsions

Description	Score
Color	
light ivory to ivory	...4
light tan	...3
dark tan	...2
brown	...1
Resilience	
good: when pressed returns to form rapidly	...5
fair: when pressed returns to form moderately rapid	...4
poor: when pressed returns to form slowly and only partially	...3
very poor: only slight suggestion of return to form	...2
none: as in mushiness	...1
Graininess	
smooth	...4
fine grain	...3
medium grain	...2
coarse grain	...1
Firmness	
firm (normal)	...5
slightly firm	...4
soft	...3
very soft	...2
mushy	...1
Binding	
good (normal)	...5
fair	...4
poor	...3
very poor	...2
none	...1

Mixer Omni Base system of Morrison et al. (1971) (M-O B system) are shown schematically in Figure 1. The two subsequent model systems which were developed by modifying the M-O B system were designated as the Mixer Omni Base 1 system (M-O B1) and the Mixer Omni Base 2 system (M-O B2). These three systems were evaluated by preparing emulsions with 50% total lipids and a meat:water ratio of 1.5:1.0.

The work of Morrison et al. (1971) showed that the emulsion yielded a CS higher than 90% but a PP score less than 21 (Table 3). The total chopping time for their system was approximately 340 sec. The first two stages (I and II, Fig. 1) of the M-O B1 and M-O B2 systems were accomplished in the same manner as described by Morrison et al. (1971). The base unit of stage III was modified by fitting two 1/8 in. thick plastic plates on opposite walls of the chopping bowl (200 ml Omni cup) to develop the M-O B1 and M-O B2 systems. In stage III for the M-O B1 system, high speed comminution was done to simulate an emulsitator by chopping three times, intermittently (15 sec each), with the Omni mixer set at 12,800 rpm.

The M-O B1 system had a total chopping time of 320 sec. The CS and PP scores on the cooked samples were 98.83% and 23, respectively. These data indicated that the plastic plates improved the degree of mixing and chopping.

The M-O B2 system differed from the M-O B1 system only by chopping continuously for 30 sec rather than three intermittent chopping times during stage III. The total chopping time was reduced to 300 sec and the CS and PP scores were 99.02% and 23, respectively. Therefore, the addition of the plastic plates allowed the emulsion to be prepared while continuously chopping during stage III.

On the basis of the work completed by Morrison et al. (1971) and the two modifications of their system (M-O B1 and M-O B2 systems), a single unit system was developed and designated as the Cutter Mixer Base system (C-M B system). This system was further modified twice (C-M B1 and C-M B2), evaluated each time, until a final operative system was developed. The basic features of the chopping unit are included in the schematic diagram of the finished model which was designated as Cutter Mixer Base 2 (C-M B2, Fig. 2). The major components and features of the chopping unit are as follows: A stainless steel cylindrical bowl, 3-3/8 in. x 4-1/4 in., was selected for use as the chopping bowl (Fig. 2, a). A 3/16 in. copper tubing was coiled around the outside of the bowl to serve as a cooling jacket (Fig. 2, b). A Forma-Temp-Baths unit was used to circulate a cooling liquid through the cooling jacket (Fig. 2, k). The bowl was nickel-copper plated to improve the cooling effect. Waring Blendor blades (Fig. 2, c) (Cat. No. 70012) were fixed in the center of the bottom of the bowl. Two rubber gaskets were used to hold the mixing blades inside the bowl. A Sunbeam blender base (model 800) was used as the motor base unit (Fig. 2, d). The bowl was mounted on a support stand (Fig. 2, e) constructed to fit on the motor base. Two scraper blades (Fig. 2, f) were developed to deliver the emulsion from

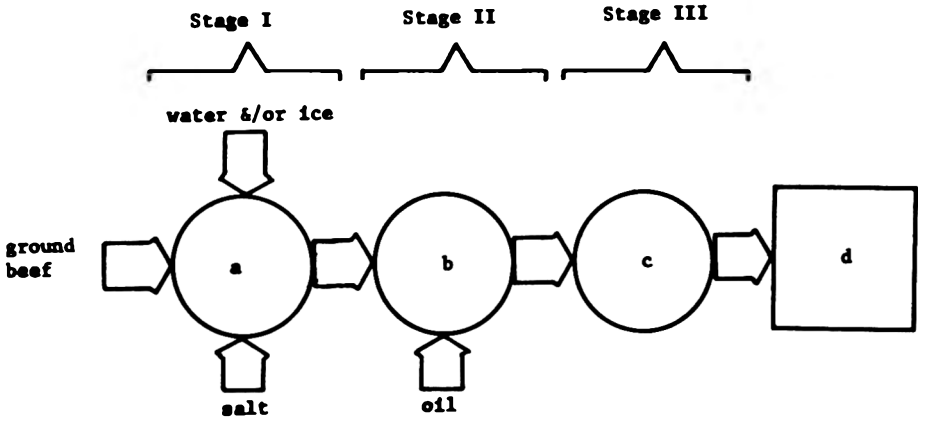

Fig. 1—Schematic diagram of the equipment, points of adding ingredients and the various stages of emulsion preparation and evaluation for the Mixer Omni Base, Base 1 and Base 2 systems. (a) Counter rotating mixer, initial mixing; (b) Counter rotating mixer, low speed; (c) Omni mixer, high speed; (d) Evaluation.

the bowl walls into the cutting blades. A Lightnin motor (model L, 1/30 h.p., 1750 rpm) was selected to power the scrapers. The direction of rotation of the scrapers was opposite to that of the mixing blades.

In the C-M B system, the CS and PP scores on the cooked samples were 98.90% and 22, respectively, which was rated as a good quality emulsion (Table 3). The chopping time was reduced from 300 to 250 sec without an appreciable change in stability. However, a substantial rise in temperature occurred. Thus, the C-M B system resulted in a good quality emulsion, as evaluated by CS and PP, but the system was not as efficient as desired. The ingredients had to be stirred several times with a rod during emulsion preparation and the oil added stepwise. Also, small quantities of the ingredients were not thoroughly mixed and remained in remote areas of the bowl.

The system was further modified and designated as the Cutter Mixer Base 1 system (C-M B1 system). In the C-M B1 system, three wax baffles (Fig. 3, b) were introduced into the bowl in order to fill the areas in which limited mixing occurred. It was observed that mixing was improved and that there was no necessity to stir the materials while preparing the emulsion. However, it was necessary to initially stir the beef, ice and salt until a thorough mixture was attained. The total chopping time was reduced from 250 to 210 sec and the CS and PP scores were 98.60% and 22, respectively (Table 3). The temperature rise during the chopping operation was substantial (22.5 ± 1.0°C) and this was attributed to the poor heat conduction of the wax in the baffles.

Following the evaluation of the C-M B1 system, major modifications were made and the completed system designated as the C-M B2 system. The completed chopping unit (C-M B2) with the final modifications is shown in Figure 2, as described previously.

To attain the desired system, the essential design features were accomplished as follows: For the areas of the bowl where the material was left unmixed, a parabolic contour design (O) (2 in. x 15/16 in.) was selected and filled with plastic metal (#98069, Sears, Roebuck and Co., Chicago, Ill.). Plastic metal was selected because of its good heat conducting and molding properties. The required quantity of the plastic metal was molded in the bottom of the bowl to give the desired parabolic contour. The required contour was generated by a mold. Immediately after molding the required shape, two stainless steel plate electrodes (Fig. 2, p), 1/4 in. x 1 in., shaped to conform to the parabolic bottom portion of the bowl, were embedded. The electrodes were connected to a resistance measuring unit. The Waring Blendor blades (Fig. 2, c) were modified so that the top and bottom blades made 20 degrees with the horizontal. The top and bottom blades were twisted counter clockwise by 8 and 2 degrees near the tips, respectively. The number of rubber gaskets for fixing the blades inside the bowl was increased from 2 to 5. An oil delivery tube (3/16 in. diam) (Fig. 2, m) was introduced into the bowl to eliminate removal of the scraper assembly from the bowl each time oil was added. A pressure pump was used to apply a slight positive pressure to the oil to attain the proper flow rate. The valve on the pressure pump was set to give an oil flow rate of 4 ml/sec. A vent hole was made near the top end of the cutting bowl to prevent development of back pressure. A thermocouple (Fig. 2, r, copper-constantan) was introduced into the bowl at a level 3/4 in. from the bottom and from the side opposite to the oil-delivery tube. The temperature sensing portion of the thermocouple was protruded 1/4 in. into the bowl and was insulated with the plastic metal, which is an electrical insulator but a good heat conductor. Therefore, the temperature measurements were not affected by the electrical charges present in the emulsion system or by the electrical signal passed through the emulsion.

A schematic diagram of the complete Cutter Mixer Base 2 system is shown in Figure 4 which shows the various components previously described. The complete C-M B2 system was operated as described below by one individual.

The cooling liquid (−6°C) was circulated through the cooling coils and after 10–20 min the emulsion preparation was started. The required quantities of beef, ice and salt were placed in the bowl. The scraper assembly was mounted on the top

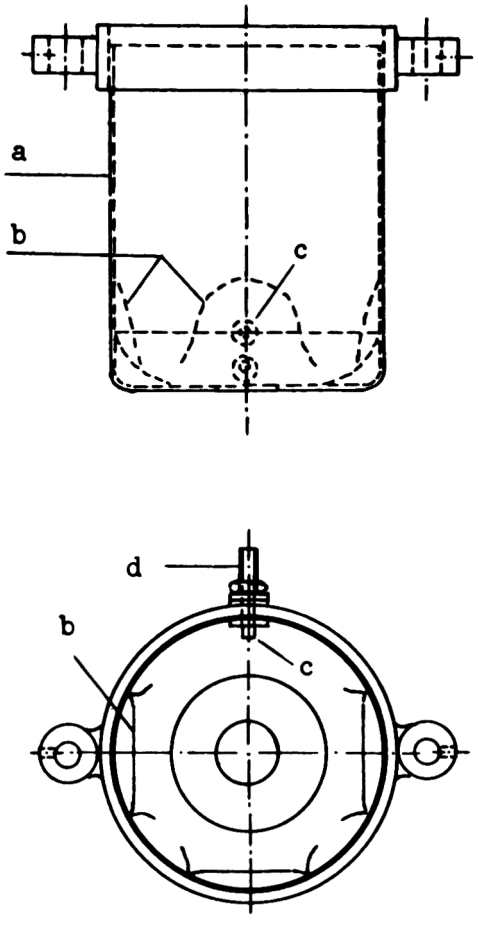

Fig. 2–Schematic diagram of the chopping unit for the Cutter Mixer Base 2 system.

Table 3–Effect of various model systems on chopping time, cook stability and physical properties of sausage emulsions[a]

Model system	No. of trials	Chopping time (sec)	Terminal chop temp (°C)	Cook stability (%)	Physical properties score
Mixer Omni Base[b] (M-O B)	–	340	15.0 ± 1.0	> 90.00	< 21
Mixer Omni Ia (M-O Ia)	6	320	19.0 ± 1.5	98.83 ± 0.00	23 ± 0
Mixer Omni Ib (M-O Ib)	9	300	16.5 ± 2.0	99.02 ± 0.39	23 ± 0
Cutter Mixer-Base (C-M B)	18	250	21.0 ± 2.0	98.90 ± 0.46	22 ± 0
Cutter Mixer I (C-M I)	14	210	22.5 ± 1.0	98.60 ± 0.55	22 ± 0
Cutter Mixer II (C-M II)	9	160	16.5 ± 1.0	98.65 ± 0.23	22 ± 0

[a]Formula: 50% total lipids at meat:water ratio = 1.5:1.0
[b]From Morrison et al. (1971)

of the bowl. The scraper motor and the motor base were started simultaneously. After a prescribed chopping time, the pressure pump was started and the valve on the separatory funnel was opened. The speed on the motor base was immediately switched from 14,900 rpm to 17,300 rpm. It was found necessary to allow 100, 60 and 20 sec before starting the pressure pump for the preparation of the emulsions with 15, 50 and 65% total lipids, respectively. Mixing was continued for 10 sec after all of the oil had been added and the time required for total chopping was recorded.

