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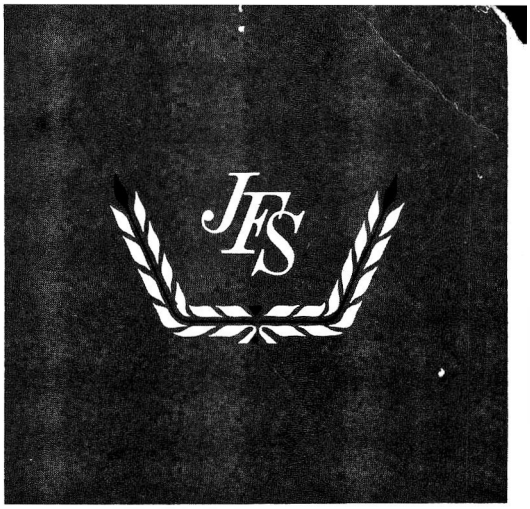

JOURNAL of FOOD SCIENCE

APPLIED SCIENCE and ENGINEERING

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A PUBLICATION OF THE INSTITUTE OF FOOD TECHNOLOGISTS

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from front cover

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ABSTRACTS:
IN THIS ISSUE

PROTEIN QUALITY OF DRY ROASTED SOYBEANS: AMINO ACID COMPOSITION AND PROTEIN EFFICIENCY RATIO. A. F. BADENHOP & L. R. HACKLER. *J. Food Sci.* 36, 1–4 (1971)– A study was undertaken to determine the effect of the dry-roasting process on the quality of soybean protein as indicated by amino acid analyses and PER. Amino acid analyses revealed losses in tryptophan, available and total lysine, cystine and histidine of 35, 31, 17, 15 and 6% respectively. These losses are reflected in both the essential amino acid index (EAAI) and PER. Average EAAI values decreased with increasing degree of roast (68.4, 67.7 and 63.8) compared with a value of 71.4 for the raw samples. The PER values for the same roasted samples were 1.70, 1.46 and 1.28 respectively. A depressed PER value of 0.61 was obtained for the raw sample because of anti-nutritional factors in the raw beans. Results indicate that the dry roasting process is a means of producing a palatable, nutritious food from soybeans. However, optimum palatability is gained at the expense of protein utilization.

FULL-FAT SOY FLOUR EXTRUSION COOKED: PROPERTIES AND FOOD USES. G. N. BOOKWALTER, G. C. MUSTAKAS, W. F. KWOLEK, J. E. MC GHEE & W. J. ALBRECHT. *J. Food Sci.* 36, 5–9 (1971)–Full-fat soy flours prepared by the extrusion process were shown to have good nutritive value, flavor, and stability. A flour cooked to a nitrogen solubility index (NSI) of 30 stored well, but the addition of tertiary butyl hydroquinone was necessary to prevent rancidity in flours cooked to 19 and 11 NSI values. The 30 NSI flour was more yellow than a commercial flour cooked to the same degree by a different process. When soy flours were compared in bread to 3 and 6% nonfat dry milk on an equivalent protein, fat, and reducing sugar basis, baking properties were similar. At 15 and 20% levels of soy flour, loaf volume decreased less with the extruded products than with nonextrusion-processed soy flour.

SOYBEAN WHEY PROTEINS–RECOVERY AND AMINO ACID ANALYSIS. J. J. RACKIS. D. H. HONIG, D. J. SESSA & J. F. CAVINS. *J. Food Sci.* 36, 10–13 (1971)– About 14 million lb of soybean whey protein of high biological value is disposed of as waste based on estimated production figures for soybean concentrates and isolates. An estimated 5–7% annual increase in consumption poses serious waste disposal problems and alternates should be sought. By simulated commercial procedures, yield and protein content of soybean whey were determined. Whey solids account for 2–28% of original nitrogen in dehulled, defatted flakes. Whole whey protein was prepared by dialysis, and whey was also fractionated by heating into heat-coagulable and supernatant proteins. Whole whey protein has a good balance of essential amino acids when compared with a system followed by the Food and Agricultural Organization of United Nations based on hen's egg protein. Heat-coagulable and supernatant proteins varied greatly: heat-coagulable fractions had 42% of the sulfur amino acid content but 181% of the tryptophan content of hen's egg protein; supernatant protein had 142% of the sulfur amino acid content but only about 60% of the isoleucine, valine, leucine and tryptophan content of hen's egg protein. All three whey protein fractions would be suitable for addition to feeds.

USE OF ULTRAFILTRATION/REVERSE OSMOSIS SYSTEMS FOR THE CONCENTRATION AND FRACTIONATION OF WHEY. R. I. FENTON-MAY, C. G. HILL JR. & C. H. AMUNDSON. *J. Food Sci.* 36, 14–21 (1971)– Existing ultrafiltration/reverse osmosis technology provides a means of fractionating and concentrating cheese whey into liquid fractions containing a number of protein:lactose ratios. Ratios ranging from 1:8 to 2:1 or higher were obtained experimentally. The ratio obtained is a function of the permeability and selectivity characteristics of the membrane and the system design and operating conditions. The experimental results indicate that mass transfer through lactose-permeable membranes is often boundary-layer controlled. Membrane permeation rates are thus strongly dependent on feed flow rate and temperature. The sanitation problems associated with the introduction of these unit operations in the dairy and food processing industries are also discussed at length.

PREPARATION OF SOYBEAN CHEESE USING LACTIC STARTER ORGANISMS. 3. Effects of Mold Ripening and Increasing Concentrations of Skim Milk Solids. D. J. SCHRODER & H. JACKSON. *J. Food Sci.* 36, 22–24 (1971)– Soybean cheeses were prepared from blends of skim milk powder and soybean milk in which the skim milk powder contributed 0, 25, 50 and 75% of total dry weight. The amount of skim milk had little effect on the flavor of the finished cheese, due to the dominating effect of the beany flavor of the soybeans. Similarly, the skim milk had little effect on the texture of the finished cheese, indicating that only a small amount of fibrous matter from the soybeans is necessary to impart a mealy texture to the product. Mold ripening resulted in desirable changes in texture, but these were offset by the development of bitter flavors.

REVERSE OSMOSIS OF COTTAGE CHEESE WHEY. 1. Influence of Composition of the Feed. C. PERI & W. L. DUNKLEY. *J. Food Sci.* 36, 25–30 (1971)– Permeation rates, retentions, and solute fluxes during reverse osmosis of whey and whey fractions were compared using two types of cellulose acetate membranes. Concentration polarization and fouling influenced performance of the membranes. Concentration polarization decreased both permeation rate and retention. Fouling decreased permeation rate, but its influence on retention was variable and depended principally on the feed, the solute, and the available driving force. Proteins and other macromolecules in whey had a greater influence on performance during reverse osmosis than smaller solute molecules.

DYNAMIC TURBULENCE PROMOTION IN REVERSE OSMOSIS PROCESSING OF LIQUID FOODS. E. LOWE & E. L. DURKEE. *J. Food Sci.* 36, 31–32 (1971)– The phenomenon of concentration polarization operates to reduce the efficiency of solvent-solute separation in the processing of foods by reverse osmosis. The viscous nature of most food concentrates aggravates the problem. Disrupting the boundary layer by mechanically inducing turbulence in the fluid stream improves the permeation efficiency. Flux improvement of up to 3-fold is possible with dynamic turbulence promotion involving plastic spheres moving randomly with the orange juice concentrate. Concentrate (and sphere) movement is pulsed alternately from one end of the membrane flow channel to the other. The spheres are confined within the flow channel to avoid the problem of moving them through the recycling pumps. A net movement of concentrate results when the pump volume is greater in one direction than the other. The permeation rate with dynamic turbulence promotion does not drop off significantly with time. The long-term effects of sphere movement on the membrane surface are not known but are believed not to be serious.

ABSTRACTS:
IN THIS ISSUE

AN EXPERIMENTAL STUDY OF MOISTURE AND TEMPERATURE DISTRIBUTIONS DURING FREEZE-DRYING. J. D. HATCHER, D. W. LYONS & J. E. SUNDERLAND. *J. Food Sci.* 36, 33–35 (1971)– Measurements of the moisture and temperature distributions were made during freeze-drying beef at a pressure of 1 torr. The transient moisture distributions measured with gamma ray techniques indicate that the phase change region would have a thickness less than 3/16 in., that no drying takes place until the phase change region reaches a given position, and that no additional drying occurs after the phase change passes a given position. A careful study of the data showed that the drying rate was influenced by heat transfer through the frozen region to the phase change position.

STABILIZATION OF CARROT JUICE BY DILUTE ACID TREATMENT. T. S. STEPHENS, G. SALDANA, H. E. BROWN & F. P. GRIFFITHS. *J. Food Sci.* 36, 36–38 (1971)– A coagulum which forms in canned juice extracted from raw carrots or carrots heated in water does not occur in canned juice extracted from carrots heated in a weak acetic acid solution prior to juice extraction. The juice from the acid-treated carrots has a bright-orange color. The canned juice from raw carrots had less pectic substances and starch and about the same amount of protein as the juice from the water-treated or acid-treated carrots, but formed the most coagulum during heat processing.

CARBOHYDRATE TRANSFORMATIONS, COLOR AND FIRMNESS OF CANNED SWEET POTATOES AS INFLUENCED BY VARIETY, STORAGE, pH AND TREATMENT. W. A. SISTRUNK. *J. Food Sci.* 36, 39–42 (1971)– There was an increase in brightness of color and firmness by holding peeled sweet potatoes 24 hr in buffers before canning. The sugars and phenolic substances were leached out during holding. There was a decrease in starch and hemicellulose as a result of storage; whereas, water- and Calgon-soluble pectin were not affected. Starch decreased with an increase in pH regardless of other variables. Water-soluble pectin increased when pH was altered up or down from the normal pH of canned sweet potatoes of approximately 6.0. In comparison, Calgon-soluble pectin and hemicellulose reacted inversely to pH. Total polyphenols decreased with an increase in pH.

EVALUATION OF OLEIC SAFFLOWER OIL IN FRYING OF POTATO CHIPS. G. FULLER, D. G. GUADAGNI, M. L. WEAVER, G. NOTTER & R. J. HORVAT. *J. Food Sci.* 36, 43–44 (1971)– Oil with iodine value ca. 90 is now available from a new variety of safflower. The low iodine value results from a reversal of oleic:linoleic acid ratio from that of ordinary safflower (iodine value ca. 145). Lower linoleic acid content causes the oil to be more stable towards oxidation at both ambient and frying temperatures. Potato chips fried in the new safflower oil were as stable toward rancidity during accelerated storage as chips fried in hydrogenated vegetable oil.

PALATABILITY OF INDIVIDUAL MUSCLES FROM OVINE LEG STEAKS AS RELATED TO CHRONOLOGICAL AGE AND MARBLING. L. E. JEREMIAH, G. C. SMITH & Z. L. CARPENTER. *J. Food Sci.* 36, 45–47 (1971)– 5 muscles from each of 243 ovine leg steaks were evaluated to determine the effects of chronological age and marbling on palatability and to identify differences in palatability among individual muscles. Significant differences in tenderness and juiciness were observed among muscles. The contribution of marbling to juiciness differed significantly among muscles, but marbling had little relationship to tenderness. As chronological age increased, tenderness decreased ($P < .01$), which might be a manifestation of the effects of drying during the cooking process.

THE INFLUENCE OF GLUCONO DELTA LACTONE ON CURED HAM COLOR AND COLOR STABILITY. T. D. PATE, R. O. SHULER & R. W. MANDIGO. *J. Food Sci.* 36, 48–50 (1971)– Pairs of hams from 72 crossbred barrows slaughtered at three weight intervals (68.2, 90.9, 113.6 kg) were assigned to three treatments of glucono delta lactone (GDL) (0, 30, or 60 g/l) and two post cure aging periods (1 or 14 days). Following post cure aging, samples from the boneless hams were exposed to light (200 ft-c) for various time intervals (0, 30, 60, 90, 120, 150, 180, 1440 min) and nitric oxide- and total-pigments extracted with acetone/water and acetone/acid respectively. There were no significant differences in color formation or color stability of hams aged 1 or 14 days. The influence of GDL and total-pigment formation and stability was non-significant at most exposure intervals.

EFFECT OF SOME CHEMICAL COMPOUNDS ON THE BUFFERING CAPACITY OF HORSEHAIR CRAB (Erimacrus isenbeckii) MUSCLE DURING INTERMOLT STAGES. T. MOTOHIRO & N. INOUE. *J. Food Sci.* 36, 51–54 (1971)– The calcium contents varied from 1.1 to 2.2 mg/g throughout the intermolt cycle; phosphoric acid and hexosamine level varied in a similar way to the calcium content and the amino acid level was almost constant. The buffering capacity was strong in "papercrab" muscle and weak in "hardcrab" muscle and was affected by the organic and inorganic reserves, so that removal by washing with water resulted in a weakening of the buffering capacity.

ACCURACY OF PREDICTING OCCURRENCE OF GREENING IN TUNA BASED ON CONTENT OF TRIMETHYLAMINE OXIDE. M. YAMAGATA, K. HORIMOTO & C. NAGAOKA. *J. Food Sci.* 36, 55–57 (1971)– To predict green tuna after cooking, a practical measurement to select green tuna using 100 pieces of iced and clipper-frozen yellowfin tuna was discussed. The most suitable sampling portions, the lowest level of trimethylamine oxide (TMAO) and the total trimethylamine content (TTMA) in tuna muscles were examined. It was recognized that the prediction of green tuna after cooking would be possible by determining TTMA content at the superficial layer of the central dorsal raw muscle (E portion). The lowest level of TMAO-N was 8–9 mg%, and that of TTMA-N was 11–12 mg% in an iced and 9 mg% in a clipper-frozen green tuna. The predictive measurement accuracy was 96% on the lowest level of TMAO-N or TTMA-N.

SALT CURE AND DRYING-TIME AND TEMPERATURE EFFECTS ON VIABILITY OF Trichinella spiralis IN DRY-CURED HAMS. W. J. ZIMMERMANN. *J. Food Sci.* 36, 58–62 (1971)– An official USDA dry-cure ham processing procedure was evaluated for efficacy in devitalizing trichinae. The procedure consisted basically of a 40-day salt cure followed by 10 days of drying at 95°F or above. Within the cure period, marked loss of viability was noted only for trichinae in muscle with brine concentrations approaching 8%. The drying temperature was the critical factor in devitalizing trichinae. 28 days' cure followed by 6 days of drying at 98°F destroyed viability of all parasites regardless of brine concentration. Other effective drying time-temperature combinations included: 125°F–4 days; 70°F–35 days and 40°F–190 days.