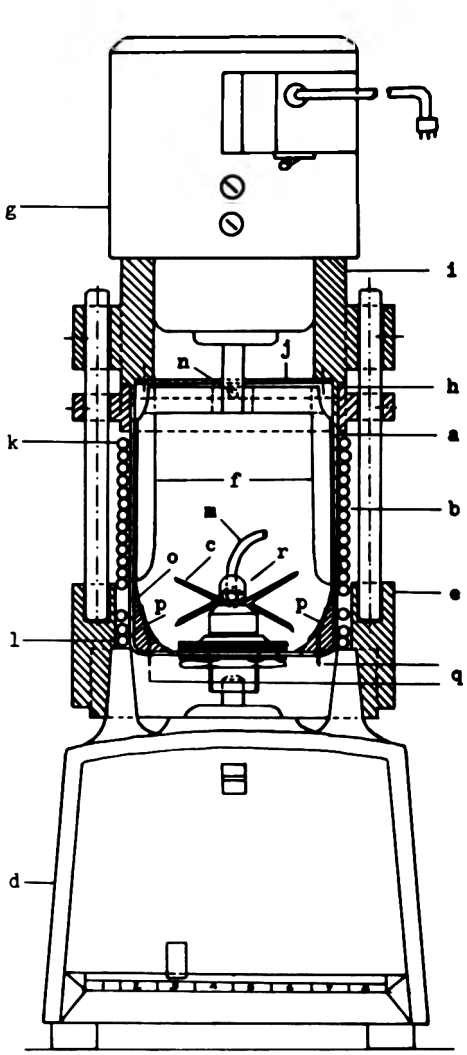

Fig. 3—Schematic diagram of the chopping bowl for the Cutter Mixer Base 1. (a) Cutter mixer bowl; (b) cooling jacket; (c) mixing blades; (d) motor base; (e) support stand; (f) scrapers; (g) scrapers motor; (h) bowl mounting bracket; (i) motor mounting bracket; (j) lid; (k) to cooling system; (l) from cooling system; (m) oil delivery tube; (n) vent hole; (o) parabolic bottom; (p) electrodes; (q) connections to resistance measuring system; (r) thermocouple.

The total chopping time in the C-M B2 system was reduced to 160 sec compared to 210 sec for C-M B1 system, with no major differences in CS and PP observed (Table 3). These results indicated that the C-M B2 system was the most efficient system of all the systems developed and resulted in very acceptable CS and PP for emulsions prepared with 50% total lipids and a meat:water ratio of 1.5:1.0.

Two more composition formulae, one at a low lipid level (15%) and the other at a high lipid level (65%), were tested in the M-O B2 and C-M B2 systems and the results were compared with the M-O B system (Table 4).

The work of Morrison et al. (1971) indicated that the emulsions with 15% total lipids and meat:water ratio 2.0:1.0 would yield a CS of less than 90% and PP scores of less than 21. The data presented in Table 4 show that the M-O B2 and C-M B2 systems yielded CS of 93.85% and 98.11%, respectively, and PP scores of 21. The CS and PP of emulsions prepared with the C-M B2 system were of good quality and the total chopping time was reduced. It is noteworthy that the chopping time was substantially less for the low lipid level (15%) than for high lipid levels (50% and 65%). These results indicated that the C-M B2 system was the most efficient system and yielded the highest CS of the emulsions with low lipid levels.

For the emulsions with 65% total lipids and meat:water ratio of 2.0:1.0, the work of Morrison et al. (1971) showed that the CS and the PP scores were less than 90% and 21, respectively. The M-O B2 system yielded the highest CS and PP scores of any of the systems. The emulsions prepared in the C-M B2 system were partially inverted as indicated by the CS (63.64%) and PP (9) scores. The investigators concluded that this was the result of high speed, short time chopping which resulted in emulsion breakdown. However, high lipid levels are not closely related to practical sausage

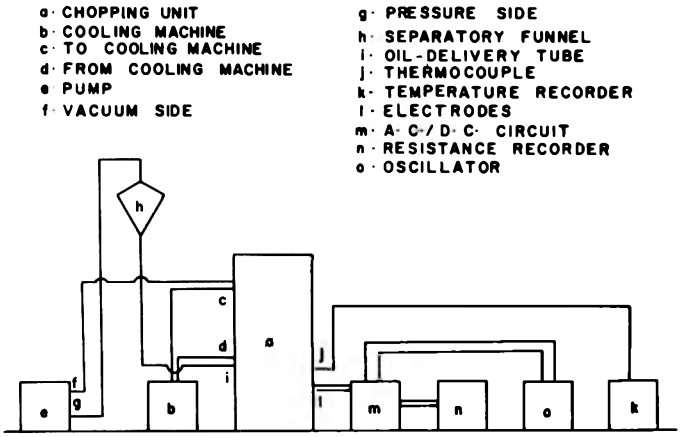

Fig. 4—Schematic diagram of the Cutter Mixer Base 2 system.

Table 4—Effect of various model systems on cook stability and physical properties of sausage emulsions

Model system	No. of trials	Chopping time (sec)	Terminal chop temp (°C)	Cook stability (%)	Physical properties score
Low lipid formula[a]					
Mixer Omni Base[b]	–	185	15.0 ± 1.0	< 90.00	< 21
Mixer Omni Base 2	9	165	13.0 ± 1.5	93.85 ± 2.64	21 ± 0
Cutter Mixer Base 2	3	160	14.0 ± 1.0	98.11 ± 0.19	21 ± 0
High lipid formula[c]					
Mixer Omni Base[b]	–	340	15.0 ± 1.0	< 90.00	< 21
Mixer Omni Base 2	6	300	20.0 ± 1.0	98.98 ± 0.26	23 ± 0
Cutter Mixer Base 2	6	140	15.0 ± 1.0	63.64 ± 2.31	9 ± 0

[a]Low lipid formula: 15% total lipids at meat:water ratio 2.0:1.0
[b]From Morrison et al. (1971)
[c]High lipid formula: 65% total lipids at meat:water ratio 2.0:1.0

production. Therefore, the C-M B2 system was selected over the M-O B2 system on the basis of results with 15% and 50% lipid levels.

Thus, with the C-M B2 system, which incorporated the features of mixing scrapers, cooling systems, oil delivery, resistance electrodes, temperature measurement and multispeed motor base, many valuable research studies can be satisfactorily conducted on sausage emulsions. These can be obtained with a minimum of time and materials.

With the addition of cooling coils and a temperature thermocouple, the maximum temperature attained during chopping can be controlled and any desired emulsion temperature can be obtained.

With the use of the oil delivery tube, emulsions with any desired level of lipid, added water, proteins or meat:water ratio can be prepared. However, the use of solid fats, such as pork or beef fats, would require the addition by removal of the lid assembly which can be accomplished with a minimum of delay.

The speeds on the cutter mixer base motor can be varied and the effect of chopping speed on the emulsion stability can be studied.

By connecting the electrodes to a resistance recording system, the resistance variation during an emulsification process can be studied and a relationship between electrical resistance and stability of emulsions can be established (Haq et al., 1971).

The effect of vacuum chopping of emulsions can be studied by utilizing the vacuum port (vent hole can be used for vacuum port) shown in Figure 4.

It is suggested that further research be conducted on the effects of process variables on the stability of emulsions. This is based upon the results obtained in this study whereby chopping time was greatly reduced while maintaining good stability with the use of frozen meat and soybean oil. The system is applicable to the study of a wide variety of ingredients. The system was developed by the use of a simple model formula. However, further investigations can be conducted with such ingredients as fresh meats, animal fats, binders, etc., to determine more precise relationships between processing variables and ingredients for practical operating conditions.

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Ms received 12/14/71; revised 2/12/72; accepted 3/4/72.

Paper No. 3645 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, N.C. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named, nor criticism of similar ones not mentioned.

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POST-IRRADIATION EVALUATION OF PATHOGENS AND INDICATOR BACTERIA

INTRODUCTION

BACTERIAL CULTURES exposed to an adverse environment commonly contain injured cells incapable of growth in selective media (Licciardello et al., 1970; Maxcy, 1970; Ordal, 1970; Sorrells et al., 1970; Speck, 1970). Recovery of some of the cells of certain species has been shown to occur when incubated in a complete medium (Ordal, 1970; Speck, 1970). Radurization of food has been shown to produce injured cells with an accompanying reduction in numbers recovered on commonly used selective media (Corry et al., 1969; Licciardello et al., 1970). The recovery to some degree is dependent on the nature of the selective medium. Since the mechanism of recovery is not understood, it is necessary to evaluate the phenomena in terms of influence of various food processing and storage operations to understand its significance in radurized foods.

Through recycling and subculturing it is possible to increase the radiation tolerance of a pure culture, which exhibits some altered morphological and physiological characteristics (Licciardello et al., 1969; Epps and Idziak, 1970). Altered cultures, however, do not show an increased infectivity. Cells injured by radiation may also be considered as altered, but the comparative infectivity of injured cells has not been studied.

Though radurization of fresh meat products is not presently accepted for commercial use, the efficacy of the process is generally recognized. The need for destruction of pathogens in meat as well as in many other fresh food products is also generally recognized. Irradiation may provide a technology to allow pasteurization and maintenance of foods in presently accepted commercial fresh forms. The adoption of irradiation processing may depend on weighing of hazards, if any, from radiation against the well documented presence of microorganisms of public health significance. A fuller understanding of these processes with the benefits and hazards is therefore desirable.

The object of this work was to determine the post-irradiation behavior of selected species of *Salmonella* and certain indicator organisms of public health significance.

MATERIALS & METHODS

Bacteria

Escherichia coli, Streptococcus faecalis and *Staphylococcus aureus* were from the departmental stock culture collection. *Salmonella typhimurium, Salmonella enteritidis, Salmonella heidelberg* and *Salmonella senftenberg* were supplied by the Communicable Disease Center, Atlanta, Ga. Cultures were maintained on Plate Count Agar (PCA) slants. Before use in the experiments, the organisms were subcultured daily at least twice in nutrient broth at 37°C (*S. faecalis* was grown at 25°C) for 20 hr to provide cultures at the stationary growth phase.

Irradiation of cultures

A cobalt-60 source similar to that described by Teeny and Miyauchi (1970) providing 17 Krad of gamma radiation per min was used. Cultures were grown to the stationary phase in Erlenmeyer flasks, dispensed into 125 × 16 mm tubes, and irradiated. Immediately after irradiation a suitable portion was removed for plating and the remainder was stored at desired temperatures for further observation.

Recovery of the organisms from culture media

All media were Difco products unless specified. Numbers of viable bacteria were determined by pour plating on PCA, which served as the standard to which selective media were compared. *Salmonella* counts on selective media were made by pour plating on Brilliant Green Agar (BGA) and by spreading on Salmonella-Shigella Agar (SSA). The selective media were Violet Red Bile Agar (VRBA) for *E. coli*, Staphylococcus Medium 110 for *S. aureus* and Citrate Azide Agar (CAA; Saraswat et al., 1963) for *S. faecalis*. To detect metabolic injury, counts of *Salmonella* and *E. coli* were also

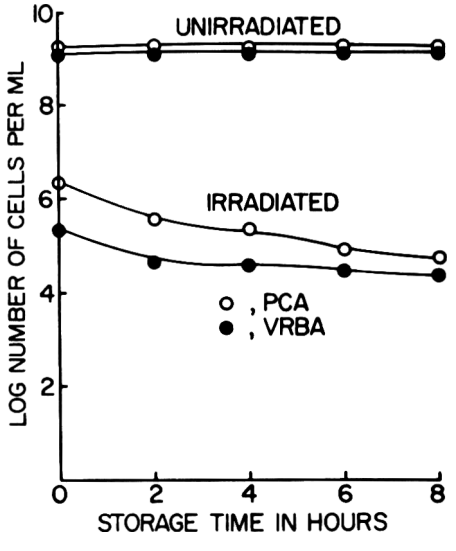

Fig. 1—The effect of irradiation (68 Krad) and storage at 37°C on injury and death of S. typhimurium *in the stationary growth phase.*

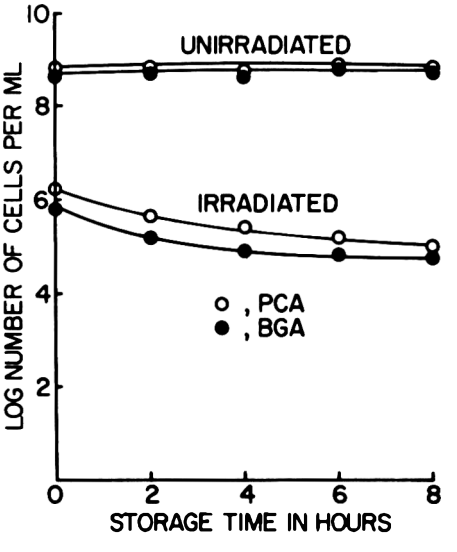

Fig. 2—The effect of irradiation (68 Krad) and storage at 37°C on injury and death of E. coli *in the stationary growth phase.*

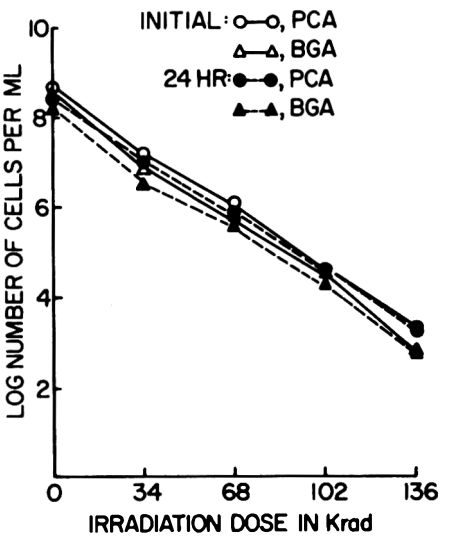

Fig. 3—The effect of dose level on the destruction and post-irradiation recovery of S. typhimurium *after 24 hr at 5°C.*

Table 1–Influence of storage and media on post-irradiation recovery of S. typhimurium

Media	Unirradiated		Irradiated	
	Initial count per ml	Count per ml after 24 hr at 5°C	Initial count per ml	Count per ml after 24 hr at 5°C
Liquid media:				
Lactose broth	1.3×10^9	1.3×10^9	2.0×10^6	1.2×10^6
Selenite-cystine broth	9.7×10^8	1.2×10^9	5.4×10^5	5.2×10^5
Tetrathionate broth	9.6×10^8	6.6×10^8	1.3×10^5	4.2×10^4
Solid media:				
Plate count agar	5.8×10^8	6.0×10^8	6.0×10^5	4.3×10^5
Brilliant green agar	5.5×10^8	5.0×10^8	4.2×10^5	2.6×10^5

made on minimal agar (MA) consisting of $NH_4H_2PO_4$, 0.3%; K_2HPO_4, 0.2%; iron as $FeSO_4 \cdot 7H_2O$, 0.5 ppm; $MgSO_4 \cdot 7H_2O$, 0.05%; glucose, 0.3%; agar, 1.5%. The pH was adjusted to 7.0 by the addition of 5N KOH. Solutions of glucose and $MgSO_4$ were autoclaved separately and added prior to plating. Difference in count obtained on the most favorable growth medium (PCA) and that obtained on a selective medium was considered to be due to injured cells not capable of growth on the latter.

A tube dilution method using Lactose Broth (LB), a common pre-enrichment medium, was used to estimate *Salmonella* by the minimum probable number (MPN) method. For comparison two common enrichment media, Selenite-Cystine Broth (SCB) and Tetrathionate Broth (TB), were used. Tubes were inoculated with serial 10-fold dilutions in pentuplicate and incubated at 37°C for 24 hr. Growth from the tubes was streaked on BGA and Bismuth Sulfite Agar (BSA) for confirmation of *Salmonella*. MPN was estimated using an appropriate table.

Results were based on a minimum of three replications of the experiments.

Determination of infectivity of uninjured and injured cells

Viable cell counts of the cultures were obtained on PCA, BGA and SSA. From 10-fold dilutions of cultures 0.5 ml portions were inoculated into yolk sac of 10-day-old viable embryonated hen's eggs. Six replicates were used for each dilution. A comparable treatment to serve as control was also made with unirradiated and with irradiated nutrient broth. Inoculated embryos were incubated at 37°C with a commonly accepted procedure of turning. The mortality of embryos was determined by candling 3 days after the challenge. The LD_{50} was estimated by the method of Reed and Munch as described by Lennette (1964). To estimate the viable cell concentration, five eggs were selected at random from each of three sets of six eggs representing 10-fold test dilutions. Growth in an egg was considered equivalent to growth in a tube which allowed use of a most probable numbers table (APHA, 1967).

RESULTS

Influence of storage and media on post-irradiation recovery

When irradiated cultures were stored at 5°C for 24 hr, fewer *S. typhimurium* were recovered than were indicated by counts immediately after irradiation. The extent of reduction in recovery was related to inhibitory properties of the medium. The greatest recovery was with the nonselective medium, lactose broth; and the least recovery was on the selective medium, tetrathionate broth (Table 1). From the irradiated samples, numbers recovered using solid media were less than with LB but more than with TB. Data for the samples immediately after irradiation were in general aggreement with the work of Licciardello et al. (1970). Their data were limited to observations immediately after irradiation.

Table 2–D_{10} values (decimal reduction dose) in Krad for Salmonella as determined by counts on different media

Salmonella	Media			
	PCA	MA	BGA	SSA
S. typhimurium	28	28	25	20
S. enteritidis	25	22	24	22
S. heidelberg	33	27	28	25
S. senftenberg	33	25	31	29

Effect of temperature on post-irradiation behavior of bacteria

The rate of post-irradiation death of *S. typhimurium* near the stationary growth phase was temperature dependent with essentially no reduction in the population in 8 hr at 5°C. At 37°C for 8 hr, however, there was a marked reduction in the population while the unirradiated culture, being in the stationary growth phase, remained unchanged (Fig. 1). At 25°C there was an intermediate death rate. The death rate as measured by PCA and by the selective medium was approximately equal. The proportion of radiation in-

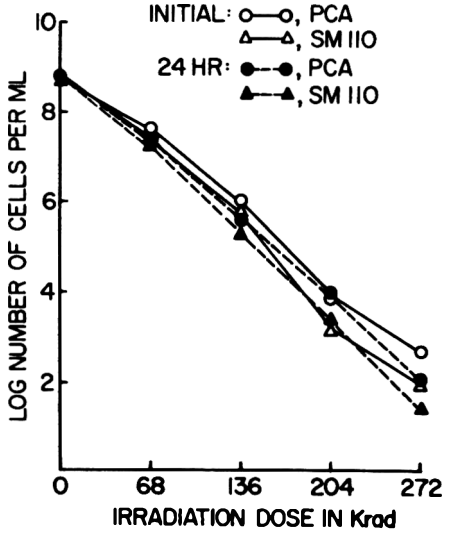

Fig. 4–Effect of dose level on the destruction and post-irradiation recovery of S. aureus *after 24 hr at 5° C.*

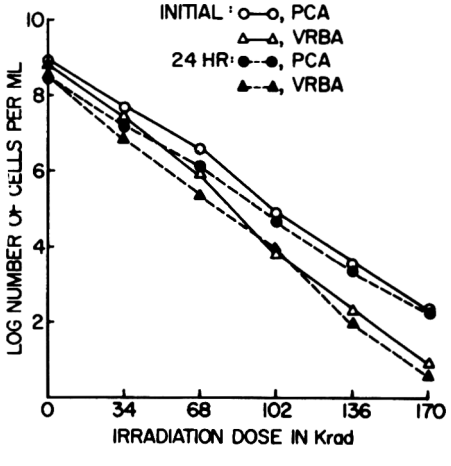

Fig. 5–Effect of dose level on the destruction and post-irradiation recovery of E. coli *after 24 hr at 5° C.*

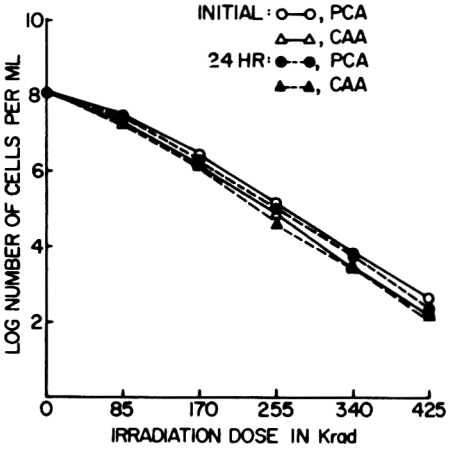

Fig. 6–Effect of dose level on the destruction and post-irradiation recovery of S. faecalis *after 24 hr at 5° C.*

Table 3—Comparative counts and LD_{50} values calculated from mortality figures of chick embryos

Culture	MPN per ml[a]	Plate count per ml PCA	BGA	SSA	LD_{50} (Number of cells)
Unirradiated	2.0×10^9	1.0×10^9	9.6×10^8	3.2×10^8	0.5
Irradiated	5.7×10^5	6.4×10^5	3.5×10^5	1.6×10^4	1.3

[a]Based on five challenges at three dilutions

jured cells remained approximately the same during storage. Death, therefore, is not limited to the injured cells but involves the population as a whole.

Comparative results with *E. coli* on PCA and on a selective medium, VRBA, were similar to those with *S. typhimurium* on PCA and on BGA. Post-irradiation death was most rapid at 37°C (Fig. 2). Even though more injured cells died during the test period, the data indicate post-irradiation death includes noninjured cells as well as injured cells.

Dose level and post-irradiation recovery of injured cells

The proportion of salmonellae considered as injured cells, unable to grow on selective media, remained constant with an increasing dose level. Approximately the same proportion of injured cells to total population for each dose level persisted after 24 hr at 5°C. Counts of *S. typhimurium* obtained with PCA and with MA were essentially the same both before and after storage. With *S. enteritidis, S. senftenberg* and *S. heidelberg*, however, slightly lower counts were obtained on MA than on PCA at higher dose levels contributing to lower D_{10} values (Table 2). Thus metabolic injury may be involved in post-irradiation recovery. The pattern of cell injury and destruction for *S. typhimurium* as determined immediately after irradiation and after 24 hr at 5°C are shown in Figure 3. Results for *S. enteritidis, S. senftenberg* and *S. heidelberg* were similar to those for *S. typhimurium*. Comparative D_{10} values for the four species of *Salmonella* using PCA, MA, BGA, and SSA are given in Table 2.

S. aureus and *E. coli* (Fig. 4 and 5) showed an increased proportion of injured cells with an increasing dose up to 272 and 170 Krad, respectively. Most of the injured cells persisted for 24 hr at 5°C. Results with *S. faecalis* (Fig. 6) showed the least cell injury of any of the cultures either immediately after irradiation or after 24 hr storage at 5°C. The D_{10} values using PCA for evaluation were 43 Krad for *S. aureus*, 26 Krad for *E. coli* and 77 Krad for *S. faecalis*.

Post-irradiation recovery and infectivity of S. typhimurium

The ability of radiation injured cells to initiate infection was estimated by determining the LD_{50} value and MPN by egg yolk challenge. The counts were also compared with PCA, BGA, and SSA. With unirradiated cultures there were more cells when determined by egg yolk challenge than by PCA. With irradiated cultures (68 Krad), however, the count was slightly larger with PCA than with egg yolk challenge. Selective media gave lower counts with irradiated cultures than either PCA or the egg yolk challenge. The results are given in Table 3 and indicate cells injured by irradiation are not as infective as unirradiated cells.