RADIATION SENSITIVITY AND BIOCHEMICAL CHARACTERISTICS OF MICROFLORA OF BOMBAY DUCK (Harpodon nehereus). U. S. KUMTA & S. S. MAVINKURVE. *J. Food Sci.* 36, 63–66 (1971)–Microbial flora of unirradiated and irradiated (0.5 Mrad) Bombay duck (*Harpodon nehereus*) was differentiated into organisms as spoilers and non-spoilers based on ability to liquify gelatin; ferment glucose; and produce indole, H_2S and urease. In spoiling unirradiated fish, there was a predominance of *Vibrio, Aeromonas* and *Proteus* spp while *Micrococci* and *Achromobacter* spp were the major surviving groups in irradiated Bombay duck stored for 15 days at 10°C. The predominant spoilers, *Proteus vulgaris* and *Aeromonas hydrophila*, produced large amounts of TMA and TVBN and were radiation sensitive as indicated by D_{10} values of 8.6 and 5.4 Krad respectively. *Micrococcus luteus* was relatively biochemically inert and radiation resistant, the D_{10} value being 88 Krad.

EFFECT OF OXYTETRACYCLINE AND CHLORTETRACYCLINE ON SURVIVAL OF THE TREMATODE Heterophyes sp. IN THE FLESH OF MULLET CAUGHT IN BRACKISH EGYPTIAN WATERS. M. G. E. HAMED & A. N. ELIAS. *J. Food Sci.* 36, 67–69 (1971)– Mullet, *Mugil cephalus,* caught from brackish Egyptian waters, serve as an intermediate host for *Heterophyes* parasite which renders such fish hazardous to health. The objective of this work was to show the effectiveness of antibiotics, used to prolong the storage life of fresh fish, as parasiticides. To achieve this, both whole mullet and small portions of infected mullet tissue were dipped in oxytetracycline (OTC) and chlortetracycline (CTC) solutions of different concentrations ranging from 30–4000 ppm. The rate of parasite destruction, very slow at low concentrations of either antibiotic, increased at much higher concentrations of antibiotic. However, a concentration of antibiotic sufficiently high to destroy the parasite completely cannot be used for the preservation of fresh fish because of the high concentration of antibiotic in the fish tissue after treatment. Although these findings are negative, they have a place in the literature.

EXTRACT RELEASE VOLUME (ERV) RESPONSES WITH ASEPTIC AND INOCULATED PORK. L. S. MILLER & J. F. PRICE. *J. Food Sci.* 36, 70–73 (1971)– The extract release volume (ERV) phenomenon, previously reported as a reliable rapid indicator of bacterial spoilage in meat, was observed for pork muscle obtained aseptically and that inoculated with pure and mixed cultures of bacteria over a 20-day storage period at 2° and 10°C. ERV values obtained using pork muscle were found to be inversely related to bacterial numbers regardless of population type (homogeneous or heterogeneous) of the microorganisms. However, the correlations between ERV and bacterial numbers did not denote reliable prediction of the bacteriological condition of pork by ERV. The correlation between ERV and bacterial numbers was much higher under conditions of mixed culture contamination than for samples contaminated with pure cultures of psychrophilic, acid-producing, or anaerobic bacteria. Minor initial differences in ERV between control (aseptic) and inoculated samples indicated that ERV responded to growth of bacteria rather than to their presence. Differences in ERV that were attributable to sample source (animal differences) by analysis of variance test indicated there was a wide range of water holding capacity in pork longissimus dorsi muscle samples. Contrary to the usual ERV response with meat spoiled under cool conditions, ERV was found to increase with storage time and bacterial growth when pork was contaminated with a putrefactive anaerobe (*C. perfringens*) and stored at 35°C. Decreases in ERV with bacterial growth or storage time could not be explained solely as a pH effect, yet pH appeared to influence the magnitude of the changes in observed ERV.

DRY ICE IN VARIOUS SHIPPING BOXES FOR CHILLED POULTRY: EFFECT ON MICROBIOLOGICAL AND ORGANOLEPTIC QUALITY. J. E. THOMSON & L.A. RISSE. *J. Food Sci.* 36, 74–77 (1971)– Comparisons were made of dry ice and water ice in three types of shipping boxes for chilled chickens: wax resin-coated corrugated fiberboard; expanded polystyrene foam; and wirebound wood-veneer. Microbial counts, CO_2 concentration and off-odor development were determined. Microbial counts on poultry stored at 0.5°C for up to 9 days were not significantly different as a function of box type or coolant. Counts on poultry stored at 4.4°C were significantly greater at 9 days on poultry stored in fiberboard boxes with dry ice than on poultry with water ice in fiberboard boxes or polystyrene boxes with dry ice; at 3 and 6 days there were no significant differences. At 5.2°C, counts were significantly smaller in polystyrene boxes with dry ice than in either wirebound boxes with water ice or fiberboard boxes with dry ice. An off-odor not characteristic of spoilage odor could develop in CO_2 atmosphere storage earlier than spoilage odor in an air atmosphere if storage temperature was low (0.5°C). At a higher temperature (5.2°C), spoilage odor in air occurred earlier than CO_2-related off-odor in CO_2 atmosphere.

COTTAGE CHEESE SHELF LIFE AND SPECIAL GAS ATMOSPHERES. C. R. SCOTT & H. O. SMITH. *J. Food Sci.* 36, 78–80 (1971)–Cottage cheese samples were stored at 3–4°C for 10–12 days in special all-glass containers with purified carbon dioxide, nitrogen and air atmospheres. Shelf-life quality of the cheese was measured by taste panel scoring and by bacterial counts of the top centimeter of product. Carbon dioxide slightly decreased the bacterial counts but it produced an acid or tart cheese. Nitrogen did not significantly decrease the bacterial counts nor affect the taste of the cheese.

FACTORS AFFECTING MACADAMIA NUT STABILITY. 3. Effects of Roasting Oil Quality and Antioxidants. C. G. CAVALETTO & H. Y. YAMAMOTO. *J. Food Sci.* 36, 81–83 (1971)– Chemical and physical changes which occurred in macadamia roasting oil during 13 weeks of continuous use did not appreciably affect kernel shelf-life. Changes in iodine number and fatty acid composition indicated there was considerable oil exchange between roasting oil and macadamia kernels. The effects of direct antioxidant application (butylated hydroxyanisole and butylated hydroxytoluene) and vacuum packing (0, 15 and 24 in.) on roasted macadamia kernel stability also were studied. Stability of antioxidant-treated kernels was greater than that of untreated kernels, regardless of vacuum level. Vacuum packing had no effect on antioxidant-treated kernels, but showed some benefit for untreated kernels.

BOUND WATER DETERMINATION USING VACUUM DIFFERENTIAL SCANNING CALORIMETRY. P. J. BECHTEL, M. P. PALNITKAR, D. R. HELDMAN & A. M. PEARSON. *J. Food Sci.* 36, 84–86 (1971)–A method has been developed for determination of bound water in dry food stuffs. The instrumentation consists of a differential scanning calorimeter (DSC), a vacuum source and a time base recorder. Samples of freeze-dried beef powder, freeze-dried egg powder, modified corn starch powder and non-fat dry milk were equilibrated to 5 different moisture contents (24, 41, 63, 82 and >99% R. H.) at a constant temperature (27°C). The samples were placed in the DSC, which was programmed for isothermal conditions, and moisture was removed by decreasing the pressure in the calorimeter. Results indicated that the energy associated with moisture losses can be calculated from the area under the curve. Relative bound water values were determined and are presented. These data are useful in determining gross energy trends in water removal from foodstuffs. Egg powder and starch powder bound water best, while egg powder and beef powder rehydrated best.

POTENTIAL OF FREEZE DRYING FOR REMOVAL OF CHLORINATED HYDROCARBON INSECTICIDES FROM EGGS. M. E. ZABIK & L. R. DUGAN JR. *J. Food Sci.* 36, 87–88 (1971)– Significant pesticide reductions of 79% for lindane, 37% for dieldrin, 57% for p,p'-DDT and 31% for o,p'-DDT-DDD occurred in freeze-dried whole eggs. Half the lindane was removed by freeze drying egg yolk, but only minimal removal of the other pesticides occurred. The success of freeze drying for pesticide removal from eggs appeared to be a function both of the vapor pressure of the pesticide and the amount of contamination in the whole egg.

ABSTRACTS:
IN THIS ISSUE

INSECT PROBLEMS OF PECAN SHELLING PLANTS AND THEIR RELATION TO INSECTS AND INSECT PARTS IN PROCESSED PECANS. J. S. GECAN, P. M. BRICKEY, W. V. EISENBERG & A. ROAF. *J. Food Sci.* **36**, 89–92 (1971)– –The incidence of various types of insect contaminants in inshell and shelled pecans and their relation to processing methods were determined in factory studies. Pecans may become infested in the field with primary and secondary insects and in the shelling plant by storage insects. Raw stock sorting by air separation proved ineffective for removing insect-infested inshell nuts. Insects and fragments in cracked infested nuts are largely removed by subsequent air separation. During processing, *Curculio caryae* larvae concentrate in midget and small pecan pieces. Hand sorting under UV light was the best procedure for removing curculio larvae from the finished product. Finished shelled pecan products contained relatively few insect fragments.

FLAVOR QUALITY OF EXPLOSION PUFFED DEHYDRATED POTATO. 3. Contribution of Pyrazines and Other Compounds to the Toasted Off-flavor. G. M. SAPERS, S. F. OSMAN, C. J. DOOLEY & O. PANASIUK. *J. Food Sci.* 36, 93–95 (1971)– –Gas chromatographic analyses of potato volatile concentrates prepared from explosion puffed and conventionally dehydrated potatoes by steam distillation, extraction of distillates with diethyl ether and solvent evaporation showed that peak heights of ten components were associated with the intensity of a toasted off-flavor formed during puffing. Eight of these and at least two other minor components were found to have toasted, baked, or burnt aromas suggestive of the off-flavor. Compounds identified or tentatively identified by mass spectrometry, retention time, and aroma included alkylpyrazines and products of proline degradation, sugar pyrolysis, and Strecker degradation reactions.

OXIDATION OF CAPSANTHIN. T. PHILIP & F. J. FRANCIS. *J. Food Sci.* **36**, 96–97 (1971)– –Oxidation of capsanthin by molecular oxygen at 40°C in the solid state is discussed. The absence of an induction period in the oxygen absorption curve indicated that oxidation does not involve the typical autoxidation pattern. A number of keto-carotenoids such as capsanthone, 3-keto-kryptocapsone, and 3-keto-β-apo-8′-carotenal were isolated in the oxidation products. Oxidation of capsanthin involves primarily the oxidation of hydroxyl groups, followed by scission of the chain at the carbon-carbon bond α to the in-chain carbonyl group.

THE NATURE OF FATTY ACIDS AND CAPSANTHIN ESTERS IN PAPRIKA T. PHILIP, W. W. NAWAR & F. J. FRANCIS. *J. Food Sci.* **36**, 98–100 (1971)–-The triglycerides present in whole ground paprika and paprika pods were extracted and hydrolyzed. The fatty acids were methylated, separated by gas chromatography and identified by mass spectrometry. The whole paprika and pods, respectively, contained approximately 66 and 45% linoleic acid, 14 and 19% palmitic acid, 12 and 14% oleic acid and 5 and 17% linolenic acid. Small quantities of myristic and lauric acids and traces of capric, stearic and palmitoleic acids also were present. Capsanthin, which amounted to 35% of the total carotenoids, occurred as the dilaurate ester. It was isolated from paprika by thin-layer chromatography after interesterification of the tryglycerides. Capsanthin dilaurate, synthesized in the laboratory, gave identical R_f value and infrared and visible spectra to those of the naturally occurring compounds.

ANTHOCYANINS IN RED ONION, Allium cepa. T. Fuleki. *J. Food Sci.* **36**, 101–104 (1971)–Eight anthocyanins were found in the bulbs of the Ruby and Southport Red Globe varieties of red onion. The major anthocyanin and the one present in second largest quantity were identified by their chromatographic, spectral and chemical properties as cyanidin 3-glucoside and cyanidin 3-diglucoside respectively. The latter was not identical with either the 3-sophoroside or the 3-gentiobioside of cyanidin; therefore, it is a "new" pigment representing a "new" glycosidic class for anthocyanins. All but one of the onion anthocyanins were cyanidin glycosides. The peonidin monoside, present only in minor quantity was identified as peonidin 3-glucoside. Peonidin 3-arabinoside, the pigment identified in the literature as the anthocyanin in the Southport Red Globe variety, was not found in any of the varieties examined.

A GAS CHROMATOGRAPHIC PROCEDURE FOR ANALYSIS OF AQUEOUS ORANGE ESSENCE. M. G. MOSHONAS & E. D. LUND. *J. Food Sci.* **36**, 105–106 (1971)– A procedure for direct GLC analysis of orange essence was developed. The procedure uses a Porapak precolumn to separate the water, methanol, acetaldehyde and ethanol from the remainder of the volatile organic constituents of the essence. Determination of the organic material in the essence can be obtained by this method from a 25 μl sample of aqueous essence. The method allows a quantitative estimation of acetaldehyde, methanol and ethanol as well as the smaller amounts of other organic compounds in the essence.

RECOVERY OF PHENOLIC WOOD SMOKE COMPONENTS FROM SMOKED FOODS AND MODEL SYSTEMS P. ISSENBERG, M. R. KORNREICH & A. O. LUSTRE. *J. Food Sci.* **36**, 107–109 (1971)– Procedures for extraction, isolation, and concentration of wood smoke phenols from commercial summer sausage, laboratory smoked pork belly, and model systems were evaluated during development of methods for quantitative determination of individual phenolic compounds in smoked food products. Extraction of summer sausage with 50% aqueous ethanol, followed by thorough washing of the residue resulted in 84% recovery of added ^{14}C-phenol. Additional losses occurred during isolation and concentration of a phenolic fraction, resulting in an overall recovery of 59% of the added phenol. Concentrates were prepared from 5% NaOH extracts and compared by gas chromatographic analysis with those prepared by aqueous ethanol extraction. A greater variety of substituted methoxy and dimethoxy phenols was found in the concentrates prepared from alkaline extracts. Recoveries of phenols from summer sausage, pork belly, and model systems simulating wet tissue and fat are compared.

MOLECULAR PROPERTIES OF POSTMORTEM MUSCLE. 10. Effect of Internal Temperature and Carcass Maturity on Structure of Bovine Longissimus. J. G. SCHMIDT & F. C. PARRISH JR. *J. Food Sci.* **36**, 110–119 (1971)– A study was carried out with light and electron microscopic techniques to discover the effect of heating and carcass maturity upon the connective tissue and myofibrillar proteins of longissimus from veal (3–6 months), A (12–20 months) and D (54–60 months) maturities. Longissimus was heated to internal temperatures of 1) 50°C; 2) 60°C; 3) 70°C; 4) 80°C and 5) 90°C. At 50°C the structural changes observed were endomysial connective tissue and myofibrillar protein shrinkage. However, at 60°C loss of M-line structure, initiation of disintegration and coagulation of thin and thick filaments and further myofibrillar protein shrinkage were observed. At 70°C, perimysial connective tissue shrinkage occurred. Also, connective tissue fibers from longissimus of maturity D were usually more heat resistant than those from veal and maturity A. Heating above 70°C caused further degradation of connective tissue and coagulation of thick filaments, but even at 90°C the principal banding features of the sarcomere were identifiable. These changes are discussed in relationship to changes in meat tenderness.