DISCUSSION

A SUBSTERILIZING DOSE of radiation kills some bacteria and injures others. Injury results in cells that are capable of recovery and growth in favorable environmental conditions but are unable to grow in the presence of inhibitory agents of selective media. The injury and sensitivity phenomena are similar for radiated cells and for cells injured by heat, freezing and bactericidal treatment (Maxcy, 1970; Ordal, 1970; Speck, 1970). Thus quantification of the total population must consider injured cells from many food processing operations.

Recovery of injured cells is dependent on post-irradiation storage temperature and is similar to the phenomenon reported by Macris and Markakis (1971) working with horse-radish peroxidase. Temperature dependence for extent of recovery was similar for salmonellae and for *E. coli*. Post-irradiation recovery should be significant in test portions with very low numbers of cells and should be considered even with test samples containing high numbers of the organisms studied. *S. faecalis* is least subject to radiation injury and least sensitive to post-irradiation storage temperature.

Since radiation injures and/or alters cells, the question of their infectivity and virulence is therefore raised by persons apprehensive of radiation. Irradiation, however, reduced the ability of *S. typhimurium* to initiate growth in egg yolk sac. Thus the numbers of cells as determined by egg yolk sac challenge were less than when determined with PCA. With unirradiated cells the numbers as determined by egg yolk sac challenge were greater than with PCA. The LD_{50} for radiated cells therefore was higher than for unirradiated cells (Table 3). Though radiation resistance may be acquired through recycling, there is no increase in virulence (Licciardello et al., 1969; Epps and Idziak, 1970). Thus it is not possible to anticipate circumstances where radiated cells would be more infective than normal cells.

While the preceding data point out additional phenomena that must be considered in enumeration of cells in foods, there is no indication that irradiation provides a unique problem of evaluating effectiveness of the process. Commonly accepted methods of pre-enrichment and recovery should be used for evaluation of foods.

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Ms received 1/22/72; revised 3/10/72; accepted 3/16/72.

Paper No. 3327, Journal Series, Nebraska Agricultural Experiment Station. Research was conducted under Project No. 16-8.

This investigation was supported in part by Contract No. AT(11-1)-2038 from the U.S. Atomic Energy Commission.

RESEARCH NOTES

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A Research Note
A SIMPLIFIED METHOD FOR THE ANALYSIS OF GLUTAMINE

INTRODUCTION

IN THERMALLY processed spinach puree, it was found that a higher quality product was obtained immediately after processing by utilizing high-temperature short-time (HTST) techniques rather than a conventional process (Lin et al., 1970). The greatest change in organic acids, color and pH were noted at 240°F with such changes decreasing with increased temperature up to 300°F. Among the acids formed, which have a direct effect on quality, pyrrolidone-carboxylic acid (PCA) showed the greatest increases at all temperatures. During storage, Lin et al. (1971) reported that PCA content again increased and, in later work, Clydesdale et al. (1971) reported an increase in glutamine during storage. Since PCA is formed directly from glutamine and is an important quality factor, it was decided to investigate conditions of hydrolysis of glutamine to PCA in order to attempt to develop a simplified procedure for the analysis of glutamine. Many methods have been proposed for the quantitative estimation of glutamine as described by Lin (1970).

These methods, in general, were laborious and time consuming. By using the Automatic Organic Acid Analyzer (AOAA) (Waters Associates, Inc., Framingham, Mass.) PCA can be separated quantitatively and a direct introduction of sample is possible. This research, therefore, was aimed at developing the appropriate techniques for hydrolysis which would quantitatively convert PCA to glutamine.

MATERIALS & METHODS

Analysis of PCA

PCA was quantitatively analyzed by an AOAA. Development of this analyzer is described by Kesner and Mutwyler (1966) and applications of this method to food materials has been described by Lin et al. (1970; 1971).

Establishment of optimum parameters for hydrolysis

Since this method was to be based on the complete hydrolysis of glutamine to PCA at 100°C, it was necessary to establish optimum parameters.

It is well known that pH is an important factor in the hydrolysis of glutamine and, for this reason, several experiments were carried out for 2 hr at 100°C with solutions of standard glutamine (L-glutamine, A grade, Calbiochem) at various pH levels.

It was also thought that the initial concentration of glutamine might affect the rate and completeness of hydrolysis to PCA. Therefore, after the optimum pH was established, experiments employing three different concentrations of glutamine were carried out. The concentrations chosen covered the range of glutamine normally found in spinach puree.

Recovery of glutamine added to spinach tissue extracts

Four samples of spinach puree with different levels of glutamine were divided into three batches. One batch was analyzed on the AOAA for PCA as described by Lin et al. (1970). The second batch of each sample was buffered to pH 6.8 with buffer solution (4.957g of potassium dihydrogen phosphate and 12.167g of sodium tetraborate in 1 liter and the pH adjusted to 6.8 with concentrated HCl) and hydrolyzed for 2 hr in a boiling water bath prior to analysis for PCA on the AOAA. To each sample in the third batch was added varying known levels of glutamine. These samples were then hydrolyzed and analyzed for PCA in the same manner as the second batch.

The amount of glutamine present in the fresh sample was found by subtracting the amount of PCA found in the first batch from the amount found in the second batch after hydrolysis. The total amount of natural and added glutamine in the third batch was found by substracting the amount of PCA present in the first batch from the amount present in the third batch after hydrolysis. Recovery of added glutamine from the spinach tissue could then be calculated from these results.

This experiment was also conducted utilizing different heating times and stirring techniques in order to define optimum physical parameters for analysis.

RESULTS & DISCUSSION

THE INTERNAL standard check for precision of PCA analysis on the AOAA was carried out as described by Lin et al. (1970).

Table 1 indicates the effect of pH on the conversion of pure glutamine to PCA. It is obvious from the Table that hydrogen ion concentration plays a very important role in the conversion of glutamine to PCA. At pH 5.4, the conversion of glutamine to PCA was about 85%. When the pH was increased from 5.4 to 5.6, the conversion only increased about 2%. When the pH was increased to 6.5, the conversion was nearly 100%. Good conversions were also obtained at pH 6.7 and 6.8, showing the optimum pH to be in the range 6.5–6.8 with the best results shown at pH 6.7–6.8. Also from the results in Table 1 the range of glutamine recovered from the samples in the pH range 6.5–6.8 was found to be 100.0–103.1%.

In order to evaluate the effect of concentration on the hydrolysis of glutamine, a separate experiment was carried out at pH 6.8 using glutamine concentrations of 7.31 mg/ml, 5.85 mg/ml and

Table 1—Effect of pH on the conversion of pure glutamine in solution to PCA

	Concentration (mg/ml)		
pH	Glutamine	Glutamine converted to PCA	% Conversion
5.40	7.31	6.28	85.9
5.40	7.31	6.21	85.0
5.60	1.17	1.02	87.5
6.50	7.31	7.54	103.1
6.70	5.85	5.89	100.6
6.80	1.17	1.17	100.0
6.80	1.17	1.19	100.7

Table 2–Conversion of glutamine to PCA in a spinach puree

pH		Glutamine concentration (mg/ml)					
Before hydrolysis	After hydrolysis	Glutamine in spinach	Glutamine added	Theoretical total	Total by analysis	Recovered	% Recovery
6.80	6.80	0.00	1.17	1.17	1.17	1.17	100.0
6.80	6.79	0.00	1.17	1.17	1.16	1.16	99.1
6.80	6.78	0.94	7.31	8.25	8.26	7.32	100.1
6.80	6.80	1.03	7.31	8.34	8.07	7.04	96.3

1.17 mg/ml (the range of glutamine concentration normally found in spinach puree). Recovery was excellent with the mean recovery being 100.1.

Pucher and Vickery (1940) converted glutamine to ammonia and PCA, followed by conversion of PCA to glutamic acid and analysis of the latter by manometric Van Slyke apparatus. In their method, it was found that the range of pure glutamine in solution recovered was 95–105% by means of an experiment similar to the one conducted in the current study.

Therefore, it may be seen that not only is the proposed method much faster and simpler but also the recovery range is much better.

Recovery of glutamine added to spinach tissue extracts

During hydrolysis of glutamine to PCA in spinach puree optimum conditions were found to require slow heating and careful stirring with a glass rod at the start of heating in order to prevent splashing of the puree. Optimum heating time at 100°C was found to be 2 hr, the same as that found for pure glutamine in solution.

Table 2 shows the results of hydrolysis of glutamine to PCA in spinach puree which in two cases contained only added glutamine and in the other two cases both added and naturally occurring glutamine. Spinach has a large enough variability in glutamine content due to age, variety, etc., that such conditions were designed into the experiment and obtained by checking different batches of spinach.

Since the pH values of spinach also vary greatly and since it has been shown that the pH of spinach puree decreases during heating due to the formation of organic acids (Lin et al., 1970) it was found necessary to buffer the samples at pH 6.8 with phosphate-borate buffer as described before. This prevented the pH value from decreasing to a point which would affect the completeness of conversion (Table 2). Fresh spinach (Table 2) showed a range of glutamine from 0.00–0.94 mg/ml. Known amounts of glutamine were then added producing a theoretical total of glutamine in the spinach puree ranging from 1.17–8.34 mg/ml. Analysis of these samples showed a range of 1.17–8.26 mg/ml representing a percent recovery of added glutamine ranging from 96.3–100.1. In a similar experiment, Pucher and Vickery (1940) obtained erratic recoveries of glutamine added to tissue extracts, but stated that "... the recovery still fell short of being quantitative but was reasonably satisfactory."

In the proposed method, there is no extraction procedure after hydrolysis. The hydrolyzed sample is placed directly on the column of the AOAA and analyzed for glutamine within 2 hr.

From the results obtained, the proposed method seems to offer simplicity and speed as well as an accurate recovery of glutamine from both pure solutions and tissue extracts.

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Contribution from the University of Massachusetts Agricultural Experiment Station, Amherst, Mass.

Supported in part by a grant from the USPH Service F.D. 0079-06 and the Glass Container Manufacturers Institute, New York, N.Y.

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A Research Note
TECHNIQUE FOR DETERMINING OXYGEN CONCENTRATION INSIDE PACKAGES

INTRODUCTION

RAPID and accurate determination of oxygen concentrations inside packages is important for evaluating the effectiveness of inert gas packaging processes. It can also be used to study package performance, to determine oxygen permeabilities, and to find the rate of oxygen uptake of food products.

Various analytical devices have been employed to determine oxygen concentrations, including the paramagnetic oxygen analyzer (Cullen and Papariello, 1970), gas chromatograph (Karel et al., 1963), polarographic instruments and galvanic cell electrodes (Mackereth, 1964; Borkowski and Johnson, 1967).

In this paper, we describe a technique to measure oxygen concentrations inside packages with an oxygen probe similar to the one developed by Johnson et al. (1964). Important features of our technique are the low cost and simplicity of the equipment required.

APPARATUS & PROCEDURE

Construction and use of the probe

The construction of this probe has been described in detail by Johnson et al. (1964) and Borkowski and Johnson (1967). The main modifications made here concern probe size and details in construction of the tip.

The outside diameter of the glass tubing is only 4 mm. This results in a smaller output than that obtained from the probes described by Johnson et al. (1964). The standard probe depicted in Figure 1a has a tip diameter of 5 mm and the epoxy cement surface is cylindrical and smooth. This probe can be used to determine the change of headspace oxygen concentration inside model packages as shown in Figure 1b. The probe is introduced into the package by a special mechanism that enables recalibration of the probe prior to measurement. This avoids the shortcomings of permanently mounted probes. This technique also allows a large number of determinations to be made with one probe. To introduce the previously calibrated probe into the package, the probe tip is positioned in the cavity of the rod assembly and then pushed into the package through the rubber seal. Only a very small amount of air is introduced into the package. After reading the oxygen concentration, the probe is pushed out by sliding the rod in the opposite direction.