EFFECT OF TEMPERATURE DURING POST-MORTEM GLYCOLYSIS AND DEPHOSPHORYLATION OF HIGH ENERGY PHOSPHATES ON POULTRY MEAT TENDERNESS. A. W. KHAN. *J. Food Sci.* 36, 120–121 (1971)–Tests made on pectoralis major muscles having post-slaughter pH values ranging between 6.1–7.0, indicated that holding poultry meat at 30 and 37°C during the onset of rigor mortis caused toughness. This toughening effect of high temperature appeared to occur when the pH level of the meat dropped from a value of about 6.3 to its ultimate low value and the adenosine triphosphate content dropped below 40% of its initial concentration. Holding temperatures at 10, 15 and 25°C during the onset of rigor mortis, or cooling to 15°C before the pH value dropped to about 6.3 produced more tender meat. After completion of post-mortem glycolysis and dephosphorylation of high energy phosphates, high temperature had no deleterious effect on tenderness. These results indicate that dephosphorylation of adenosine triphosphate at high temperature affects the mode or extent of stiffening of the muscular tissue and prevents tenderization.

PROTEIN EXTRACTABILITY OF TURKEY BREAST MUSCLE EXHIBITING DIFFERENT RATES OF POST-MORTEM GLYCOLYSIS. D. R. LANDES, L. E. DAWSON & J. F. PRICE. *J. Food Sci.* 36, 122–124 (1971)–Changes in pH and in protein extractability were determined in turkey breast muscles from anesthetized and nonanesthetized birds during the period from 0–72 hr post-mortem. Shear values were measured for cooked muscles 72 hr post-mortem. The anesthetic caused the pH and extractability of proteins to remain fairly constant during the first hr post-mortem, in contrast to rapid changes in muscles from control birds. Total extractable nitrogen, total soluble fibrillar protein nitrogen and soluble actomyosin nitrogen extracted from muscle of control birds increased during post-mortem aging. Muscles from anesthetized birds were more tender than those from control birds.

RESPONSE TO ELECTRICAL STIMULATION AND POST-MORTEM CHANGES IN TURKEY PECTORALIS MAJOR MUSCLE. R. T-I MA, P. B. ADDIS & E. ALLEN *J. Food Sci.* 36, 125–129 (1971)– Muscle samples were obtained from 20 turkey hens 5 min postexsanguination for the determination of sarcomere length, response to electrical stimulation and time course of rigor mortis. Additional samples were obtained from the opposite (undisturbed) pectoralis major muscle at 1, 2, 6 and 24 hr post-mortem for the determination of sarcomere length. Hens that displayed a long time course of rigor mortis had a low threshold of response (9v) and a long duration of response (106 sec) to electrical stimulation. Conversely, muscles which became inextensible within 50 min displayed a high threshold (123v) and low duration (8 sec). Increase in sarcomere length occurred within 1 hr post-mortem in the fast-rigor group and 6 hr in slow-rigor birds. Further experimentation revealed that postexsanguination struggling decreased the time course of rigor, elevated threshold and shortened duration of response to stimulation. The results suggest that in some presently-used commercial turkey processes some birds are frozen in a pre-rigor, short-sarcomere state.

EFFECT OF A PORCINE PANCREATIC COLLAGENASE ON MUSCLE CONNECTIVE TISSUE. L. D. SATTERLEE. *J. Food Sci.* 36, 130–132 (1971)–Porcine and bovine pancreas as well as porcine spleen, liver and kidney were analyzed for possible collagenolytic activity. Both the porcine and bovine pancreas extracts possessed collagenolytic activity at pH 5.5, 37°C. The ability of the porcine pancreas extract to break down connective tissue was not due to tryptic or chymotryptic activity, but was due to a combination of collagenolytic and elastolytic activity. The collagenase was partially purified using Sephadex chromatography and was shown to degrade collagen into small soluble peptide fragments.

MYOSIN STABILITY IN INTACT CHICKEN MUSCLE AND A PROTEIN COMPONENT RELEASED AFTER AGING. C.-S. C. WU & R. N. SAYRE. *J. Food Sci.* 36, 133–137 (1971)– Myosin was extracted with Hasselbach-Schneider solution from both red (myosin-R) and white (myosin-W) chicken muscle aged for 30 min to 24 hr post-mortem. Chromatography on DEAE-Sephadex of myosin-W from aged muscle separated an unusual protein fraction, component T, which differed from myosin by (1) no ATPase activity; (2) a higher sedimentation coefficient; and (3) ultraviolet absorption spectra, extinction coefficient and tyrosine-to-tryptophan ratio. Following chromatography, the ATPase activities and sedimentation patterns were similar for myosin from unaged and aged muscle.

METMYOGLOBIN FORMATION IN BEEF MUSCLES AS INFLUENCED BY WATER CONTENT AND ANATOMICAL LOCATION. D. A. LEDWARD. *J. Food Sci.* 36, 138–140 (1971)– Decreased water content of semitendinosus muscle led to increased metmyoglobin formation, but only at degrees of dehydration that led to marked discoloration due to other causes e.g., heme concentration. Metmyoglobin formation was very dependent on the anatomical location of the muscle ($P < 0.001$). The susceptibility to metmyoglobin formation for the four muscles studied was biceps femoris > semimembranosus > longissimus dorsi = semitendinosus.

USE OF SONIC AND ULTRASONIC MEASUREMENTS ON BOVINE BONE TO ESTIMATE CHRONOLOGICAL AGE. C. E. DAVIS, E. E. FINNEY & D. R. MASSIE. *J. Food Sci.* 36, 141–143 (1971)–Sonic and ultrasonic measurements were made on bovine bone to explore their possible value in objectively determining chronological age of a beef animal carcass. The sonic resonant frequency of the bone and the transmission rate of an ultrasonic pulse through the bone were measured on the right large metacarpal bone of slaughtered beef animals of different ages of the same breed. Thirty-four bones from animals approximately 6, 12, 18, 24, and 30 months of age were measured. The simple correlation coefficient between animal age and the sonic resonance parameter, $(FL)^2$ was 0.875 and between age and the ultrasonic velocity parameter, $(cm/\mu sec)^2$ was 0.866.

AVAILABILITY OF AMINO ACIDS IN SARCOPLASMIC FISH PROTEINS COMPLEXED WITH SODIUM HEXAMETAPHOSPHATE. G. A. PELROY & J. SPINELLI. *J. Food Sci.* 36, 144–146 (1971)– No significant differences were found in the availability of 4 essential amino acids in sarcoplasmic fish proteins and sarcoplasmic fish proteins complexed with sodium hexametaphosphate under various conditions of phosphate concentration and pH. Assays of samples dried at 75°C showed that nearly 100% of the methionine, threonine and tryptophan and 85% of the lysine were available. Moist heat treatment for up to 24 hr reduced availability of all 4 of the amino acids by equivalent amounts in both the complexed and noncomplexed samples. The results indicate that the nutritional characteristics of sarcoplasmic fish proteins are unimpaired by complexing with sodium hexametaphosphate.

DIETARY EFFECTS ON BEEF COMPOSITION. 4. Processing and Palatability Attributes. G. DUBE, V. D. BRAMBLETT, R. D. HOWARD, B. E. HOMLER, H. R. JOHNSON, R. B. HARRINGTON & M. D. JUDGE. *J. Food Sci.* 36, 147–154 (1971)– Influence of dietary regimen of 104 half-sib Angus steers allotted to 1 of 13 combinations of hay, corn silage and corn concentrate, and of slaughter weights ranging from 284–454 kg (live weight) on processing qualities and palatability of beef was studied. Feeding regimens had a greater effect than did slaughter weights on qualities studied. Meat from corn-silage vs. hay-fed animals was more tender by both shear values and panel scores and was more juicy and flavorful. In the groups weighing 409 and 454 kg, the effects of diet during the intermediate period were considerably less pronounced than those of the early feeding period.

TEXTURE OF SEMI-SOLID FOODS: SENSORY AND PHYSICAL CORRELATES. W. F. HENRY, M. H. KATZ, F. J. PILGRIM & A. T. MAY. *J. Food Sci.* 36, 155–161 (1971)– Texture profile analyses (TPA) were conducted on a series of commercial desserts (vanilla puddings, custards, gelatin and toppings). Textural attributes were obtained under compression and under tension. Additional attributes relating to tensional force, stringiness, and work of extension, were also measured. 15 sensory attributes related to texture were also evaluated by a taste panel. Both the physical and sensory data were analyzed by factor analysis and by multiple regression to arrive at relationships between the physical and sensory textures.

ABSTRACTS:
IN THIS ISSUE

QUANTITATIVE DETERMINATION OF COMPOSITION OF MODELS OF FOOD SYSTEMS BY THE INFRARED ATTENUATED TOTAL REFLECTANCE TECHNIQUE. J. M. WILSON, I. BEN-GERA & A. KRAMER. *J. Food Sci.* 36, 162–165 (1971)–Attenuated total reflectance (ATR), offering a possible rapid infrared spectrophotometric method for quantitative measurements of major nutritional components in foods materials, was employed in this study for evaluating composition of model systems representing solid foods: system #1–starch and protein; system #2–starch, protein and fat. Using a band ratio technique, correlations were determined between the ratio of intensities for carbohydrate and protein peaks, and their proportion in the systems studied. When the log of the ratic of these two peaks was plotted against the composition, correlation coefficients of .9699 and .9358 for systems #1 and #2 respectively were obtained. Errors assignable to the systems' composition and those inherent to the ATR technique are discussed.

INFRARED SPECTROSCOPIC DETERMINATION OF DEGREE OF UNSATURATION OF FATS AND OILS. R. G. ARNOLD & T. E. HARTUNG. *J. Food Sci.* 36, 166–168 (1971)– Infrared spectra of various fats and oils were determined. Ratios of absorbance at 3.3μ (olefinic C–H stretching band) to absorbance of other characteristic triglyceride absorption bands were calculated. Relationships between these ratios and unsaturation, as estimated by iodine value, were determined. Analyses of 25 fats and oils showed that the ratio of absorbance at 3.3μ to absorbance at 3.5μ (aliphatic C–H stretching band) and iodine value were linearly related and exhibited a correlation coefficient of 0.98. Estimation of degree of unsaturation of 19 additional fats and oils revealed an average deviation of ± 0.97 iodine value units or ± 1.12% between measured values and values calculated from infrared absorption patterns.

IDENTIFICATION AND CHARACTERIZATION OF SOME OXIDIZING ENZYMES OF THE MC FARLIN CRANBERRY. H. T. CHAN JR. & H. Y. YANG. *J. Food Sci.* 36, 169–173 (1971)– Peroxidase, catalase, and polyphenolase were found in frozen cranberries. Phenol binding agents were necessary to obtain high enzyme activity. Optimum pH's for cranberry peroxidase, catalase, and polyphenolase were 6.0, 7.5–9.2, and 7.0, respectively. Heat inactivation of cranberry peroxidase and polyphenolase was found to follow first order kinetics, while that of catalase was neither first order nor zero order.

THE β-N-ACETYLGLUCOSAMINIDASE ACTIVITY OF EGG WHITE. 1. Kinetics of the Reaction and Determination of the Factors Affecting the Stability of the Enzyme in Egg White. J. W. DONOVAN & L. U. HANSEN. *J. Food Sci.* 36, 174–177 (1971)–Enzymatic activity of β-N-acetylglucosaminidase, which occurs naturally in chicken egg white, was characterized to establish conditions suitable for routine assay for this enzyme in egg products. A variation in enzyme content of approximately 3-fold was observed in individual fresh eggs. The enzyme has a pH optimum between 3.0 and 3.4, and a K_m of approximately 0.6 mM for the substrate p-nitrophenyl-N-acetyl-β-D-glucosaminide. Activation energy for hydrolysis of this substrate is 10.7 ± 0.8 kcal/mole. The enzyme is stable for at least several hours at ambient temperature from pH 6.8 to approximately 8.8. Above pH 8.8, inactivation is first order with respect to time. Enzyme activity in shell eggs decreases fairly rapidly at ambient temperature; loss of activity probably results from increase in pH of egg white which occurs normally upon loss of carbon dioxide. Eggs held at 4°C retain activity much longer.

THE β-N-ACETYLGLUCOSAMINIDASE ACTIVITY OF EGG WHITE. 2. Heat Inactivation of the Enzyme in Egg White and Whole Egg. J. W. DONOVAN & L. U. HANSEN. *J. Food Sci.* 36, 178–181 (1971)– The kinetics of the heat inactivation of β-N-acetylglucosaminidase in egg white and whole egg at neutral pH is first order in the temperature range 58–62°C. Rate constants for enzyme inactivation by heat are reported for both egg white and whole egg. The activation energy for heat denaturation is 91 kcal/mole in egg white and 73 kcal/mole in whole egg. Reduction of enzymatic activity upon heating can be used as an indication of whether egg white or whole egg has been maintained at the time-temperature combinations approved for pasteurization by the U.S. Department of Agriculture. This test should be useful as a process control tool.

CHARACTERISTICS OF PROGENY OF ETHYLENE OXIDE TREATED Clostridium botulinum TYPE 62A SPORES. R. A. SAVAGE & C. R. STUMBO. *J. Food Sci.* 36, 182–184 (1971)–Spores of *Clostridium botulinum* Type A were exposed to a mixture of ethylene oxide (12%) and dichlorodifluoromethane (88%) to determine if the ethylene oxide (ETO) resistance, toxin producing ability and spore producing ability would be retained in progeny of successive generations exposed to ETO. Through four successive generations of ETO survivors, no difference in any of these characteristics was observed.

JFS APPLIED SCIENCE and ENGINEERING

A.F. BADENHOP[1] and L. R. HACKLER
New York State Agricultural Experiment Station
Department of Food Science & Technology, Cornell University, Geneva, N.Y. 14456

PROTEIN QUALITY OF DRY ROASTED SOYBEANS: AMINO ACID COMPOSITION AND PROTEIN EFFICIENCY RATIO

SUMMARY—A study was undertaken to determine the effect of the dry-roasting process on the quality of soybean protein as indicated by amino acid analyses and PER. Amino acid analyses revealed losses in tryptophan, available and total lysine, cystine and histidine of 35, 31, 17, 15 and 6% respectively. These losses are reflected in both the essential amino acid index (EAAI) and PER. Average EAAI values decreased with increasing degree of roast (68.4, 67.7 and 63.8) compared with a value of 71.4 for the raw samples. The PER values for the same roasted samples were 1.70, 1.46 and 1.28 respectively. A depressed PER value of 0.6 was obtained for the raw sample because of anti-nutritional factors in the raw beans. Results indicate that the dry roasting process is a means of producing a palatable, nutritious food from soybeans. However, optimum palatability is gained at the expense of protein utilization.

INTRODUCTION

THE ROASTING OF peanuts, tree-nuts, coffee, and other products includes a rapid dehydration followed by a partial pyrolysis. The process is carried out mainly for the desirable flavor changes produced in the product. In some cases the process also imparts other beneficial changes, such as improved texture and color. Also, as in the roasting of soybeans, the process may improve nutritional quality by destroying substances such as trypsin inhibitor.

There are at least two types of roasting processes. The simplest and most direct form is often termed "dry roasting" and involves heating of the product in air and/or a mixture of gases from the heat source. Dry roasting is employed in the coffee and cocoa processing industries, to a considerable extent in the peanut processing industry, and to some extent in the tree-nut processing industry. The other type involves the heating of the product in an oil or fat and is often termed "oil roasting" or "deep-fat frying." Oil roasting is used extensively in the peanut and tree-nut processing industries, as well as in other industries such as the potato chip processing industry.

In the case of peanuts and other tree-nuts, a desirable texture can be achieved merely by roasting the shelled raw product without any pretreatment other than perhaps a preliminary drying or curing operation or, in some cases, a blanching operation to loosen and remove skins or hulls. The roasting of soybeans without some pretreatment results in a product with an extremely hard texture.

The roasting of soybeans is not a new process. Soybeans have been roasted for centuries in Japan for the production of kinako. Kinako was originally made in the home by roasting soybeans in an earthenware pan over an open flame. The resulting roasted beans were then ground into a powder which was used as a coating or ingredient in other foods (Fukushima, D., 1968, personal communication).

In this country, considerable attention was directed toward the soybean as a source of food during the early 1940's, when, because of World War II, various sources of high protein food were quickly becoming limited. In this connection considerable work was done on the production of a roasted soy nut which could be salted and consumed like other nutmeats, or utilized in candy products in place of peanuts. The Borden Soy Processing Company used a procedure which involved soaking the beans followed by a dry roasting process in order to produce a soy nut with a desirable tender texture (Anon., 1948). Another product was made by deep-fat frying soybeans which had been subjected to a previous soaking step (Hale, 1941 and Blumenthal, 1947).

A previous report (Badenhop et al., 1968) described the processing operation of dry roasting as applied to soybeans, and presented the evaluation of the resulting soy nut product in terms of taste panel acceptability and the results of color, texture, and bulk density measurements. That report indicated that the most acceptable sample, in terms of flavor and texture, was obtained by roasting soybeans with an initial moisture of approximately 53% to an end point of 180°C.

The beneficial effects of a mild heat treatment of soybean protein used to destroy growth inhibiting substances such as trypsin inhibitor have been well established and need no elaboration here. Several workers have determined the effects of excessive heat on the nutritional properties of soybean protein. Worthy of note is the work of Rios Iriarte and Barnes (1966), Hacklet et al. (1963) and Riessen et al. (1947). Rios Iriarte and Barnes (1966) found that the overheating of soybean protein caused a destruction of the amino acids lysine, arginine, and cystine as measured chemically by the analysis of acid hydrolysates. They did not analyze for trytophan. The only factors found responsible for the decreased nutritive value of excessively heated soybean protein were a destruction of cystine and a decrease in nitrogen absorbability. Hackler et al. (1965) evaluated the effect of heating soy milk on the nutritional quality of the protein. They found that heating soy milk @ 121°C for 32 min or longer resulted in a lowering of

[1]Present address: Cooperative Extension Service, College of Agriculture, University of Georgia, Athens, Ga. 30601.

Table 1–Description of samples.

Sample number	Time of Soak @ 50°C (min)	Approx. moisture content after soak (as is basis) %	Degree of roast expressed as final temp. °C	Moisture content after roasting (as is basis) %
1	80	43	170	2.85
2	80	43	180	2.16
3	80	43	185	1.83
4	100	48	170	2.81
5	100	48	180	2.27
6	100	48	185	1.81
7	144	53	170	2.83
8	144	53	180	2.41
9	144	53	185	1.94
10	240	59	170	3.82
11	240	59	180	2.81
12	240	59	185	2.41
13	0	12	Not dried	–
14	80	43	Freeze dried	–
15	240	59	Freeze dried	–

the PER value and a decrease in available lysine. Stillings and Hackler (1965) also studied the effects of deep fat frying on the amino acid content of tempeh and found that the content of most amino acids declined after deep fat frying for 7 min. Lysine and cystine were found to be most susceptible to heat destruction. Riessen et al. (1947) observed that excessive heat treatment of soybean protein decreased the liberation of each of the essential amino acids by pancreatic enzymes, but decreased the liberation by acid of cystine, arginine and tryptophan without affecting the release of the other essential amino acids.

In the roasting process the temperatures attained are considerably higher than needed to destroy trypsin inhibitor and may indeed be detrimental to the nutritional quality of the protein. For this reason the present investigation was conducted to determine the effect of the roasting process on the quality of soybean protein as indicated by amino acid analyses of acid hydrolysates and protein efficiency ratio (PER) determinations.

EXPERIMENTAL

Sample preparation

Certified seed soybeans (var. Harosoy-63) were size-graded, using a Clipper Mill (Model M-2B), to a size range of 16–18 mesh. Samples of beans were then soaked in water at 50°C for four different periods of time (80, 100, 144, and 240 min). The soaking operation increased the moisture content of the beans to levels of 43, 48, 53, and 59%, respectively. After soaking, the beans were drained and held in a closed container for 1 hr in order to allow for moisture equilibration within the beans. Small gas-fired coffee sample roasters manufactured by the Jabez Burns Company (now part of Blaw Knox Corporation) were used for the roasting operation. The temperature of the roasters prior to charging was approximately 400°C. The end point of the roast was determined by holding a thermometer in the tumbling beans during the final minutes of roasting. Samples from each of the four moisture levels were roasted to end points of 170, 180, and 185°C. The average temperature of the beans after roasting was also determined by dumping batches directly from the roasters into a Dewar flask and observing the maximum temperature. These were 160, 168, and 175°C respectively. The time of roast for all of the samples (average time = 31 min 55 sec) was approximately equalized by adjusting the size of the charge to the roasters so that the weight of moisture removed per batch was nearly constant for all of the samples. In addition to the 12 roasted samples, 3 samples of unroasted beans were prepared for comparison. One of these was untreated and the other two were prepared by soaking beans for 80 and 240 min, after which they were drained and freeze dried. A simplified description of the 15 samples prepared for this study is given in Table 1.

Chemical analyses

The soybean samples were analyzed for protein (N × 6.25) by the micro-Kjeldahl procedure as outlined in AOAC (1960). Samples were prepared for amino acid analysis by acid-hydrolysis of the protein using 6N HCl at 110°C for 22 hrs. The hydrolysates were fil-

Table 2–Amino acid composition g/16g N and essential amino acid indices of roasted samples and unheated controls.[a]

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aspartic acid	10.8	11.3	10.5	11.8	11.1	10.9	11.3	10.9	10.3	10.4	11.0	10.9	11.5	11.0	11.1
Threonine	3.95	4.51	4.21	3.91	4.30	3.97	4.09	4.24	3.74	3.81	4.36	3.90	4.66	3.71	3.88
Serine	4.86	4.84	4.67	5.00	4.99	4.78	4.96	4.76	4.84	4.87	4.90	4.85	4.99	5.31	4.91
Glutamic acid	18.0	18.6	17.3	18.2	18.6	17.9	18.4	18.0	17.9	16.5	18.2	17.7	18.4	17.2	16.7
Proline	4.30	5.36	5.56	5.06	5.27	4.74	5.50	5.02	4.82	5.09	5.11	4.69	5.44	4.76	4.97
Glycine	3.92	4.13	3.77	4.12	4.07	4.06	4.05	4.02	4.02	3.78	4.07	3.91	4.26	3.79	3.92
Alanine	4.11	4.32	4.00	4.28	4.27	4.25	4.28	4.26	4.21	3.91	4.17	4.05	4.23	3.91	3.97
Valine	4.08	4.28	4.01	4.23	4.18	4.11	4.31	4.14	4.11	3.91	4.18	3.98	4.30	3.95	4.12
Cystine	1.45	1.24	1.21	1.43	1.24	1.21	1.28	1.30	1.16	1.31	1.18	1.23	1.52	1.40	1.42
Methionine	1.06	1.08	0.95	1.05	1.01	0.92	1.07	1.06	1.07	0.95	1.12	1.01	1.04	0.94	1.04
Isoleucine	3.74	3.86	3.58	3.93	3.74	3.68	3.84	3.81	4.51	3.52	3.80	3.68	3.94	3.67	3.63
Leucine	7.40	7.40	7.16	7.75	7.54	7.51	7.43	7.56	7.42	7.02	7.37	7.48	7.80	7.65	7.44
Tyrosine	3.25	2.89	2.84	3.06	3.17	2.92	3.08	3.12	3.10	3.12	3.64	3.10	3.02	3.04	3.00
Phenylalanine	4.30	4.39	3.98	4.36	3.82	4.52	4.48	4.37	4.53	4.12	4.79	4.40	4.64	4.38	4.28
Lysine	5.18	4.88	4.21	5.41	4.92	4.52	5.40	4.93	4.61	4.94	5.07	4.64	6.19	5.63	5.79
Histidine	2.38	2.47	2.26	2.41	2.39	2.40	2.42	2.41	2.39	2.34	2.45	2.24	2.50	2.65	2.38
Arginine	7.03	7.07	6.58	7.20	7.01	6.89	7.20	6.75	6.94	6.88	6.80	6.66	7.47	7.20	7.42
Tryptophan	1.20	1.00	0.94	1.14	1.10	0.90	1.15	1.05	0.74	1.13	1.11	1.01	1.51	1.66	1.53
Available Lysine	3.65	2.83	2.14	3.43	3.10	2.08	3.41	2.85	2.70	3.33	2.97	2.85	4.11	4.58	4.14
Essential Amino Acid Index	68.4	66.9	63.1	69.2	66.6	64.1	69.0	67.4	64.4	64.9	68.7	57.8	73.8	69.6	70.1

[a]See Table 1 for description of samples.

Table 3–Average amino acid composition g/16g N of roasted soybean samples and unheated controls.

	170°C Avg.	180°C Avg.	185°C Avg.	Avg. of all roasted samples	Avg. of all unheated samples
Aspartic acid	11.1	11.1	10.7	11.0	11.2
Threonine	3.94	4.35	3.96	4.08	4.08
Serine	4.92	4.85	4.79	4.86	5.07
Glutamic acid	18.1	18.3	17.7	18.1	17.4
Proline	5.15	5.19	4.95	5.10	5.06
Glycine	3.97	4.07	3.94	3.99	3.99
Alanine	4.15	4.26	4.13	4.18	4.04
Valine	4.13	4.20	4.05	4.13	4.12
Cystine	1.37	1.24	1.20	1.27	1.50
Methionine	1.03	1.07	0.99	1.03	1.01
Isoleucine	3.76	3.80	3.86	3.81	3.75
Leucine	7.40	7.47	7.39	7.42	7.63
Tyrosine	3.13	3.21	2.99	3.11	3.02
Phenylalanine	4.32	4.49	4.36	4.39	4.43
Lysine	5.23	4.95	4.50	4.89	5.87
Histidine	2.39	2.43	2.32	2.38	2.54
Arginine	7.23	7.39	7.06	7.23	7.45
Tryptophan	1.16	1.07	0.90	1.04	1.57
Available Lysine	3.45	2.94	2.44	2.94	4.28
Essential Amino Acid Index	68.4	67.7	63.8	66.5	71.4

Table 4–Effect of roasting process on the nutritive value of soybean protein as indicated by PER values.

Sample No. used as diet supplement	Average daily weight gain (g)[a]	Average daily feed consumption (g)[a]	PER[a,b]	Diet Protein %
1	1.63 ± .15	9.33 ± .67	1.51 ± .05	9.19
2	1.52 ± .14	8.77 ± .31	1.51 ± .10	9.13
3	1.12 ± .08	8.51 ± .74	1.17 ± .10	9.50
4	1.95 ± .13	9.61 ± .38	1.87 ± .07	8.63
5	1.58 ± .10	9.08 ± .48	1.50 ± .04	9.25
6	.81 ± .07	7.15 ± .29	0.99 ± .07	9.13
7	1.77 ± .12	9.34 ± .39	1.59 ± .06	9.44
8	1.33 ± .10	8.49 ± .27	1.30 ± .06	9.50
9	1.06 ± .06	7.97 ± .22	1.24 ± .06	8.63
10	2.18 ± .15	10.68 ± .24	1.83 ± .10	8.88
11	1.58 ± .11	8.69 ± .34	1.51 ± .06	9.56
12	1.29 ± .20	8.18 ± .52	1.73 ± .18	7.00
13	0.58 ± .11	6.01 ± .40	0.80 ± .14	8.81
14	0.32 ± .14	5.42 ± .45	0.48 ± .18	8.06
15	0.45 ± .19	6.24 ± .24	0.57 ± .09	10.06

[a]Each datum represents the mean for each group of rats ± the standard error of the mean.

[b]Adjusted to a casein standard with a PER of 2.5.

tered, dried, and taken up in pH 2.2 sodium citrate buffer. Amino acid composition of the samples was determined by ion-exchange column chromatography using a Technicon amino acid auto analyzer (Model NC-3). Cystine was analyzed by conversion to cysteic acid using the method of Moore (1963) followed by chromatography. Available lysine was determined using the method of Rexen and Christensen (1967). Tryptophan was determined using the method of Horn and Jones (1945).

Animal feeding study

Protein efficiency ratios (PER) were determined by growth studies using male weanling albino rats. The soybean supplements were used as the sole protein source in the test diets. These diets were fed ad libitum to groups of 10 rats for 28 days. A casein control was also included and the resulting PER values were adjusted to the casein diet standardized to a PER of 2.5. Complete details of the experimental methods used in the rat growth studies have been reported previously, Hackler et al. (1963).

RESULTS & DISCUSSION

TOTAL AMINO ACID analyses (Table 2) indicate that the effect of roasting on the amino acid composition of soybean protein is confined mainly to the destruction of total and available lysine, cystine and tryptophan. Even these amino acids were not consistently affected by the degree of roast. Total lysine content was found to be inversely related to degree of roast in all but the three samples roasted from 59% moisture. Available lysine, however, was inversely related to the degree of roast in the samples from all 4 moisture levels. Cystine content was inversely related to degree of roast in the samples roasted from 43 and 48% but not those from 53 and 59% moisture. Tryptophan content was inversely related to degree of roast in the samples from all 4 moisture levels.

In order to show the combined effects of the roasting process on the essential amino acids, the essential amino acid index (EAAI) was also calculated according to the method of Mitchell (1954). These values for the 15 samples (Table 2) indicate that the EAAI is inversely related to the degree of roast in all but the three samples with the highest initial moisture content.

When the four samples which were roasted to each of the three degrees of roast are averaged, one obtains the values contained in Table 3. Tryptophan, cystine, total lysine and available lysine are all inversely related to the degree of roast. Serine also shows this trend. Also contained in Table 3 are the average of all 12 roasted samples and the average of the three unheated samples. If the difference between these two values is considered to be the average effect of the roasting process on the amino acid composition, tryptophan appears to be the most heat-labile amino acid. Tryptophan content was found to be decreased by 35% during roasting. Following in order of heat lability are available lysine, total lysine, cystine and histidine, decreased by 31, 17, 15, and 6% respectively. Since the most heat-labile amino acids are all essential, or semi-essential, the effect of roasting is reflected in the EAAI values calculated using the averages in Table 3. The EAAI drops from 68.4 for the 170°C samples to 67.7 for the 180°C samples and 63.8 for the 185°C samples. Finally a comparison of the average EAAI of all 12 roasted samples with that of the 3 unheated samples shows a decrease from 71.4 to 66.5 due to the roasting process.