The rod assembly is made as follows: swell thin-walled 4-mm (OD) tygon® (registered trademark of Norton) PVC flexible tubing for several days in chloroform. When the tubing is

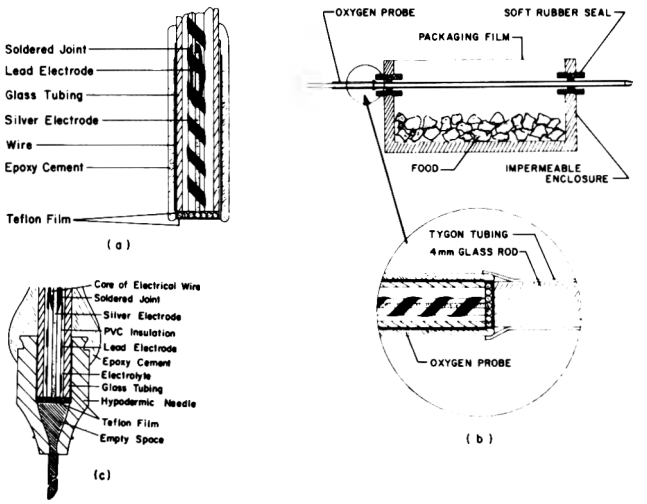

Fig. 1—Schematic representation of (a) standard probe tip, (b) mechanism for probe introduction into model package and (c) needle tip probe.

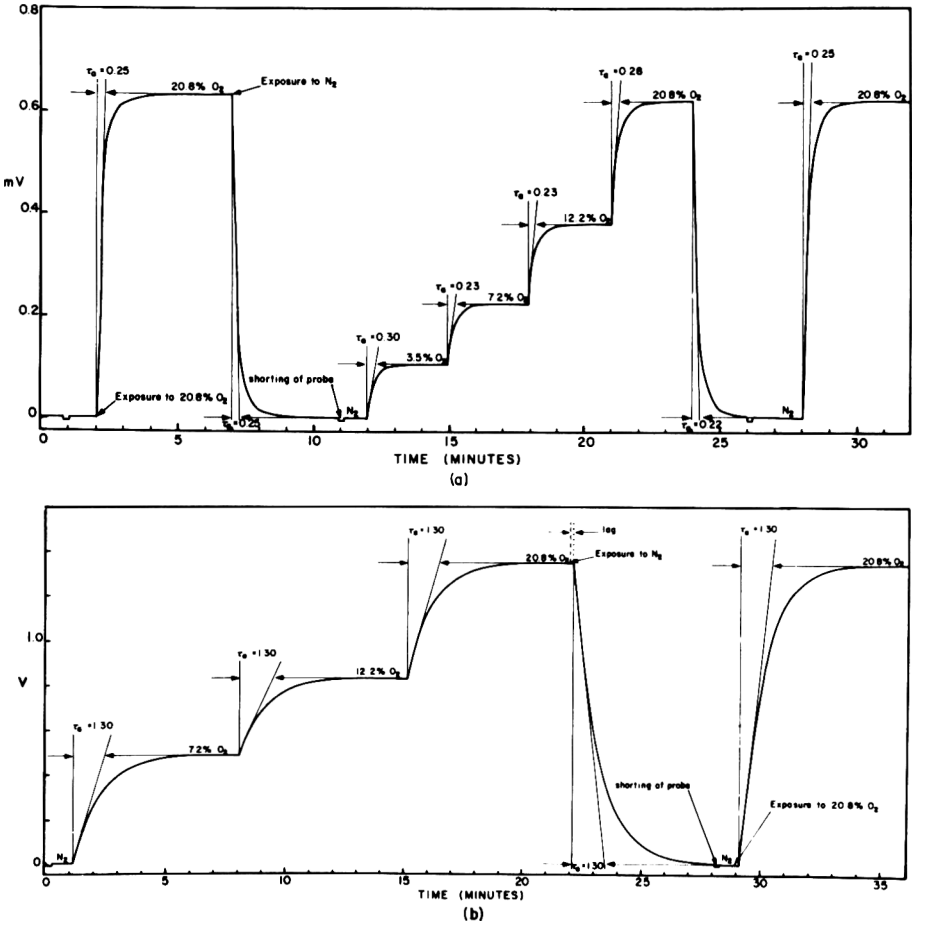

Fig. 2—Response curves of oxygen probes (a) standard probe and (b) needle tip probe.

soft, pull it over the 4-mm glass rod. The end is pulled over a 5-mm (OD) glass tubing which has a shape similar to the probe tip. Then let the tygon tubing dry for several days. Due to its swelling in chloroform, the tygon loses plasticizer and, upon evaporation of the chloroform, becomes rigid and retains the probe cavity after removal of the 5-mm glass tubing.

If the volume of the package and the film area are known, then the technique described can be used to determine the oxygen permeability of the film in the absence of sample. If the whole package is impermeable, then the rate of oxygen uptake by the product can be determined from the change in headspace concentration. Finally, if the package is permeable to oxygen and moisture and contains sample, then the technique will determine the headspace oxygen concentration and moisture content. Consequently, the rate of oxygen uptake will change with time depending on the particular initial conditions and the package parameters. Except for the construction of the idealized package shown in Figure 1b, this model represents all the processes that occur during storage of food products in permeable packages.

Alternate construction of probe tip

An alternative construction of the probe tip is shown in Figure 1c. Here a 10-mm tip of a no. 18 hypodermic needle is used. The oxygen diffuses through the needle tip toward the membrane of the electrode. This probe can be used to determine oxygen concentrations inside containers with appropriate rubber stoppers which can be perforated directly with the probe tip without introducing a significant amount of air.

To determine oxygen concentrations inside flexible packages, a small piece of soft rubber sheet can be glued to the film and the probe tip introduced through this rubber seal.

RESULTS & DISCUSSION

THE MAIN disadvantage of the probe with the needle tip is its slow response. Figure 2 shows the response of the two types of probes to step changes in oxygen concentration. It can be seen that the response is essentially first order with time constants of 0.25 and 1.30 min. The probe with a 10-mm needle tip has a lag of 0.15 min.

Since the probes are inexpensive and easy to build, it is convenient to make a large number of determinations with several probes but the same measuring circuit. Approximate measurements can be made by measuring the current generated by the probe directly with a microammeter with internal resistance less than 2000 Ω. Such an instrument costs about $30 and the current generated is approximately 5 μA. A more convenient and accurate method consists of recording the millivoltage generated by the probe across a small resistance. With a resistance of 200 Ω, the potential generated is typically 1–5 mV at atmospheric oxygen concentration. Figure 2 was obtained with the latter circuit.

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Ms received 1/8/72; accepted 1/24/72.

This work was supported by PHS Grant No. FD-00140 from the Food & Drug Administration.

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A Research Note
TEMPERATURE GRADIENT FREEZE-DRYING MICROSCOPE STAGE

INTRODUCTION

IN AN EFFORT to help open new pathways to the understanding of the freeze-drying process, the authors have been involved in a three phase program of freezing, drying and rehydration (Rosano et al., 1972). In this program the effects of each of the above stages on various model systems such as solutions, colloids and gels are being studied. It is hoped that such a study will help compliment studies by Luyet and Rasmussen (1969); Rey (1964) and Meryman (1966) yielding a general theory on the mechanism of freeze drying. Although the above authors have contributed monumentally to this topic, and King (1971) has recently written an excellent review on freeze drying of foods, some questions still need to be answered more fully: i.e., (1) What is the effect of the rate of freezing on structure, color, drying times, rehydration properties and shrinkage? Can a general correlation be made? (2) How do additives (gases included) influence processing conditions and the overall freeze-dried material?

A valuable aid in our study has been the use of a freeze-drying microscopic stage. Whenever possible it is extremely useful and interesting to observe physical and structural changes of the specimen under actual conditions.

Much work has been done using isothermal freeze-drying stages (Cheuffard, 1967; Chamot and Mason, 1958). Although these types of stages are suitable for nucleation formation and identification studies, they have limited use for investigation of the solidification and liquid removal kinetics. In addition, it should be noted that in any large scale freeze-drying process a temperature gradient will exist. When freezing, a temperature gradient exists since the substance cannot be cooled evenly due to its bulk thickness. For example, in tray freeze drying the sides and bottom of the tray containing the substance are the coldest because of their direct contact (conduction) with the refrigerated plates. Therefore a temperature gradient exists between the walls of the tray and the warmer region near the center of the substance. Secondly, in the sublimation stage of the freezing process, heat must be added to cover the heat of sublimation requirements of the ice being vaporized. This is generally accomplished by heating one side of the vessel while still keeping the overall system temperature below the freezing point of the substance. Once again, a temperature gradient is produced due to the thickness of the material and the uneven distribution of heat energy (Rey, 1964).

This cell is advantageous both as a research tool and as an engineering aid because a temperature gradient exists perpendicular to the observer and is within the thin film of material being freeze dried. Secondly it provides variable controls for adjusting the temperature of the cell and the vacuum pressure. Finally, this cell is inexpensive and easily constructed while still producing good results.

EXPERIMENTAL

Description of the stage

Our stage is composed essentially of a completely wettable slide inserted into a circular cold plate and connected to a vacuum chamber. The stage is shown in Figure 1. It has a distinct advantage over the conventional isothermal stages in that a radial temperature gradient exists perpendicular to the observer. This allows the viewer to examine the freezing front as it starts at the circumference of the viewing area and propagates in a radial direction to the center of the sand blasted slide.

A sand blasted glass slide is used since the liquid solution will easily spread on its surface to produce a thin transparent film of uniform thickness. No cover slip is used to cover the specimen surface.

The sand blasted slide fits into a recess on the copper slab which serves as the cooling medium. One end of the copper slab is placed into a cold reservior (i.e., dry ice-acetone mixture or liquid nitrogen). A heating coil attached to the cold plate regulates the temperature.

The cold plate rests on the top of the vacuum chamber. Holes in the cold plate serve as vacuum ports which connect the vacuum chamber and the specimen surface.

Plexiglas plates are used to seal the top of the cold plate and the bottom of the vacuum chamber while still allowing light to be transmitted to the specimen or the sand blasted slide. Only the copper plate surrounds, supports and conducts heat away from the slide.

To prevent condensation on the windows, dry nitrogen is gently flushed across the top of the cell.

The temperature of the specimen may be monitored by placing a fine microthermocouple on the sand blasted slide in the area desired. Rubber gaskets provide a seal for the thermocouple between the Plexiglas and the cold plate.

Since a temperature gradient exists in the design of this cell, a thermocouple measures the average temperature for the region in which it is located. However, in many cases it is advantageous to know exactly what the temperature is at a particular location in the cell for a defined cold plate temperature. To determine this accurately a series of calibrations are made using salts which form eutectic mixtures (constant freezing points) as references (NRC, 1928). The theory behind this is that a eutectic

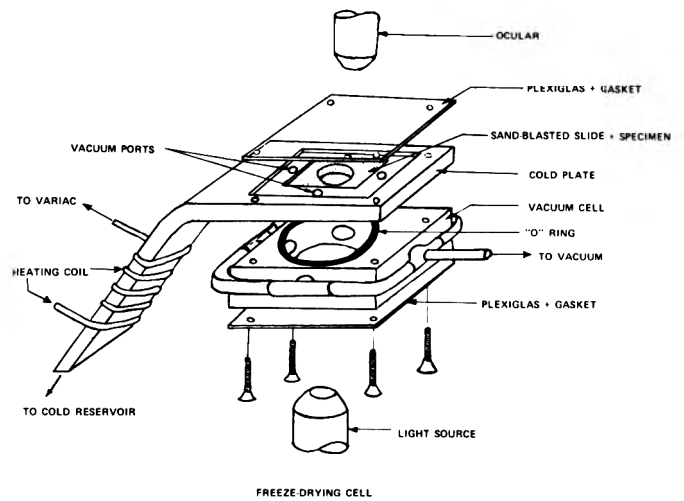

Fig. 1—Temperature gradient freeze-drying microscope stage.