The results of the animal feeding studies (Table 4) indicate that PER is inversely related to the degree of roast. This relationship was noted for samples 4, 5, and 6 and 7, 8, and 9, roasted from moisture contents of 48 and 53% respectively. Samples 1, 2, and 3, roasted from 43% moisture, showed no change in PER between the 170°C and 180°C roasts. For both samples the PER was 1.51. The PER dropped to 1.17 for sample 3, roasted to 185°C. The only other deviation from inverse relationship was noted in sample 12 (185°C roast from 59% moisture). This sample produced a PER of 1.73, a higher value than that obtained from the 180°C roast of the same initial moisture. The explanation for this high value may be the abnormally low protein content of the diet used for measuring the PER value. The average PER values for all samples roasted to each of the three degrees of roast were 1.70, 1.46, and 1.28 for the 170, 180, and 185°C end points respectively.

The values for the average daily weight gain and average daily feed consumption

show that they are both inversely related to the degree of roast from all four initial moisture levels.

The PER values for the 12 roasted samples averaged 1.48 as opposed to an average PER of 0.61 for the unroasted samples. While the roasting process improves the nutritive value of soybeans by destroying the activity of trypsin inhibitor, the resulting PER values are not as high as those obtained by more gentle heating. PER values greater than 2.00 are easily obtained for soybean protein that has been properly heat treated (autoclaving at 121°C for 5–10 min) in the presence of sufficient moisture (Hackler et al., 1965). Such a treatment will achieve the desired destruction of trypsin inhibitor activity without significant destruction of the essential amino acids. The higher temperatures attained during the roasting process result in the destruction of some of the essential amino acids. The loss of essential amino acids contributes to the lowering of the PER value.

Based on these results and the data reported previously on the quality and acceptability of roasted soybeans (Badenhop et al., 1968), it is apparent that the dry roasting process is a simple method of producing an edible food from soybeans. Roasted soybeans have an appearance and flavor resembling roasted Spanish peanuts. The texture is quite different, because of the grossly different proportions of fat and protein in the two seeds. One would expect the presence of the hull to have an undesirable effect on the mouth feel of the roasted beans; their presence is of little consequence, however, since they break up easily during mastication. More of a mouth feel problem is caused by the inherent dryness of the product presumably a result of the relatively low fat content. Even this problem is minor in terms of over-all acceptability. Both mouth feel and flavor can be improved considerably by coating the roasted beans with vegetable oil or margarine and salt to produce a salted nut-like product similar to Spanish peanuts.

Roasted soybeans can serve as a high protein snack food or be used as a valuable protein-enriching adjunct in the candy industry. Another possibility is the grinding of roasted soybeans with the addition of sufficient oil to produce a peanut butter analog.

When the similarity of roasted soybeans to roasted peanuts is contrasted with the raw product cost ratio (ca. 1 to 5), extensive product development using roasted soybeans as an ingredient would seem to merit considerable attention.

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G. N. BOOKWALTER, G. C. MUSTAKAS,
W. F. KWOLEK[a], J. E. MC GHEE and W. J. ALBRECHT
USDA Northern Regional Research Lab., ARS, Peoria, Illinois 61604

FULL-FAT SOY FLOUR EXTRUSION COOKED: PROPERTIES AND FOOD USES

SUMMARY—Full-fat soy flours prepared by the extrusion process were shown to have good nutritive value, flavor, and stability. A flour cooked to a nitrogen solubility index (NSI) of 30 stored well, but the addition of tertiary butyl hydroquinone was necessary to prevent rancidity in flours cooked to 19 and 11 NSI values. The 30 NSI flour was more yellow than a commercial flour cooked to the same degree by a different process. When soy flours were compared in bread to 3 and 6% nonfat dry milk on an equivalent protein, fat, and reducing sugar basis, baking properties were similar. At 15 and 20% levels of soy flour, loaf volume decreased less with the extruded products than with nonextrusion-processed soy flour.

INTRODUCTION

FOR MANY PEOPLE in the world both the quality and the quantity of protein in their daily diet are inadequate. This deficiency can be corrected by high-protein supplements. Foremost among the supplements in availability, low cost, and quality are soybean products. Both defatted soy flour and full-fat soy flours are being used in dietary supplements for preschool children in developing countries (USDA, 1969).

Conventional full-fat processed soy flour is produced from beans that have undergone a direct steam-heat treatment (Horan, 1966). Another method for cooking soybeans (Mustakas et al., 1964; 1970) is extrusion at elevated temperatures. The dry, dehulled soybeans are treated in a steam-jacketed conveyor for 6–8 min to a final temperature of 218–220°F to inactivate lipoxygenase (Mustakas et al., 1969). The dry heat-treated (DHT) meats are moistened to about 20% and continuously extrusion-cooked at about 275°F (1.25 min retention time) to inactivate antinutritional factors and then are dried and ground as described by Mustakas et al. (1970). Protein efficiency ratios (PER) of extrusion-cooked soy flours increased slightly over the range of conditions for tests A to D (Table 1); available lysine values also changed only slightly, while trypsin inhibitor, urease activity, and nitrogen solubility index (NSI) decreased considerably. As indicated by change in peroxide values (PV), oxidative stability was excellent over a wide range of extruder conditions. However, at extreme processing conditions (Table 1, tests D and E) oxidative stability was inadequate, a condition indicating damage to the natural antioxidants.

Specific objectives of the experiments reported here were to investigate further nutritional aspects, color properties, effect of antioxidants on oxidative stability of extrusion-cooked soy meats, and performance of soy flour in sponge-dough bread.

MATERIALS & METHODS

Experimental soy flours

Hawkeye soybeans were cracked and dehulled to produce soy meats in the range of 12–30 mesh. The soy meats were dry heat-treated (DHT at 218–220°F for 6–8 min) followed by extrusion cooking at various conditions (15 and 20% moisture, 1.25 and 2.0 min retention time, 250–300°F).

Analytical methods

PV's were determined on hexane-extracted oils from whole soy flours by the standard AOCS (1966) procedure. Urease activity was run on defatted soy flours following the official AACC (1962) method. For the measurement of water-soluble protein, NSI was measured on the defatted soy flours by a modification (pH 7.2) of the method of Smith et al. (1966).

Amino acid values were obtained by analyses of the acid hydrolysates of the protein by the accelerated system of Benson and Patterson (1965). The cysteine and cystine were determined as cysteic acid using the method of Moore (1963). Tryptophan was determined by a procedure of Spies (1970).

Other analyses were made according to standard AACC (1962) procedures.

Color values

Visual observations were made for color; β-carotene and total xanthophyll values were obtained by the method of Moore and Ely (1941). Color was measured with a Hunter Model D-25 color difference meter. Color comparisons between a commercial, nonextruded, and a laboratory extrusion-cooked full-fat soy flour were made on samples that had been cooked to about the same degree as determined by NSI and urease activity.

Antioxidant tests

To study the effect of antioxidants at the upper range of cooking conditions as indicated by NSI and urease activity, three samples of full-fat soy meats were extrusion-cooked at 20% moisture as follows:

Flour	Retention time (min)	Temp (°F)	NSI (%)	Urease activity (pH units)
1	1.25	275	30	0.6
2	2.0	275	19	0.2
3	2.0	300	11	0.1

Two Eastman Chemical products, Tenox 4 (20% butylated hydroxyanisole, 20% butylated hydroxytoluene, and 60% corn oil) and Tenox 7 (28% butylated hydroxyanisole, 12% propyl

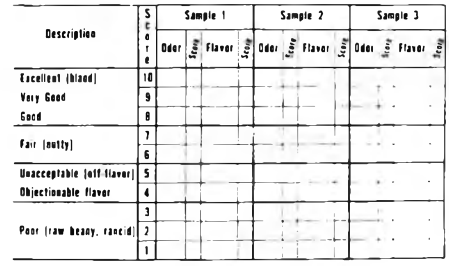

Fig. 1—Score sheet used by a taste panel to evaluate full-fat soy flour prepared under different extrusion conditions.

Table 1—Protein efficiency ratios (PER) and analytical values for full-fat soy flour prepared with various extruder conditions.[a]

Test No.	Extruder conditions: Moisture (%)	Retention time (min)	Temp (°F)	PER[b]	Available lysine (g/16g N)	Trypsin inhibitor inactivation (%)	Urease activity (pH change)	NSI[c] (%)	Peroxide values (meq/kg) oil: 0	12 mo (77°F)
A	15	1.25	275	1.8	6.1	12	1.0	50	1.5	0.8
B	20	2.0	250	1.9	6.3	43	0.9	36	2.0	1.2
C	25	1.25	275	2.0	6.3	62	0.2	21	6.7	1.0
D	20	2.0	275	2.1	6.3	89	0.1	21	7.6	63.0
E	20	1.25	300	1.9	6.2	98	0.0	16	4.7	213.0

[a]Source:-Mustakas et al. (1970).
[b]Adjusted to casein = 2.5.
[c]NSI = nitrogen solubility index.

[a]Biometrical Services, USDA Northern Regional Research Lab., ARS, Peoria.

Table 2–Formula variations of bread doughs containing full-fat soy flours compared to nonfat dry milk on an equivalent protein, reducing sugar, and fat composition.

Dough ingedients	700g Flour basis			
	3.0% Nonfat dry milk	2.6% Full-fat soy flour	6.0% Nonfat dry milk	5.3% Full-fat soy flour
Wheat flour (14.0% m.b.)	280.0	280.0	280.0	280.0
Salt	14.0	14.0	14.0	14.0
Water	173.0	173.0	194.0	194.0
Dextrose hydrate	38.0	44.1	30.4	42.6
Nonfat dry milk[a]	21.0	...	42.0	...
Extrusion-cooked full-fat soy flour	...	18.4	...	36.9
Soybean oil added	18.4	14.4	18.4	10.4
α-Monoglycerides	0.5	0.5	0.5	0.5
Hydrogenated soy oil flakes	2.1	2.1	2.1	2.1

[a]Nonfat dry milk: protein, 36.0%; fat, 0.7%; moisture, 3.0%; lactose, 51.0%.

Table 3–Formula variations of bread doughs containing 5, 10, 15, and 20% full-fat soy flours.

Dough ingedients	700g Flour basis			
	Full-fat soy flour			
	5%	10%	15%	20%
Wheat flour (14.0% m.b.)	280.0	280.0	280.0	280.0
Salt	14.0	14.0	14.0	14.0
Water	194.0	229.0	264.0	285.0
Dextrose hydrate	38.0	38.0	38.0	38.0
Full-fat soy flour	35.0	70.0	105.0	140.0
Soybean oil added	10.4	2.6	...	...
α-Monoglycerides	0.5	0.5	0.5	0.5
Hydrogenated soy oil flakes	2.1	2.1	2.1	2.1

gallate, 6% citric acid, 20% glyceryl monooleate, and 34% propylene glycol), and one experimental sample, 100% tertiary butyl hydroquinone (TBHQ) were used as antioxidants.

In one of the two methods used in adding these antioxidants, each was dispersed in ethanol, sprayed onto extrusion-cooked flour (100 ml/2 kg) while being mixed in a Hobart mixer, and air-dried at room temperature (75°F) for 16 hr. Controls without antioxidants were treated the same at each process variable. In the second method of addition an emulsion of 1g Tenox 7 and 1450g water was blended with 20 lb soy meats before they were extrusion-cooked under each set of conditions used in this study.

Experimental samples with and without antioxidants were packaged in closed 6-oz glass containers and stored in temperature-controlled cabinets at 100 and 120°F and also at room temperature (approximately 77°F). Reference samples were stored at 0°F. Withdrawals from storage were made at various times. The stored samples were evaluated for flavor by organoleptic tests and for oxidative stability by PV's.

Organoleptic evaluation

Coded, randomized samples, three at a time, were presented to an experienced eight-member taste panel and rated for odor and flavor using the 10-point score sheet shown in Figure 1.

Statistical evaluations were made of the experimental data after completion of each taste panel series. Analysis of variance (Snedecor, 1959) and Duncan's multiple range test (Duncan, 1955) were used to study results at each time interval.

Bread baking tests

To determine baking potential of extrusion-cooked full-fat soy flour, tests were made using flours prepared at three levels of heat treatment as indicated by NSI and urease activity.

Process	Moisture (%)	Retention time (min)	Temp (°F)	NSI (%)	Urease activity (pH units)
DHT only	–	–	–	68	1.6
Ext. 1	15	1.25	250	51	0.7
Ext. 2	20	2.0	250	26	0.2

Commercial hard wheat flour used for bread baking tests analyzed: moisture, 13.3%; protein, 11.8%; ash, 0.45%; absorption, 61.0%; malt index, 550; mechanical tolerance index, 35; peak time, 5.5; and 5.5 ppm potassium bromate.

Commercial dextrose hydrate, nonfat dry milk, α-monoglycerides, soybean oil, and fully hydrogenated soybean oil flakes were incorporated into all bread doughs. All ingredients conformed to commercial standards of identity.

Bread baking tests were carried out according to the standard sponge-dough, pound-loaf method (AACC, 1962) except for the dough formulation. The sponge formulation (700g flour basis) for all tests was:

Wheat flour (14.0% m.b.)	420.0g
Water	268.0g
Yeast, compressed	14.0g
Yeast food	3.5g

The yeast food formula was: ammonium chloride, 9.7%; potassium bromate, 0.3%; calcium

Table 4–Proximate analysis of extruded full-fat soy flour.

Component	%
Protein, (N × 6.25)	41.0
Crude fat	22.5
Crude fiber	1.7
Ash	5.1
Moisture	3.4
Granulation	
% through 100 mesh	96.5

Table 5–Essential amino acid patterns. (mg/g total essential amino acids).

Amino acid	Full-fat soy flour[a]	Whole egg protein[b]
Isoleucine	114	129
Leucine	182	172
Lysine	169	125
Aromatic AA	220	195
Sulfur AA	63	107
Threonine	94	99
Tryptophan	33	31
Valine	124	141

[a]Flour 2, PER 2.1, NSI 21, urease activity 0.1.

[b]FAO-WHO (1965).

Table 6–Hunter color values, β-carotene, and total xanthophyll content of commercial and experimental full-fat soy flours cooked to about the same degree.

Full-fat soy flour	Indicators of heat treatment		β-Carotene (mg/100g)	Total xanthophyll (mg/100g)	Hunter color values		
	NSI (%)	Urease activity (pH units)			L (lightness)	a (+ red) (– green)	b (+ yellow) (– blue)
Extrusion-cooked (moisture 20%, 1.25 min ret., 275°F)	30	0.6	0.15	1.00	80	–1.9	27
Commercial sample (nonextrusion cooked)	33	0.3	0.08	0.76	88	–2.1	20

sulfate, 25.0%; sodium chloride, 25.0%; starch, 40.0%.

The full-fat soy flours (DHT; Extrusions 1 and 2) were added in the proportions shown in the dough formula (Table 2), the sponge and dough being combined after fermentation at the remixing stage of bread dough preparation. Full-fat soy flours (2.6 and 5.3% levels) were compared to nonfat dry milk (3 and 6% levels) on an equivalent protein, reducing sugar (AOAC, 1965), and fat composition.

Two full-fat soy flours, DHT and Extrusion 1, were added in the proportions shown in the dough formula (Table 3), the sponge and dough being combined after fermentation at the remixing stage. Baking tests were made with soy flour in the formula at levels of 5, 10, 15 and 20%, based on flour. The fat content was decreased in the formula as additional fat was added in the form of full-fat soy flour as shown in Table 3.

The experimental bread was evaluated for odor and flavor, grain, texture, crust color, and crumb color according to standard cereal laboratory practice as used by Rainey and Horan (1961). Bread volume and baking absorption were obtained according to standard AACC (1962) procedures.

RESULTS & DISCUSSION

Nutritional aspects

Extrusion-cooked full-fat soy flour (Table 4) is a rich source of both protein and oil in foods which do or do not require further cooking. The protein is of good quality as indicated by PER (Table 1) and by the essential amino acid patterns (Table 5) when compared to egg protein, the provisional standard of the Food and Agriculture Organization of the United Nations. This full-fat soy flour contains an excess of lysine and is well balanced in other amino acids, except for the sulfur amino acids. In foods low in protein or deficient in one or more of the essential amino acids, full-fat soy flour can be used to improve both the quantity and quality of protein. Cereal foods low in lysine (Inglett et al., 1969) can be improved nutritionally by fortification with full-fat soy flour.

Color properties

By visual observation, extrusion-cooked full-fat soy flour appears more yellow in color than a similar commercial flour prepared by another process. When color comparisons were made between these two flours cooked to about the same degree (30 vs. 33 NSI), values for β-carotene, total xanthophyll, and Hunter b values were higher for the extrusion-cooked than for the commercial full-fat soy flour (Table 6). The yellow color may be advantageous in some food applications where formula enrichment is accomplished with a minimum of change in the inherent product color. An example of this is soy-fortified corn meal.

Effect of antioxidants

The effect of antioxidants on three full-fat soy flours cooked to three degrees of heat-treatment are shown in Tables 7 to 9. The mean flavor scores (MFS) and mean peroxide values (MPV) for the various antioxidants of extruder series of flours 1, 2 and 3 are summarized from Tables 7, 8 and 9 as follows:

Table 7–Peroxide values and flavor scores of full-fat soy flour No. 1 (NSI 30, urease activity 0.6) extruded at 20% moisture, 1.25 min retention 275°F, with and without antioxidants.

Storage		No antioxidant		Tenox 7 0.05% added				Antioxidant added after cooking			
				Before cooking		After cooking		Tenox 4 0.05%		TBHQ[c] 0.02%	
Temp. (°F)	Time (days)	PV[a]	FS[b]	PV	FS	PV	FS	PV	FS	PV	FS
...	0	1.1	7.8	0.6	7.7	1.1	7.7	1.2	7.8	1.9	7.4
120	28	0.8	7.2	1.2	7.7	1.0	7.5	0.8	7.3	1.5	7.4
120	56	0.8	6.5	0.7	7.3	1.3	7.0	1.6	6.9	1.7	7.1
100	90	0.6	7.7	0.6	8.1	0.8	7.4	0.8	7.5	0.6	7.6
100	182	0.6	7.3	0.8	7.2	0.6	7.6	1.8	7.7	2.0	7.5
77	182	3.9	6.9	0.6	7.9	1.8	7.0	3.3	7.1	1.4	7.2
77	365	1.2	6.9	1.0	7.4	1.2	7.3	1.3	7.3	0.8	7.3

[a]PV = peroxide values, expressed as meq/kg oil.
[b]FS = flavor scores, each based on 15 flavor scores by a trained panel of eight tasters.
[c]TBHQ = tertiary butyl hydroquinone.

Table 8–Peroxide values and flavor scores of full-fat soy flour No. 2 (NSI 19, urease activity 0.2) extruded at 20% moisture, 2.0 min retention, 275°F, with and without antioxidants.

Storage		No antioxidant		Tenox 7 0.05% added				Antioxidant added after cooking			
				Before cooking		After cooking		Tenox 4 0.05%		TBHQ[a] 0.02%	
Temp. (°F)	Time (days)	PV[a]	FS[a]	PV	FS	PV	FS	PV	FS	PV	FS
...	0	23	7.5	29	7.7	22	7.7	14.5	7.9	11.7	8.0
120	28	27	7.4	59	7.5	18	6.8	10.5	6.8	5.2	6.7
120	56	76	4.6	117	3.7	24	4.9	22	5.7	5.5	7.3
100	90	74	5.5	78	4.5	48	7.9	20	6.9	3.9	7.8
100	182	189	4.6	86	2.4	95	6.3	38	6.4	9.8	7.5
77	182	91	5.7	120	4.7	77	5.8	33	6.8	16	7.6
77	365	164	4.5	196	2.9	158	4.6	66	6.2	13	7.6

[a]See footnotes Table 7.

Table 9–Peroxide values and flavor scores of full-fat soy flour No. 3 (NSI 11, urease activity 0.1) extruded at 20% moisture, 2.0 min retention, 300°F, with and without antioxidants.

Storage		No antioxidant		Tenox 7 0.05% added				Antioxidant added after cooking			
				Before cooking		After cooking		Tenox 4 0.05%		TBHQ[a] 0.02%	
Temp. (°F)	Time (days)	PV[a]	FS[a]	PV	FS	PV	FS	PV	FS	PV	FS
...	0	24	7.7	27	7.6	16	7.8	18	7.6	9.5	7.5
120	28	21	7.0	68	6.7	14	7.3	14	7.1	5.7	7.6
120	56	61	4.6	90	2.9	16	5.1	17	5.5	9.4	7.0
100	90	65	6.8	121	5.5	17	7.6	25	7.6	5.6	7.7
100	182	117	5.7	232	2.8	51	6.3	117	7.0	13	7.3
77	182	99	5.9	134	4.9	31	6.8	60	6.2	16	7.3
77	365	158	2.8	184	1.7	69	5.7	91	6.0	6.7	7.1

[a]See footnotes Table 7.

Antioxidant addn before or after processing	Flour 1		Flour 2		Flour 3	
	MPV[1]	MFS[1]	MPV	MFS	MPV	MFS
No antioxidant	1.0	7.1	87	5.4	73	5.4
Tenox 7 before	0.8	7.6	101	4.2	127	4.1
Tenox 7 after	1.1	7.3	54	6.0	27	6.5
Tenox 4 after	1.4	7.3	27	6.5	39	6.5
TBHQ after	1.2	7.4	8	7.4	9	7.3

[1] Each based on six storage conditions.

In the flour 1 series (NSI 30, urease activity 0.6) there were no significant differences in flavor or PV associated with the three factors—antioxidant, temperature, and length of storage. As indicated by MFS of 7.1 and above and MPV of 1.4 and below, flour 1 was stable with or without antioxidants.

In the series for flour 2 (NSI 19, urease activity 0.2) and flour 3 (NSI 11, urease activity 0.1), which received more heat-treatment than flour 1, there were highly significant (95% level) interactions between antioxidant, storage time, and temperature both for flavor scores and PV's. Flours 2 and 3 were unstable without antioxidants. Addition of Tenox 7 before processing acted as a prooxidant and caused further autoxidation of the fat with resultant flavor scores lower than the control. Addition of either Tenox 4 or Tenox 7 after processing improved both PV and flavor scores, but both were significantly poorer than those of the flour 1 series. The presence of TBHQ in either the flour 2 or 3 series was associated with good product stability, as indicated by significantly lower peroxide values and significantly higher flavor scores. The presence of TBHQ in the flour 2 or 3 series resulted in flavor scores not significantly different from those of the flour 1 series.

Table 10—Characteristics of sponge-dough bread containing dry heat-treated (DHT) full-fat soy flours (Extrusion 1 and 2) compared to nonfat dry milk on an equivalent protein, reducing sugar, and fat composition basis.

Sponge-dough bread exp. variables (wheat flour basis)	Baking characteristics[a]			
	Baking absorption (%)	Loaf vol (cc)	Grain (cells)[b]	Crumb color[c]
3.0% Nonfat dry milk	63	2735	SR	100
2.6% Full-fat soy flour (DHT)	63	2725	SR	99.5
2.6% Full-fat soy flour (Ext 1)	63	2735	SR	99.5
2.6% Full-fat soy flour (Ext 2)	63	2700	SR	99.0
6.0% Nonfat dry milk	66	2675	E	99.5
5.3% Full-fat soy flour (DHT)	66	2675	E	99.0
5.3% Full-fat soy flour (Ext 1)	66	2675	E	99.0
5.3% Full-fat soy flour (Ext 2)	66	2650	E	99.0

[a] All bread samples were similar (velvety texture, golden brown crust color, normal odor and flavor).
[b] SR = slightly round, close thin; E = elongated, close thin.
[c] 100 = White; 99.0 = creamy white.

Sponge-dough process bread

Results of baking with extrusion-cooked full-fat soy flour are found in Tables 10 and 11. The soy flours pre-

Table 11—Baking characteristics of sponge-dough bread containing 5, 10, 15 and 20% of full-fat soy flours prepared by DHT and extrusion cooking (Extrusion 1).

Sponge-dough bread exp. variables (wheat flour basis)	Baking characteristics						
	Baking absorption (%)	Loaf vol (cc)	Grain (cells)	Texture	Crust color	Crumb color[a]	Odor and flavor
5% DHT	66	2685	Elongated close thin	Velvety	Golden brown	99.0	Normal
5% Ext 1	66	2685	Elongated close thin	Velvety	Golden brown	99.0	Normal
10% DHT	71	2600	Elongated close thin	Velvety	Sl. dark golden	98.5	Normal
10% Ext 1	71	2610	Elongated close thin	Velvety	Sl. dark golden brown	98.5	Normal
15% DHT	76	2165	Round sl. open med.	Velvety	Med. dark golden brown	97.0	Acceptable sl. nutty
15% Ext 1	76	2365	Elongated close med.	Velvety	Med. dark golden brown	98.0	Acceptable sl. nutty
20% DHT	79	1810	Round open thick	Coarse	Dark golden brown	96.0	Acceptable sl. nutty
20% Ext 1	79	2150	Elongated sl. open medium	Velvety to sl. coarse	Dark golden brown	97.5	Acceptable sl. nutty

[a] 99.0 = Creamy white; 96.0 = creamy yellow.

pared from dry heat-treated (DHT) soy meats and Extrusions 1 and 2 were compared to 3 and 6% nonfat dry milk on an equivalent protein, reducing sugar, and fat composition basis (Table 10). Bread containing either 6% nonfat dry milk or equivalent (5.3%) DHT or Extrusion 1 and 2 full-fat soy flours had 3% greater baking absorption and slightly smaller loaf volumes than bread containing either 3% nonfat dry milk or equivalent (2.6%) full-fat soy flours. There were no differences in baking absorption, loaf volume, texture, crust color or odor and flavor in bread made with either 3% nonfat dry milk or 2.6% DHT or Extrusion 1 and 2 full-fat soy flours. Crumb color of bread containing nonfat dry milk was slightly whiter than bread containing either the DHT or Extrusion 1 and 2 full-fat soy flours. The Extrusion 1 flour produced the creamiest crumb color at the 2.6% level. There were no differences in baking absorption, loaf volume, texture, crust color or odor and flavor in bread made with either 6% nonfat dry milk or 5.3% DHT or Extrusion 1 and 2 flours. Crumb color of bread containing nonfat dry milk was slightly whiter than bread containing either DHT or Extrusion 1 and 2 flours. At the 2.6 and 5.3% levels there were no significant differences in bread made with DHT or Extrusion 1 and 2 flours.

The DHT and Extrusion 1 full-fat soy flours were compared in bread at levels of 5, 10, 15, and 20%. Baking absorption increased by 1% for each 1% increase in soy flour in the formula except for an increase of 0.87% at the 20% level. At the 5 and 10% levels there were no substantial differences in baking characteristics between bread containing either DHT or Extrusion 1 flours. A slight decrease in loaf volume was found as the amount of soy flour was increased from 5 to 10% and loaf volume decreased sharply at the 15 and 20% levels. However, bread volume decreased to a much lesser extent with the Extrusion 1 than with the DHT flour. At the 5 and 10% levels, there were no substantial differences in grain, texture or odor and flavor. As soy flour in the formula was increased from 5 to 20%, crust color became progressively darker: crumb color was creamy white at the 5% level and became creamy yellow at the 20% level. At the 15 and 20% levels, grain became slightly open in the DHT bread with a slightly darker crumb color than that of the Extrusion 1 bread. At the 15 and 20% levels bread flavor was acceptable, but a slightly nutty flavor was detected.

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J. J. RACKIS, D. H. HONIG, D. J. SESSA and J. F. CAVINS
USDA, Northern Regional Research Laboratory, ARS, Peoria, Illinois 61604

SOYBEAN WHEY PROTEINS—RECOVERY AND AMINO ACID ANALYSIS

SUMMARY—About 14 million lb of soybean whey protein of high biological value is disposed of as waste based on estimated production figures for soybean concentrates and isolates. An estimated 5–7% annual increase in consumption poses serious waste disposal problems and alternates should be sought. By simulated commercial procedures, yield and protein content of soybean whey were determined. Whey solids account for 2–28% of original nitrogen in dehulled, defatted flakes. Whole whey protein was prepared by dialysis, and whey was also fractionated by heating into heat-coagulable and supernatant proteins. Whole whey protein has a good balance of essential amino acids when compared with a system followed by the Food and Agricultural Organization of United Nations based on hen's egg protein. Heat-coagulable and supernatant proteins varied greatly: heat-coagulable fractions had 42% of the sulfur amino acid content but 181% of the tryptophan content of hen's egg protein; supernatant protein had 142% of the sulfur amino acid content but only about 60% of the isoleucine, valine, leucine and tryptophan content of hen's egg protein. All three whey protein fractions would be suitable for addition to feeds.

INTRODUCTION

IN A MARKET survey, Eley (1968) reported that estimated annual production of food-grade, soybean protein concentrates and isolates was 17–30 million and 22–35 million lb, respectively. He also stated that consumption of these products is increasing 5–7% annually. In addition, about 55 million lb of soy protein isolate is manufactured for industrial use.

Soybean whey, the spent solubles derived during the preparation of protein isolates, accounts for nearly one-third of the original defatted flakes. The whey is in the form of a dilute solution. Smith et al. (1962) found that soybean whey contains about 4.3 mg protein/ml and has a high biological oxygen demand (BOD) of 13,730 ppm after a 5-day incubation. Because of its high BOD, whey presents a serious waste disposal problem. Usually, whey has been disposed of as sewage or by spraying on unused land. Only with processing operations involving aqueous alcohol extraction can whey be more easily concentrated so that it can be incorporated into feeds.