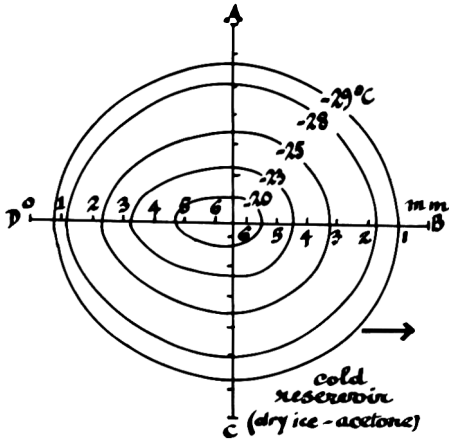

Fig. 2—Freeze drying isotherm profiles stage.

solid-liquid interface indicates a specific temperature.

Procedure for the stage calibration

A microscope sand blasted glass slide is placed on the cold plate. The plexiglas top is placed on the cell to prevent condensation between the cold plate and glass slide. The system is allowed to come to equilibrium.

One drop of the salt solution is spread on the slide. Care must be taken to prevent any liquid from seeping between the cold plate and the sand blasted slide thus altering the heat transfer.

When the system reaches equilibrium, the distance between the cold edge and solid eutectic front is measured.

Results are graphed on polar coordinate paper.

RESULTS & DISCUSSION

Calibration results

Using a dry ice-acetone mixture for the cold reservoir and eutectic solutions of NaCl, KI, NH_4CNS, NaBr and $Zn(NO_3)_2$ a calibration was made. The positions of the solid eutectic fronts when plotted on polar coordinate paper produced a series of isotherms shown in Figure 2. It is evident that the isotherms are circular on the cold sink side while being egg shaped on the OD axis. This is primarily due to the environmental conditions and its effect on the shorter side of the cold plate. The ambient temperature during this experiment was 21°C.

Good reproducibility exists for this calibration and similar ones at different cold plate temperatures provided the slide sits evenly in its recess and no liquid exists between the cold plate and slide. The ambient conditions can vary as much as 4°C without any serious error.

In conclusion, it should be noted that this stage contains all the necessary elements for varying the temperature, pressure and the rates of freezing and freeze drying for a particular system. Observations of the actual freeze-drying process (freezing and freeze drying) have been made visually and recorded on Polaroid photographs, 35 mm slides and on 16 mm movies (using actual and time-lapsed photography). Thus a simplified method is provided for studying the complicated three dimensional heat transfer processes found in industrial freeze drying as a two dimensional phenomenon.

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Ms received 10/21/71; revised 1/14/72; accepted 1/16/72.

The authors thank Mr. John Russo for his help in the design and construction of the freeze-drying cell.

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A Research Note

INFLUENCE OF PROCESSING PROCEDURES ON TOTAL COUNT, PRESUMPTIVE COLIFORMS AND Clostridium perfringens IN BEEF FOR FURTHER COOKING

INTRODUCTION

"BEEF FOR further cooking" is a fabricated beef product prepared from boneless beef cuts and trimmings which are chopped, mixed and stuffed into a fibrous casing. The product is utilized by roast beef sandwich shops to replace boneless beef from the round. "Beef for further cooking" allows the sandwich shop to obtain more uniform sandwiches and is generally more economical. The name is derived in that the product must be further cooked at the sandwich shop before it may be served. The manufacturing process does not include any specific procedures for the reduction or elimination of microorganisms.

This study was undertaken to determine if the number of organisms in this product changed during processing. The results would indicate the magnitude of public health hazard presented by this product.

EXPERIMENTAL

Product preparation and sampling

"Beef for further cooking" used in this study had the following formulation: 28.5% shank meat, 28.5% knuckles, 9.5% skirts, 9.5% rib fingers, 9.5% trimmings, 1% salt, 0.5% plant protein and 3% water containing sodium tripolyphosphate sufficient to provide 0.5% in the finished product. About one-third of the meat was ground through a 2.4 mm grinder plate and the remaining was ground through a 2.54 cm grinder plate. The ground components were then mixed and the remaining ingredients added during mixing.

After mixing, the product was stuffed into fibrous casings which held approximately 4 kg of product. The stuffed product was submerged in water for 15 min at 88°C to coagulate the surface protein, transferred into 1°C water for 30 min and frozen in a blast freezer (−23 to −28°C). Nineteen batches of product were examined at mixing, after water-chilling and after freezing for total aerobes, presumptive coliforms and *Clostridium perfringens*. A 100-g sample was collected aseptically at each processing point and packed with ice in an insulated container for shipment from the cooperating processor to the university laboratory.

Bacterial enumeration

A meat homogenate was prepared from each sample by placing 50g of the product in 450 ml sterile water and blending for 90 sec at 21,000 rpm. Subsequent 10-fold serial dilutions were made and utilized for enumeration of total aerobes, presumptive coliforms and *C. perfringens*. The enumeration of total aerobes was accomplished by the pour plate method (APHA, 1960) using nutrient agar (Difco) and incubating at 37°C for 24 hr. The 37°C incubation temperature was utilized to enumerate those organisms which would be of most concern if the product were either mishandled or cooked to the rare state and held at serving temperatures for a period of time. The results were recorded as number of organisms per gram of tissue. Presumptive coliforms were determined by the most probable number method (MPN) (APHA, 1960) using lauryl sulfate (tryptose) broth and three tubes per dilution. After incubation at 37°C for 36 hr the results were converted to MPN using an appropriate table (USDHEW, 1969). *C. perfringens* were enumerated utilizing the MPN method after 12–14 hr anaerobic incubation at 37°C in cooked meat broth (Difco). The presence of *C. perfringens* was determined by a series of identification steps including black colony development on sulfite-polymyxin sulfadiazine (SPS) agar (Difco); gram-positive pleomorphic rods after growth on blood agar; nonmotility, nitrate reduction and lack of indole reduction after growth in motility indole nitrate media; lactose fermentation in lactose broth; and lecithinase activity on McClung-Toabe egg yolk agar (Lowis, 1971; Hall et al., 1969; Dowell and Hawkins, 1968; Angelotti et al., 1962; McClung and Toabe, 1947).

Statistical analysis

Analysis of variance, Duncan's multiple range and simple correlations were calculated according to generally accepted methods (Snedecor, 1956).

RESULTS & DISCUSSION

THE MEAN LOGARITHMIC count and standard deviation of total aerobes, presumptive coliforms and *C. perfringens* may be observed in Table 1. The total number of aerobes changed very little during the processing of "beef for further cooking." The mean counts and overall range of log 4.06–6.94 are in general agreement with reported values for fresh beef products (Rey et al., 1970; Ayres, 1960).

The number of presumptive coliforms increased slightly from mixing through heating and cooling, then a significant ($P < .05$) decrease was noted upon freezing. This decrease probably resulted from loss of vegetative cell viability during freezing (Major et al., 1955). The means and overall range of 0–4.86 are in agreement with those reported by Rey et al. (1970) for fresh beef.

C. perfringens counts were lower than the others and although the counts varied through the processing, the changes were not significant. The range of counts for this organism was 0–1.95 which is in general agreement with those reported by Rey et al. (1970). 35 of the 57 samples had no detectable *C. perfringens*.

Significant ($P < .01$) overall correlation values of 0.73, 0.42 and 0.34 were found between total aerobic count and presumptive coliforms, total aerobic count and *C. perfringens* and presumptive coliforms and *C. perfringens*, respectively. Similar correlation values (overall $r \pm .1$) were found for each comparison within the three processing groups. These values indicate that as one type of bacteria

Table 1—Mean logarithm and standard deviation of total aerobes, presumptive coliforms and C. perfringens counts/gram

Manufacturing point	Type of organism: Total aerobes $\bar{X}$	Total aerobes s	Presumptive coliforms $\bar{X}$	Presumptive coliforms s	*Clostridium perfringens* $\bar{X}$	*Clostridium perfringens* s
Mixer	5.18	0.50	2.38	0.92	0.60	0.68
Water chill	5.24	0.60	2.85	0.94	0.64	0.73
Freezer	4.94	0.70	1.95	1.08	0.26	0.53

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increases, so do the other types.

Except for the decrease in presumptive coliforms upon freezing, no significant changes in total aerobes, presumptive coliforms or *C. perfringens* were noted. Based on these three types of organisms the processing of "beef for further cooking" does not cause this product to be more of a public health hazard than that reported for fresh beef cuts.

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Ms received 1/31/72; revised 3/16/72; accepted 3/16/72.

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A Research Note
TOCOPHEROL SUPPLEMENTATION AND LIPID STABILITY IN THE TURKEY

INTRODUCTION

MECCHI ET AL. (1953) stated that the tocopherol content of the fat is the principal, if not the only, component causing chicken fat to be more stable than turkey fat. When these researchers added tocopheryl acetate to turkey, the stability of fat increased by four- to sixfold compared with that from birds on a control diet. Mickelberry (1970) injected male turkeys with various antioxidants, including d-α-tocopherol, at 5 min or 24 hr before sacrifice, but found no significant increase in lipid stability as determined by peroxide values. Webb et al. (1971) studied the effects of feeding dl-α-tocopheryl acetate, Ethyoxyquin and BHT on rancidity development during frozen storage of precooked broiler parts. These researchers found that feeding broilers 5 or 10 I.U. of vitamin E per pound of feed for 36 days preslaughter held TBA numbers below those of controls.

The experiment reported here was set up to establish the effect of method and level of tocopherol supplementation in stabilizing turkey lipids during the cooking process.

MATERIALS & METHODS

THE FOLLOWING treatments of dl-α-tocopherol acetate were applied to 8-wk-old large white turkeys:

(1) None added, control diet;
(2) 10 I.U. per pound of ration;
(3) Weekly subcutaneous injections equivalent to 10 I.U.;
(4) 100 I.U. per pound of ration; and
(5) Weekly subcutaneous injections equivalent to 100 I.U.

At 20 wk for hens and 24 wk for toms, 10 birds were selected at random from each treatment and slaughtered at the Iowa State University processing facility. Carcasses were chilled in ice slush in a walk-in cooler for 18 hr, after which two 100g samples of breast and thigh tissues were taken from each bird. These samples were immediately packaged in Cryovac bags, evacuated and frozen at −25°C in a blast freezer. The remaining carcasses were packaged similarly and held briefly at 4°C. They were roasted in groups of five, one representing each treatment. Roasting was done at 162°C to an internal breast temperature of 82°C in a rotary hearth oven. Samples of breast and thigh tissues were taken for immediate TBA determination (Tarladgis et al., 1960).

Table 1—Mean TBA numbers

	Treatment (Tocopherol, I.U.)				
		Oral		Injected	
	Control	10	100	10	100
Male					
Breast					
Raw	0.37	0.37	0.16	0.20	0.10
Cooked	0.79	0.54	0.31	0.35	0.24
Thigh					
Raw	0.35	0.37	0.13	0.14	0.08
Cooked	1.26	1.28	0.51	0.62	0.33
Female					
Breast					
Raw	0.23	0.36	0.21	0.26	0.10
Cooked	0.72	0.56	0.31	0.34	0.17
Thigh					
Raw	0.25	0.30	0.29	0.22	0.11
Cooked	1.46	0.95	0.52	0.88	0.25

RESULTS & DISCUSSION

WE SUBJECTED the TBA data to an analysis of variance and further made an analysis of means (Table 1) as dictated by orthogonal comparisons. Significant effects ($p < 0.01$) were found due to tocopherol treatment, meat type (breast or thigh) and cooking, but not to sex (Table 2). Also of interest was the significant ($p < 0.01$) Treatment x Cooking interaction.

Based on the results of orthogonal comparisons of adjusted means, significantly lower TBA numbers were associated with:

(1) Tocopherol treatment, oral or injection;
(2) Injection of tocopherol compared with oral application;
(3) Application of 100 I.U. of tocopherol compared with 10 I.U.; and
(4) Cooked meat from tocopherol-treated turkeys compared with cooked meat from control turkeys.