Meager information exists concerning the value of soybean whey as a product. Smith et al. (1962) found that edible gums and detergents precipitate protein from soybean whey. The removal of protein lowers the BOD 8–18%. In addition, the complexes with edible gums retain the enzymatic activity of the original proteins. Whey proteins may be a source of edible protein because their essential amino acids are well balanced (Rackis et al., 1961). Soybean whey solids prepared by various procedures have been recovered, various whey protein fractions prepared and their amino acid contents determined. The yields from these different experiments have also been evaluated.

EXPERIMENTAL

Preparation of soybean whey

From certified, seed-grade soybeans (Amsoy variety) were prepared dehulled, defatted flakes with a nitrogen solubility index (NSI) of 93%. NSI is defined as the ratio of water-soluble nitrogen to total nitrogen × 100 as determined under conditions of the test procedure BA11-65 (AOCS, 1965). Soybean whey was obtained from these flakes in several ways which, in principle, follow the processes employed in commercial manufacture (Meyer, 1967). Byproducts of the manufacture of concentrates and isolates were prepared as shown in Figures 1 and 2. In this report, we designate these various soluble byproducts as wheys.

Commercial procedures for preparing products similar to protein concentrate-A by extraction of defatted soybean meal with aqueous alcohol solvents are described by O'Hara and Schoepfer (1965). Protein concentrate-B prepared by the acid leach process is patented (Sair, 1959). McAnelly (1964) described details of the hot-water leach process (protein concentrate-C). Figure 2 shows a process for producing protein isolates based on a patent issued to Circle et al. (1959).

Preparation of whey protein fractions

Starting with soybean whey solution (Fig. 2), whole whey protein, heat-coagulable protein and supernatant protein were prepared by the procedure outlined in Figure 3. Precipitation of heat-coagulable protein from whey solutions heated to 80°C at pH 7–8 does not occur until the solution is adjusted to a pH of about 5.

Amino acid analyses

Protein samples were hydrolyzed as described previously (Rackis et al., 1961). Amino acid content was determined by the procedures of Benson and Patterson (1965). Cystine after oxidation to cysteic acid was determined by the method of Schram et al. (1954). A forerun of 100 ml of 0.01N acid was required. Procedure N of Spies and Chambers (1949) was followed to analyze for tryptophan.

RESULTS & DISCUSSION

Analysis of whey solids

Yield, plus nitrogen and protein con-

Table 1—Yield, nitrogen and protein of various preparations of soybean whey solids.

Preparation[a]	Yield (g/100g meal)	Nitrogen content (%)	Nitrogen as protein (%)	Protein content[b] (%)	Whey nitrogen[c] (% of total meal nitrogen)
A	23.2	0.87	12.6	0.7	2.2
B	31.3	3.24	75.2	15.2	11.1
C	36.3	6.92	91.2	39.5	27.6
D	31.1	3.28	75.0	15.4	11.2

[a]See Figures 1 and 2 for details. All values are dry basis and represent averages of duplicate analysis on two different preparations of soy flakes.

[b]Protein N × 6.25 after correction for nonprotein nitrogen (Becker et al., 1940).

[c]Dehulled, defatted soybean (Amsoy variety) contained 9.09% nitrogen.

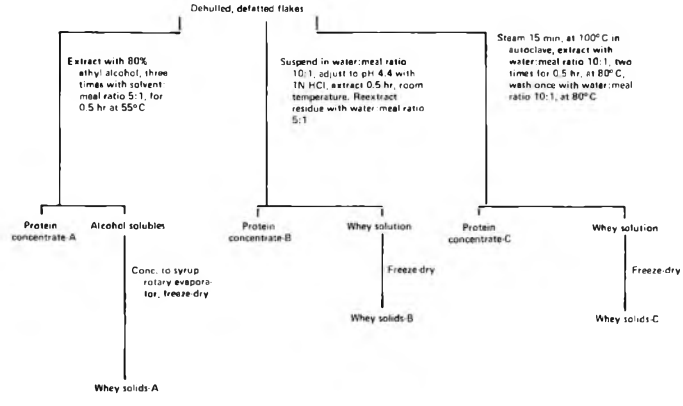

Fig. 1—Whey solids prepared by the soy protein concentrate process.

Table 2–Typical proximate analysis of soybean whey solids.[a]

Constituent	%
Carbohydrate[b]	62.1
Total protein (N × 6.25)	20.5
Actual protein[c]	15.4
Ash	5.5
Others (by difference)	11.9

[a]See Figure 2 for details of preparation. All values are dry basis.

[b]Phenol-sulfuric acid procedure (DuBois et al., 1956).

[c]After correction for nonprotein nitrogen not precipitable with 0.8N trichloroacetic acid.

Table 3–Calculated yearly production of soybean whey protein.

Whey solids (million lb)	Protein content[a] (%)	Yield of protein (million lb)
84[b]	15.4	12.94
6[c]	0.7	0.04
6[c,d]	15.2	0.91
Total protein produced		13.89

[a]Calculated value after correction for nonprotein nitrogen (See Table 1).

[b]Based on the estimated average production of 84 million lb of protein isolate and a yield of isolate:whey of 1:1 (preparation D, Fig. 2).

[c]Based on the estimated average total production of 24 million lb of protein concentrate, a yield of 2:1 concentrate:whey and the assumption that each concentrate produced by preparations A and B of Figure 1 represents one-half of the total production of concentrates.

[d]Preparation B (Fig. 1).

tent of whey solids prepared by various procedures, is given in Table 1. In determining protein content, correction for nonprotein nitrogen was made by precipitating the whey proteins with 0.8N trichloroacetic acid and then determining the nitrogen in the supernatant (Becker et al., 1940). Free amino acids and peptides usually account for 50% of the nonprotein nitrogen (unpublished data). Phospholipids, purine and pyrimidine bases, nucleosides and nucleotides, as well as other nitrogenous constituents, are also present.

As shown in Table 1, the least amount of nitrogen was extracted with the aqueous alcohol procedure (preparation A), whereas the largest amount was extracted with hot water (preparation C). In this latter procedure, about 91% of the nitrogen in the whey solids exists as protein. The nitrogen in preparation C accounts for 28% of that in the original flakes. With preparations B and D, about 11% of the total nitrogen in the starting flakes is discarded as whey.

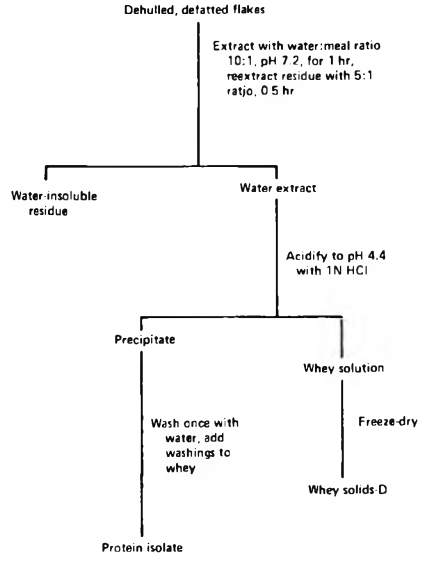

Fig. 2–Whey solids obtained by the soy protein isolate process.

The loss of nitrogen as whey (preparation D) with the Amsoy soybeans used in these studies is similar to the loss from most varieties. By analyzing 23 strains of soybeans, Smith et al. (1966) found that whey, prepared by procedures similar to Figure 2, represented 9.0–15.3% of the total nitrogen in dehulled, defatted flakes. However, the established soybean varieties had a narrower range from 10.2–14.4% of total nitrogen.

A typical proximate analysis of soybean whey solids (preparation D, Fig. 2) is given in Table 2. Total carbohydrate content is about 62%. Oligosaccharides–sucrose, raffinose and stachyose–account for at least 80% of the total carbohydrate. Actual protein content is about 15.4%. The other constituents, calculated by difference, represent a large number of components, each of which is present in minute amounts. Whey solids prepared by procedure B, Figure 1, have a similar composition.

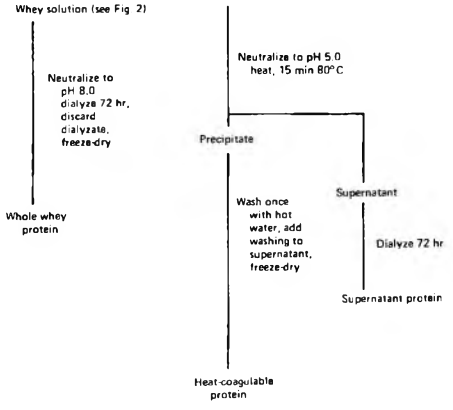

Fig. 3–Preparation of soybean whey protein fractions.

Calculated yearly production of soybean whey protein is shown in Table 3. For purposes of calculation, total production of soy protein concentrates was assumed to be half protein concentrate-A and half concentrate-B.

As a result of these calculations and assumptions, it would appear that up to 14 million lb of protein of high biological value may be discarded annually. Since Eley (1968) estimates that soy protein concentrates and isolates are increasing 5–7% annually, antipollution laws may serve as an incentive to recover whey protein.

Isolation and recovery of whey protein fractions

Since whey solids-B and -D (see Table 3) represent most of the protein in waste disposal, an analysis was made of the proteins in these preparations that could be recovered as useful products. From a practical consideration, reverse osmosis and heat coagulation probably would be combined. However in our laboratory studies, dialysis was used to remove low-molecular-weight substances, including nonprotein nitrogenous constituents.

As shown in Table 4, the whole whey protein fraction from dialysis of whey preparation D contains about 81% protein and represents 8.2% of the total protein of the original flakes based on the amount of protein recovered after dialysis. With the data given in Table 1, the protein in preparation D accounts for about 8.7% of the total protein when corrected for nonprotein nitrogen. Whey can be fractionated into two major protein fractions (heat-coagulable and supernatant proteins) with a yield ratio of about 1.3:1, respectively. The heat-coagulable protein has a high state of purity as indicated from its protein content. Although the data are not shown, comparable yields of heat-coagulable and

supernatant proteins were obtained with whey solids prepared by procedure B, Figure 1.

On the basis of data in Table 1, the calculated yield of whole whey protein from preparation C would be 14.3g/100g of flakes and would represent 26.5% of the protein in the original dehulled, defatted flakes. The increased whey protein yield of preparation C can be accounted for by the appreciable solubility of denatured protein in hot water (Circle et al., 1959). Although the flakes were heat-treated before extraction with hot water, some of the heat-coagulable protein would also be extracted with hot water since the pH of the extract was 6.4.

Table 4—Yield and protein content of soybean whey protein fractions.

Fraction[a]	Yield (g/100g meal)	Protein content (% N × 6.25)	% Of meal protein[b]
Whole whey protein	5.44	81.4	8.22
Heat-coagulable protein	2.60	98.5	4.75
Supernatant protein	2.40	83.2	3.70

[a]See Figure 3 for details. All values are dry basis and represent averages of duplicate analysis on two different preparations of flakes.
[b]Dehulled, defatted flakes contained 54.0 protein (% N × 6.25) after correction for 5% non-protein nitrogen.

Table 5—Amino acid composition of whey proteins (g/16 g N).

Amino acid	Soybean meal	Supernatant	Heat-coagulable	Whole whey	Standard deviation[a] (S)
Lysine	6.83	8.89	7.72	8.38	0.420
Histidine	2.64	3.35	2.71	3.49	0.317
Ammonia	1.63[b]	1.26[b]	1.06[a]	1.38[b]	
Arginine	6.87	6.45	6.38	6.77	0.297
Aspartic acid	11.23	14.57	13.38	13.92	0.557
Threonine	4.47[b]	7.08[b]	4.53	5.35	
Serine	5.67[b]	7.34[b]	5.77[b]	6.66[b]	
Glutamic acid	18.28	15.72	13.34	14.76	0.555
Proline	4.76	4.99	6.01	5.36	0.352
Glycine	4.38	4.31	4.93	4.67	0.159
Alanine	4.50	5.64	4.96	5.24	0.123
Cystine	1.50[c]	3.38[c]	0.62[c]	2.28[c]	
Valine	5.51	3.44	7.10	5.28	0.381
Methionine	1.58	2.45	1.79	2.33	0.385
Isoleucine	5.03	3.07	6.45	4.59	0.102
Leucine	7.56	4.54	9.95	7.21	0.119
Tyrosine	3.82	3.85	4.53	4.16	0.184
Phenylalanine	5.02	2.53	5.77	4.16	0.210
Tryptophan	1.35[d]	0.82[d]	2.20[d]	1.53[d]	

[a]For any given amino acid, the least significant difference is 1.7S. Thus, two values differing by more than this amount are significantly different, at least at the 95% level.
[b]Value obtained by extrapolation to zero-hydrolysis time.
[c]By the procedure of Schram et al. (1954).
[d]By the procedure of Spies and Chambers (1949).

Table 6—Essential amino acid patterns of whey proteins.[a]

Essential amino acid	Supernatant	Heat-coagulable	Whole whey protein	Soy flour
Isoleucine	60	105	78	91
Valine	61	99	82	91
Leucine	66	114	92	103
Cystine + methionine	136	45	95	67
Threonine	179	90	119	106
Phenylalanine + tyrosine	82	104	94	106
Lysine	178	122	148	128
Tryptophan	65	139	110	100

[a]Calculated as % of hen's-egg protein pattern (mg/g total EAA).

Amino acid composition of soybean meal and whey protein fractions

Data are reported in Table 5 as grams of amino acid per 16g of nitrogen. Values represent averages of duplicate determinations on both 48- and 72-hr hydrolyzates. The analyses were repeated on a second hydrolyzate from each sample. Standard deviations represent an analysis of variance of all results for an amino acid. The amino acid analysis of dehulled, defatted soybean flakes is included for comparative purposes.

In determining the order of limiting essential amino acids and in assessing biological values of whey proteins, the Food and Agricultural Organization of the United Nations (FAO) system (1965) was adopted in which each individual amino acid is reported as milligrams per gram of total essential amino acids. These values were then converted to percentages of hen's egg protein pattern (Table 6). The total sulfur amino acids (cystine plus methionine) are the first limiting acids in soybean flakes. Isoleucine and valine are also below the level in hen's egg protein.

The limiting amino acids in whole whey protein are: isoleucine, valine and leucine. The heat-coagulable protein is deficient in total sulfur amino acids, whose content is only 42% of that present in hen's egg protein. In the supernatant protein, isoleucine, valine, leucine and tryptophan are the first limiting amino acids (60–68% of hen's egg protein).

All the soybean protein fractions, particularly supernatant protein, have an excess of the important amino acid, lysine. The supernatant protein is high in threonine and in sulfur amino acid, whereas the heat-coagulable protein is especially rich in tryptophan. Because several difficulties were encountered in determining tryptophan, a detailed study of the tryptophan assay is at present under investigation and will be reported elsewhere.

On the basis of known results, whole whey protein has a good balance of essential amino acids, which explains the high-protein efficiency ratios of rat diets containing toasted whey proteins (Rackis et al., 1963). On the other hand, a great imbalance of essential amino acids occurs when the whey is fractionated into heat-coagulable and supernatant protein fractions. Further research is necessary to determine functional uses for the heat-coagulable protein. Finding outlets for this protein fraction would enhance the potential of heat coagulation as a means by which a portion of the wastes in soybean processing could be recovered.