From these data we conclude that tocopherol supplementation, oral or injection, to the turkey offers considerable potential in reducing the rate of oxidative deterioration of lipids during meat cookery.

Table 2—Analysis of variance of TBA numbers

Source	df	Mean squares
Sex (S)	1	0.0002
Treatment (T)	4	2.08**
Error A	40	0.03
Meat (M)	1	1.95**
Error B	50	0.04
Cooking (C)	1	9.07**
Error C	20	0.05
T x C	4	0.71**
Error	80	0.03

**Significant $p < 0.01$

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Journal Paper No. J-7133 of the Iowa Agriculture & Home Economics Experiment Station, Ames, Iowa, Project No. 1864.

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A Research Note
REDUCTION OF ANTITHIAMINE ACTIVITY IN CRAYFISH BY HEAT TREATMENTS

INTRODUCTION

THE PRESENCE of antithiamine substances in fish has been known since Chastek-paralysis (an acute dietary deficiency disease) was induced by inclusion of raw carp in the fox's diet (Green et al., 1942). Thiaminase occurs in many species of fish and shellfish (Yudkin, 1949; Fujita, 1954; Deutach and Hasler, 1943; Sealock and Goodland, 1944; Wooley, 1941; Krampitz and Wooley, 1944; Krzeczkowski, 1968; Krzeczkowski, 1970; Greig and Gnaedinger, 1971). Many of the above researchers have noted the enzymatic characteristics of the antithiamine factor. Recently, Kundig and Somogyi (1967) found a thermostable antithiamine factor in carp viscera. Moreover, Hilker and Peter (1968) reported that the antithiamine activity of skipjack tuna was not enzymatic. Thus it appears that different antithiamine factors exist in different species of fish and also in the same fish. Studies by Hilker and Peter (1968) and Tang and Hilker (1970) indicated that more than one antithiamine factor was present in tuna.

In Louisiana and other areas of the United States, fresh water crayfish (*Procambarus clarkii*) are becoming a commercially important food product. The authors have found antithiamine substances in crayfish. Since crayfish are a gourmet item, the presence of antithiamine factors poses no serious problem. However, the presence of thiaminase in waste material which may ultimately be converted into animal feed could constitute a drawback to the use of crayfish meal in animal rations which are consumed on a daily basis.

This investigation was conducted to study the effects of boiling as well as drying on antithiamine activity in crayfish (*P. clarkii*).

EXPERIMENTAL

THE ASSAY METHOD was similar to that of Gnaedinger (1965) with the exception that potassium ferricyanide was used instead of cyanogen bromide. Gnaedinger (1965) also suggested that cysteine (1×10^{-2} M) added to the KCl solution used in extraction served as an enzyme activator. However, preliminary work with the assay procedure showed no improvement with crayfish samples; therefore, the addition of cysteine was omitted.

Treatment of samples

20 lb of live crayfish were obtained from a local fish market in Baton Rouge, La. and divided into four groups, each receiving one of the following treatments: maintained fresh, boiled 5 min, boiled 30 min, or oven dried at 100°C for 24 hr. Each crayfish was separated into two anatomical parts for analysis: abdomen and cephalothorax. The flesh material in the abdomen (tail meat) and cephalothorax was removed from the shell and ground in a meat grinder using a 1/8-in. plate.

Preparation of crayfish extracts

Thiaminase activity was expressed as micrograms of thiamine hydrochloride destroyed per minute per gram of sample, expressed on a dry weight basis. Consequently, moisture determinations were made on each of the ground materials in order to convert data from a wet weight basis to a dry weight basis.

Seven 5-g samples (wet wt) from each of the crayfish materials were homogenized with 95 ml of cold 5% KCl for 1 min at high speed in a Waring Blendor. The homogenate was centrifuged at 1500 rpm for 5 min at 1°C. The supernatant was filtered through gauze and the residue discarded.

Reaction of extract with thiamine

95 ml of each extract and 5 ml of a standard thiamine solution (0.02 mg thiamine hydrochloride per ml) were brought to incubation temperature by submerging them separately in a 37°C water bath for 10 min before mixing. At the start of the assay (0 time), the thiamine solution was added to the extract and two 10 ml aliquots immediately taken and transferred to 25 ml volumetric flasks containing 12 ml of hot 0.1N HCl, which had been preheated in a boiling water bath. The flasks were heated for an additional 10 min in the boiling water to coagulate the protein, cooled rapidly to room temperature under tap water, and diluted to 25 ml with 2.5M sodium acetate. One of these samples was to serve as a standard, and the other as a blank. After the reaction had proceeded for 30 min, two additional 10 ml aliquots were taken and treated in like manner.

Oxidation of thiamine to thiochrome

Each 25 ml collection was filtered and 5 ml portions placed in 250 ml separatory funnels. 1 ml of the ferricyanide solution (1 ml of 1% ferricyanide with 24 ml of 15% NaOH) was added to each separatory funnel except the blank, to which 1 ml of 15% NaOH was added. 15 ml of isobutyl alcohol was added, and each separatory funnel was shaken vigoriously for 2 min and then let stand for 5 min to separate the liquid phases. The aqueous layer was discarded, and the butanol layer was shaken with 1.5g of anhydrous sodium sulfate until clear.

Measurement of Thiochrome fluorescence

The clear solution in each separatory funnel was decanted into cuvettes, and the fluorescence was read with a Coleman Photofluorometer (Model 12-C) in combination with a Coleman Galv-o-meter (Model 22). A 365 mμ primary filter and a 430 mμ secondary filter were used.

RESULTS & DISCUSSION

Distribution of antithiamine activity in crayfish

The tail meat or abdomen section was found to be devoid of the thiamine

Table 1—Antithiamine activity in fresh and heat-treated crayfish cephalothorax and abdomen

	Cephalothorax				Abdomen			
	Fresh	Boiled 5 min	Boiled 30 min	Oven dried	Fresh	Boiled 5 min	Boiled 30 min	Oven dried
Amount of thiamine destroyed (μg/min/g)	3.70 ± 0.318	1.02 ± 0.142	0.89 ± 0.068	0.52 ± .054	0.00	0.00	0.00	0.00
Specific activity units/g[a]	.0110	.0030	.0027	.0015	0.00	0.00	0.00	0.00
% Reduction due to heat treatment	–	72.3	75.8	85.9	–	–	–	–

[a]A unit is defined as micromoles of thiamine destroyed per minute.

destroying factor; however, the material found in the cephalothorax region was quite active (3.70 μg/min/g). The results presented in Table 1, are not surprising in view of the fact that many investigators have demonstrated that the antimetabolite is more prevalent in some tissues than others (Sealock et al., 1944; Hilker and Peter, 1968; Tang and Hilker, 1970; Greig and Gnaedinger, 1966; Krzeczkowski, 1968). Sealock et al. (1944) found the greatest concentration of activity in four tissues, namely, the spleen, liver and pancreas, gastrointestine and gills—a fact which suggests that the antithiamine factor plays an important role in metabolic processes. In crayfish samples examined, the digestive glands (liver) destroyed 21.46 μg of thiamine/min/g of sample compared to 3.70 μg/min/g for the entire cephalothorax. Since the digestive glands constitute only 15% of the total cephalothorax, it can be inferred that these glands contained over 87% of the total antithiamine activity in the fresh crayfish samples analyzed.

Heat inactivation

Percent reduction in specific activity due to various heat treatments is shown in Table 1. Boiling the crayfish for as long as 30 min or oven drying at 100°C for 24 hr failed to destroy all the activity in the samples. Therefore, either there is a heat stable antithiamine factor in crayfish cephalothorax or the assay procedure is inadequate. Recently Kundig and Somogyi (1967), Hilker and Peter (1968), and Tang and Hilker (1970) found thermostable antithiamine factors in carp viscera and skipjack tuna. If there is a thermostable factor in crayfish, the total activity in the fresh samples would result from the presence of at least two factors, one enzymatic and one nonenzymatic. It is incorrect to assume that after heat treating, the activity remaining is a true measure of the amount of total activity represented by the nonenzymatic factor. The effects of heat on the rate of the nonenzymatic factor reaction would have to be known before attempting to divide the total activity between the enzymatic and nonenzymatic factor.

Deutsch and Hasler (1943), Sealock et al. (1943), Melnick et al. (1945), and Gnaedinger and Krzeczkowski (1970) studied antithiamine substances in fish and reported complete heat inactivation under their experimental conditions. Other researchers (Kundig and Somogyi, 1967; Hilker and Peter, 1968; and Tang and Hilker, 1970) have been unable to effect complete inactivation by heat treatments. The number of assay procedures are as numerous as the number of researchers, a fact which may or may not be responsible for some of the differences encountered. Consequently, further investigations on the nature of the antithiamine factor(s) in crayfish are in progress using several assay procedures.

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Ms received 8/2/71; revised 2/7/72; accepted 2/14/72.

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A Research Note

A SIMPLE METHOD TO DETERMINE THE WATER-HOLDING CAPACITY OF MUSCLE FOODS

INTRODUCTION

WATER-HOLDING CAPACITY (WHC) of muscle foods has been utilized as an index of palatability (Miller and Harrison, 1965), microbial quality (Jay, 1967) and manufacturing potential (Saffle, 1968). Several methods for measuring this property of muscle foods have been developed. Grau and Hamm (1953) subjected meat samples on a filter paper to a press at 10,000 psi and measured the area occupied by moisture on the filter paper after the treatment. This method was modified by Miller and Harrison (1965) to consider the area of the sample as well as the area of the moisture imprint. Wierbicki et al. (1957) used a centrifuge and measured the amount of juice extracted from the meat which had been placed upon fritted glass disc in a divided centrifuge tube. Miller et al. (1968) made some technical improvements on this method using stainless steel centrifuge tubes with 1 mm holes in the bottom, a wetted filter covering these holes and centrifuging 20g of ground meat at 12,000G to extract loosely bound water.

The method described in this note utilizes the basic concepts of Miller et al. (1968) with modifications which allow significant reduction in sample size and eliminate the need for specially designed equipment.

MATERIALS & METHODS

THE MEAT PRODUCT used in this study was the tail meat of lobster (*Homarus americanus*) at different stages of processing. A Waring Blendor was used to prepare the meat samples. The Waring-Blendor tops and lids were precooled by placing 25–40g of dry ice pulver into the blender top and operating the blender for 10 sec prior to the introduction of samples. The lobster meat was cut into small pieces, mixed with 3–4 times its weight of pulverized dry ice in the stainless steel blendor top and mixing at maximum speed for several 10-sec periods. Between blendings all residue was removed from walls of the blendor cup with a spatula and returned to the bottom of the blendor top. Blending continued until the mixture became a uniform fine powder. This usually required 3–5 blending periods.

About 0.5–1.0g (more than sufficient to absorb all expressed juices) of molecular sieve (80–100 mesh; Applied Science Labs., Inc., State College, Pa.) were roughly measured into a 10-ml polycarbonate centrifuge tube. Two discs of filter paper (Whatman No. 42) were cut to a slightly larger diameter than the centrifuge tube so that they had to be squeezed down to the surface of the molecular sieve. (The filter paper discs can be made to fit more tightly by pre-wetting with 2–3 drops of water before weighing.) The tube and contents were then weighed to ± 0.001g. Dry ice-muscle powder, containing approximately 1g of meat, was then placed on the top of the filter paper. After thawing and evaporation of all CO_2 (usually 5–10 min) the amount of meat was determined by weighing on an analytical balance. The tube was then centrifuged at 12,000G for 10 min in a refrigerated centrifuge (MSE Hi-speed 25; Measuring & Scientific Equipment, London). After centrifugation the "meat-cake" was removed with forceps from the top of the filter paper and the amount of water lost by the meat was determined by weighing the tube and its contents. The water holding capacity may be expressed either as grams of water lost per gram of meat, or after determining the total moisture content of the sample material, the amount of water retained by the meat after centrifugation.

Lobsters were cooked for 10 min in boiling 2.5% NaCl brine and quick frozen in an air blast freezer. WHC of raw lobster meat was evaluated immediately after sacrificing.

Table 1—Grams of liquid expressed from 1-g portions prepared from two different cooked and frozen lobster tails during centrifugation for 10 min at 12,000G

Lobster tail	Samples 1	2	3	4	5	6	7	8	Mean	Standard deviation
I	.456	.457	.459	.470	.451	.474	.444	.445	.457	.011
II	.468	.474	.484	.464	.482	.467			.473	.008

RESULTS & DISCUSSION

RESULTS of experiments to test the reproducibility of the method are shown in Table 1. The standard deviation is lower than that reported by Miller et al. (1968) in their experiments for improvement of the centrifugal method. The centrifugal force applied was that which was recommended by Miller et al. (1968). Great increases in centrifugal forces will increase the amount of water lost from the meat but a recent publication by Bouton et al. (1971) showed that a considerable fraction of water is still retained in mutton after ultracentrifugation at 120,000–190,000G for 30–60 min. Miller et al. (1968) showed that the amount of water expressed increased rapidly as centrifugal force increased up to 12,000G. A 25% increase in G force above 12,000G (15,000G) did not alter the amount of expressed juices.

Table 2 shows the differences in WHC between raw, cooked and cooked frozen-thawed lobster meat, measured by the previously described method. The difference in WHC between raw and cooked samples is highly significant ($P < 0.01$), whereas freezing and immediate thawing results in WHC differences which are not statistically significant. The standard deviation is somewhat higher than was experienced in the testing of the method since the results are means of duplicates

Table 2—Water-holding capacity of raw, cooked and cooked frozen-thawed lobster meat. Water retained is expressed as percent of total wet weight of the samples

Product	Total moisture %	Liquid removed[a] %	Water retained %
Raw	77.0	14.4 ± 1.8	62.6
Cooked	76.9	31.1 ± 2.8	45.8
Cooked frozen-thawed	75.5	33.3 ± 2.3	42.2

[a]Means ± standard deviation; n = 8

from four different lobster tails and the inherent biological variation affects the data.

The data show that the method is useful for determining differences in water-holding capacity resulting from different treatments of a muscle food product.

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Ms received 10/31/71; accepted 2/3/72.

A paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers The State University of New Jersey, Dept. of Food Science, New Brunswick, New Jersey.

This study was supported in part by a fellowship awarded to Bjorn Dagbjartsson from the International Atomic Energy Agency, Vienna, Austria.

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A Research Note
THE POSSIBLE IDENTIFICATION OF AN IRRADIATION TREATMENT OF FISH BY MEANS OF ELECTRICAL (ac) RESISTANCE MEASUREMENT

INTRODUCTION

EXPERIMENTS and theory on the electrical properties of biological tissue have shown that there is an ohmic and a capacitive contribution. The capacitive part may be due to the cell membranes or, in the opinion of other investigators, to the protoplasm. After death of the organism the action of endogenous enzymes causes a degradation of cell constituents, and the capacitive part of the tissue impedance diminishes. That fact was utilized by Hennings (1962) when he built an electronic device ("Fish-Tester") for the measurement of elapsed storage time of fish. The instrument is commercially available, and it is used at several veterinary offices at fisheries' ports (Reichstein, 1966). In a series of shipping studies in the USA the instrument was used to determine the initial freshness of the fish (Kaylor et al., 1968).

MATERIAL & METHODS

PRELIMINARY experiments had shown that the readings of the "Fish-Tester" were altered by an irradiation treatment of fish. That effect was followed up in a series of experiments with trout, a species freshly available locally. The effect of different doses on the readings was confirmed and at the same time an ac bridge assembly was set up to measure the impedance of the trout samples at several frequencies in the range from 1–100 kHz.

The bridge assembly was balanced in the arm opposite to the fish sample by calibrated resistor and capacitor connected in parallel. The impedance thus obtained is dependent of frequency. The impedance was calculated for each individual fish at the frequencies used. That value is equivalent to the impedance of the fish sample multiplied by the bridge ratio. Following the equation given by Hennings (where m and n are frequencies, and m < n)

$$Q(m,n) = \frac{Z(m) - Z(n)}{Z(n)} \cdot 100$$

the so-defined Q-values for combinations of ac frequencies were computed. The bridge ratio does not appear in the equation as it cancels in the numerator and the denominator. The Q-values are normalized by the impedance in the denominator and therefore are independent of the length of the measuring distance, i.e., the thickness of the fish sample. The Q-values were then averaged separately for each combination of frequencies and for the same treatments. A group of treatments contained seven to ten fishes, and the experiment was repeated several times.

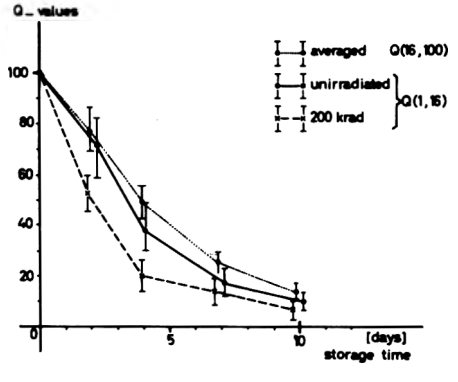

Fig. 1—Q-values for irradiated and unirradiated trout

RESULTS & DISCUSSION

"FISH-TESTER" readings are based on the simultaneous use of an ac current at 1 and 16 kHz. The corresponding Q-values computed from the bridge measurements agreed with the "Fish-Tester" readings after correction by response and calibration function of that instrument. Although the frequencies of 1 and 16 kHz were chosen by Hennings as suitable with salt water fish they proved to be useful with trout for the estimation of the time elapsed since slaughter for an interval of up to 10 days.

Q-values computed from bridge measurements at 16 and 100 kHz [in the following denoted as Q(16,100)-values] appeared to be relatively independent of the dose of irradiation treatment. Therefore these values were averaged for all treatments. From these means of the Q(16,100)-values the estimation of storage time on ice is possible (dotted curve in Fig. 1), regardless of irradiation.

At a radiation dose of 200 krad the Q(1,16)-values (dashed curve in Fig. 1) were significantly different from the Q(1,16)-value for the unirradiated trout (solid curve in Fig. 1). The results shown stem from one experiment, each Q(1,16)-curve is based on ten fish and the appropriate standard deviations are shown by vertical bars. Replications of the experiments gave fairly close agreement.

During the first day after slaughter all Q-values are rather scattered over a wide range and the rigor mortis leads to values higher than 100, i.e., beyond the range of the "Fish-Tester." Therefore the bridge readings leading to Q-values higher than 100 were cut off. Consequently in Figure 1, all Q-curves initiate at the common point of 100 and no standard deviations are presented at this point. With the disappearance of rigor mortis the readings become meaningful after the second day of storage. Up to the seventh day of storage the difference between irradiated and unirradiated trout is statistically significant at an error probability level ranging from 0.1–5%.

It is therefore proposed to estimate the age of trout from the Q(16,100) values, and then to use the radiation sensitive Q(1,16)-values to determine a radiation treatment and possibly also the dose.

Radiation doses higher than 200 krad lead to an even more marked decrease with respect to the values for unirradiated fish. With lower doses the difference is not significant. We hope to be able to recognize a radiation treatment with a dose of about 100 krad (which dose is likely to be used in commercial applications) by the use of better suited frequencies. The method is now well established for trout and may lead to a revised version of the "Fish-Tester," operating by simultaneous use of three frequencies and allowing the estimation of storage time and dose of radiation treatment from corresponding calibration curves. Further research will aim at adapting the method to economically important sea fish species.

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Ms received 10/31/71; revised 2/4/72; accepted 2/14/72.

Work supported by Research Contract No. 929/RB of the International Atomic Energy Agency, Vienna.

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A Research Note

EFFECT OF PRE- AND POST-MORTEM HANDLING ON REFLECTANCE CHARACTERISTICS OF CANNED SKIPJACK TUNA

INTRODUCTION

A PROJECT was carried out under the auspices of the National Marine Fisheries Service (formerly the Bureau of Commercial Fisheries) to study the effects of controlled pre- and post-mortem handling on the quality of canned skipjack. Previous reports by Crawford (1970) and Crawford et al. (1970) considered in detail the results from two experiments which attempted to relate the history of the fish after capture to post-mortem biochemical assay and finally to quality of the canned product.

EXPERIMENTAL

OUR PARTICIPATION involved measuring reflectance characteristics of canned samples from fish subjected to all of the controlled environmental conditions described by Crawford et al. (1970). In previous reports, (Little, 1969a, b, c) we showed (1) that CIE-Y values correlate directly with visual color scores; and (2) that the magnitude of the chromaticity shift (Δx, Δy) on reduction with Na dithionite is a measure of the relative concentration of denatured globin hemochrome. Therefore, we were interested in looking for factors responsible for exerting a significant effect on the reflectance characteristics and thus on the color of canned skipjack tuna.

Considerable delay occurred before results from coded cans could be interpreted in terms of handling variables, post-mortem changes and sensory evaluations of the corresponding samples. Consequently, our observations are presented as an addendum to the reports previously cited.

Table 1—Effect of holding temperature and time on Y value and chromaticity shift—mean value for all samples held at T°F-X hr

Holding temp/time	Y	Δx	Δy
Controls	26.7	5.0	− 9.4
32°F–6 hr	29.2	4.9	− 8.3
60°F–6 hr	27.6	3.6	−10.6
78°F–6 hr	28.0	2.4	− 9.5
78°F–9 hr	27.2	1.2	− 8.7

RESULTS

REFLECTANCE data were obtained on samples representing 34 combinations of pre- and post-mortem handling conditions, rested or stressed before death, held at various temperatures for specified intervals of time postmortem, canned immediately or after freezing, along with appropriate controls not subjected to post-mortem holding conditions.

On the basis of Y value alone, the samples showed remarkable consistency in color evaluation with an over-all mean value of 28, a range of 22–33, with 82% of the samples clustering between 26 and 30 and the remaining 18% distributed equally between higher and lower values. According to the Federal Standard of Identity for canned tuna, samples are designated light if they fall within the range 5.3–6.2 Munsell Value units, equivalent to range in Y value of 22.7–33.6. Thus, over 90% of the samples discussed here fell within the upper range of the scale for light meat tuna.

It was apparent that none of the handling practices prior to canning was sufficiently severe to affect systematically and significantly the reflectance values of the canned fish; therefore, differences among samples must be ascribed to individual variation.

Data on the magnitude of chromaticity shift (Δx, Δy) on reduction with Na dithionite told a somewhat different story. When we examined the effect of increasing temperature of the holding bath post-mortem on the reflectance measurements, we observed a systematic decrease in magnitude of chromaticity shift, particularly in Δx, on increasing holding temperature and finally on increasing time at the highest temperature to which the fish were subjected. The data presented in Table 1 show the mean values for Y, Δx and Δy for all fish subjected to the same post-mortem holding conditions.

Since the x-chromaticity coordinate is a measure of the proportion of 'red' reflectance, in this case, a decrease in Δx signifies a decrease in the formation of the bright red ferro-hemochrome. Thus, when the amount of pigment available for reduction in the canned fish is shown to decrease with increasing holding temperature prior to processing, it becomes attractive to relate these observations to the hypothesis that post-mortem denaturation of myoglobin inhibits conversion to hemochrome, the pigment responsible for the typical color of canned tuna (W.D. Brown, private communication).

The observations reported here provided the only presumptive evidence of systematic change in muscle chemistry under the experimental conditions employed in the study. These changes, however, did not exert a measurable effect on quality evaluation based on Y value or on visual color score. The possibility exists that the range in treatment was not sufficient to identify conditions of abuse leading to marked deterioration in quality of canned tuna.

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Ms received 12/16/71; accepted 1/24/72.

This study was carried out under contract to the National Marine Fisheries Service, Contract 14-17-0001-2091.

The author thanks Joachim Wolf-Priessnitz for his technical assistance.

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