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ROUALEYN I. FENTON-MAY and CHARLES G. HILL JR.
Department of Chemical Engineering, University of Wisconsin, Madison, Wisconsin 53706
and CLYDE H. AMUNDSON
Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706

USE OF ULTRAFILTRATION/REVERSE OSMOSIS SYSTEMS FOR THE CONCENTRATION AND FRACTIONATION OF WHEY

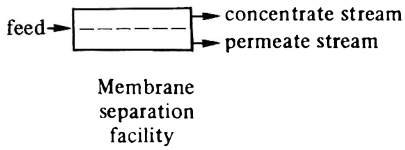
SUMMARY—Existing ultrafiltration/reverse osmosis technology provides a means of fractionating and concentrating cheese whey into liquid fractions containing a variety of protein:lactose ratios. These ratios may range from about 1:8 (raw whey) through 3:5 (a "skim milk equivalent") to 2:1 or higher. If a two- or three-stage ultrafiltration system were used with water injection between stages, a product with a protein:lactose ratio of 20:1 could be obtained. The exact protein:lactose ratio in the concentrate stream is a function of the permeability and selectivity characteristics of the membrane, and the system design and operating conditions. Some of the sanitation problems associated with the introduction of these new unit operations in the dairy and food processing industries are also treated at length.

INTRODUCTION

WITHIN THE LAST decade a number of membrane separation processes have evolved from laboratory curiosities to practical pilot plant scale operations. Two such processes, reverse osmosis and ultrafiltration, have been shown to be particularly useful in the concentration and fractionation of whey. Dunkley (1969) has reviewed the work reported prior to the January 1969 symposium on Reverse Osmosis in Food Processing. More recently, McDonough and Mattingly (1970) described the performance of a commercially available reverse osmosis unit for whey concentration and discussed the economics of this process. Horton et al. (1970) have also described the development of a two-stage process using reverse osmosis and ultrafiltration for the fractionation and concentration of whey.

Both reverse osmosis and ultrafiltration are based on the ability of various polymeric membranes to discriminate between molecules on the basis of molecular size and/or chemical composition. While these terms are often used interchangeably, the following distinction should be made between these processes. Reverse osmosis is that process in which virtually all species except water are rejected by the membrane. The osmotic pressure of the feed stream in such a system will often be quite high. Consequently, in order to achieve satisfactory water flux rates through the membrane, such systems often utilize hydrostatic operating pressures of 600 psig or greater. On the other hand, the term ultrafiltration refers to that membrane separation process in which the membrane is permeable to relatively low molecular weight solutes and solvent, but is impermeable to higher molecular weight species. The permeability and selectivity characteristics of these membranes may be controlled during the fabrication process so that they will retain only molecules above a certain molecular weight (the "molecular weight cut-off"). This cut-off point may be as large as several hundred thousand for membranes designed for the fractionation of high molecular weight species.

Both reverse osmosis and ultrafiltration may be represented schematically in the following manner:



The permeate stream contains only those species to which the membrane is permeable. The concentrate stream will contain the entire amount of all species to which the membrane is impermeable and will be appropriately depleted in those species which have passed through the membrane in the permeate. The percent rejection (R) of a particular species at the membrane is defined as

$$R = [C_i - C_p/C_i]\ 100$$

where C_i is the concentration of the species of interest in the feed to a part of the membrane separation system and C_p is the concentration of that species in the permeate from the same part of the system.

In order to meet the stringent sanitary requirements of the food industry (and especially of the dairy industry), most ultrafiltration/reverse osmosis equipment used in food processing applications is based on a configuration in which the membrane is cast on the inside of a porous tube. This tube may vary in diameter from ½-in. to 2 in. It provides the necessary mechanical support to enable the membrane to withstand the stresses imposed by the hydrostatic pressure employed in the process. In a complete system many such tubes of varying lengths may be connected together in some form of series/parallel arrangement in order to provide the required membrane area for the desired degree of water removal.

The purposes of this paper are to present the results of research on the feasibility of using reverse osmosis and ultrafiltration membranes for the concentration and fractionation of various cheese wheys and to comment on some of the practical problems which must be overcome before these systems can find more widespread acceptance by the dairy industry.

Products available from the fractionation of whey

One may produce a variety of useful product concentrates from raw whey by using different combinations of ultrafiltration and reverse osmosis membranes and different sequences of processing operations. The flexibility of these systems permits the production of liquid concentrates having different protein:lactose ratios depending on the desired end use of the product. In order to fractionate whey one may use a membrane which is permeable to lactose, ash and short-chain polypeptides, but impermeable to the whey proteins. The osmotic pressure difference between the concentrate and permeate streams in such ultrafiltration systems will be very low, thus permitting the system to be operated at hydrostatic pressures as low as 10 psig. Such membranes have essentially zero rejection of lactose and mineral salts. The concentrations of these species in the permeate stream will thus be nearly the same as their concentrations in the feed stream. Thus if 80% of the water were removed from the feed by ultrafiltration, the same proportion of the lactose and ash would be removed with the water in the permeate. Such a process produces the highest possible protein:lactose ratio in the product, the ratio depending only on the composition of the raw whey and the degree of volumetric reduction attained in the process. The fractional volumetric reduction obtained will in turn depend on the area of membrane used in the system

Table 1—Compositions of whey concentrates which can be obtained by ultrafiltration.

Product Composition (wt percent of)	Percent water removal 0	80	90	95	97.5
	Initial whey	Whey products			
Protein	0.67	3.3	6.3	11.9	21.2
NPN compounds	0.2	0.2	0.2	0.2	0.2
Lactose	5.0	4.9	4.7	4.4	4.0
Lactic acid	0.2	0.2	0.2	0.2	0.2
Ash	0.5	0.5	0.5	0.4	0.4
Total solids	6.6	9.1	11.9	17.1	26.0
Protein:lactose ratio	1:7	2:3	7:5	3:1	5:1

Table 2—Two procedures for the production of a "skim milk equivalent" from raw whey.

Composition (wt percent of)	Initial whey	Product compositions Procedure A[a] Stage I	Stage II	Procedure B[b]
Protein	0.67	2.2	10.3	10.3
NPN compounds	0.2	0.7	0.6	0.6
Lactose	5.0	16.7	15.3	14.8
Lactic acid	0.2	0.7	0.6	0.6
Ash	0.5	1.7	1.5	1.5
Total solids	6.6	22.0	28.3	27.8

[a]Procedure A uses a reverse osmosis system as its first stage in order to remove 75% of the initial water content and an ultrafiltration system as its second stage for removal of 80% of the remaining water and low molecular weight constituents. (Overall water removal = 95%.)

[b]Procedure B achieves a 95% reduction in the water content using ultrafiltration membranes which give a 15% rejection of the non-protein constituents of the whey.

and the conditions at which the unit operates.

Table 1 indicates the product compositions which may be obtained from a typical whey at various degrees of water removal by ultrafiltration. The calculations on which this table is based assume complete rejection of the whey proteins and zero rejection of all other constituents of the whey. The percent water removal which may ultimately be obtained by ultrafiltration is largely governed by the rheological properties of the concentrated protein solution. These properties determine the hydrodynamic characteristics of the product, the character of the protein boundary layer which controls mass transfer through the membrane and thus the extra membrane surface area which will be required for additional increments of water removal.

From Table 1 it may be seen that the product fraction with 80% water removal has a protein:lactose ratio which corresponds closely to that of skim milk. This "skim milk equivalent" has a concentration of only 8.6% total solids. A more concentrated product could be obtained by first concentrating the whey to 21% total solids using a high pressure reverse osmosis system and then removing 80% of the remaining water, lactose and ash by ultrafiltration. An alternative method would be to use an intermediate pressure system with a membrane rejection of 15% of the non-protein components of whey. The composition of the liquid concentrates produced by these methods from the typical raw whey are shown in Table 2.

EXPERIMENTAL

Equipment

All of the membrane separation equipment used in this research was based on ½-in. diameter tubular configurations, and all were manufactured by Havens International. The backing material is epoxy-bonded fiberglass on which a modified cellulose acetate membrane is cast. These tubes, each about 8 ft long, are connected with "U" turn connectors to give a compact modular configuration surrounded by a shroud which serves as a permeate collector. The older Havens modules contained seven such tubes per module (6.5 ft^2 of membrane) while the newer modules contain 18 (17.2 ft^2 of membrane). In addition some of the 18-tube modules have ¼-in. O.D., helically wound polyethylene volume displacement rods (VDR) in each tube to serve as detached turbulence promoters. For a given volumetric throughput, these rods serve to increase the effective superficial velocity of the feed over the membrane.

The 7-tube modules (series 101, 102 and 103) and 18-tube modules (series 300 and 310) contained reverse osmosis membranes with different selectivity and permeability characteristics. These modules exhibited a total solids rejection of 94–99.5% for raw cottage cheese whey, the exact value depending on the membrane and operating conditions. The pure water permeability of these modules ranged from 7 gfd (600 psig module average pressure, 68°F) for the 101-series to 18 gfd for the more permeable 103 and 300 series. The series 215 modules used high permeability ("loose") ultrafiltration membranes with a pure water permeability of 14 gfd (180 psig, 68°F) and rejection characteristics which are discussed in more detail later.

Pressure was provided by a Manton-Gaulin variable speed drive homogenizer, using a 1-gal, nitrogen-charged, bladder-type accumulator to damp out pulsations in the flow.

Procedure

Cottage, Cheddar, and sweet wheys were obtained from the University of Wisconsin Dairy. The cottage whey was allowed to stand to settle out suspended casein fines as these are known to clog some membranes. This removal of suspended fines could also be effected by continuous centrifugal clarification. The sweet and Cheddar wheys were defatted using a centrifugal separator at 110°F. The whey was adjusted to the desired operating temperature in a hollow jacketed stainless steel vat and maintained at that temperature by circulating hot or cold water through the jacket.

In order to provide a means of characterizing membrane performance over the several months during which this research was carried out, the following calibration was performed each day for the membranes used in that day's experiments. The permeability of each membrane to pure water at fixed conditions of temperature, pressure, and feed flow rate was determined before any experiments with whey were initiated. The pure water permeability provides a measure of the deterioration in membrane performance over long periods of time.

In no case did the pure water permeability decline more than 5% for a total of up to 300 hr of intermittant operation over a period of 1 yr. This decline was noted only for series 103 and 300 modules.

Two basic types of experiments were carried out:

1. The performance of the unit at a fixed speed composition was investigated using a continuous recycle of both permeate and concentrate to the feed tank. Other operating parameters (pressure, temperature, flow rate) were then investigated in systematic fashion.

2. In order to investigate the effect of variations in feed composition on the performance of the system, only the concentrate was recycled to the feed tank. The result of this procedure is to produce a feed which becomes progressively more and more concentrated as the experiment proceeds. By maintaining the other independent process variables at fixed values, one may then investigate the effect of feed composition on the performance of the unit.

After completion of a series of experiments, the modules were flushed with tap water, under pressure, for 30 min or until there was no taste or odor to the exit water stream. In most recent work, the modules were flushed with water adjusted to pH 3.5 with hydrochloric acid, and allowed to stand overnight. If operation was continued the next day, the modules were again flushed with tap water and subjected to the start-up procedure described. If, on the other hand, the modules were to be out of use for more than 24 hr, they were flushed with water at pH 4.5 and stored in that condition. This cleansing procedure was judged adequate from an engineering standpoint insofar as it prevented deterioration of the module performance. However, the odor of the storage water after 2 or 3 days indicated that this procedure was inadequate from the viewpoint of sanitation. The use of additional cleansing agents and/or bacteriostatic storage solutions is currently under investigation.

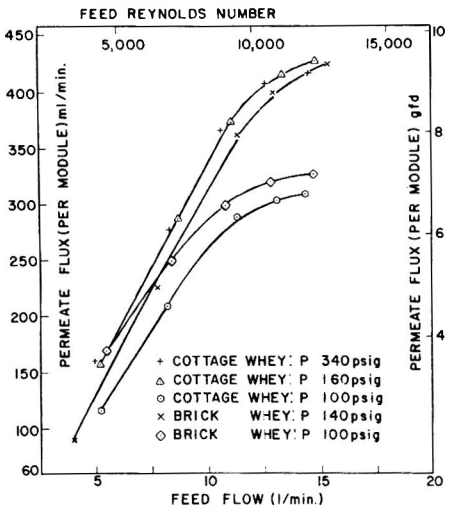


Fig. 1—Variation of permeate flux with feed flow rate at various pressures for raw whey in a single ultrafiltration module. Havens module type 215 operating at 66°F. Brick whey composition: 6.6% total solids, 0.67% protein, 5.2% lactose. Cottage whey composition: 6.2% total solids, 0.67% protein, 4.8% lactose.

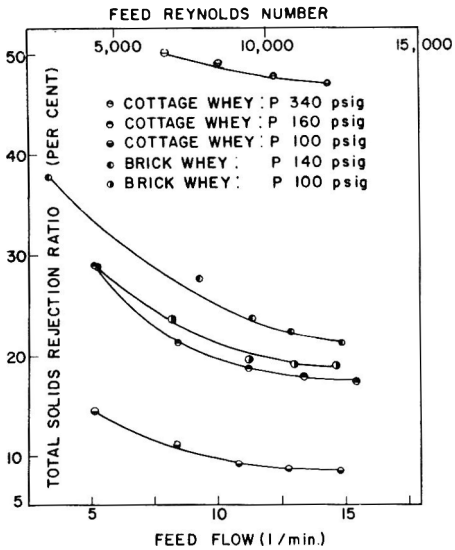


Fig. 2—Variation of total solids rejection ratio with feed flow rate at various pressures. Havens module type 215 operating at 66°F. Feed as in Figure 1. Lactose rejection is approximately T.S. rejection—10.0%.

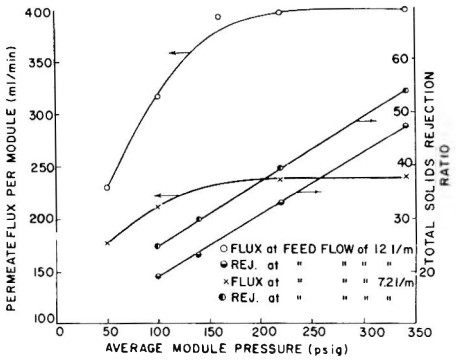


Fig. 3—Variation of permeate flux and total solids rejection with pressure. Havens module 215 operating at 66°F. Feed: defatted Brick cheese whey.

Analytical methods

Samples of feed, concentrate and permeate from each module were analyzed for solids by the Mojonnier method, ash by combustion at 550°C, lactose by the phenol-sulfuric acid method (Marier and Boulet, 1959), total nitrogen by a micro-Kjeldahl procedure (Bradstreet, 1965) and non-protein nitrogen by precipitation with 24% trichloracetic acid. The protein factor used in conjunction with the Kjeldahl method was 6.38.

In addition, in a few experiments, each sample was analysed for calcium by atomic absorption spectrophotometry (Willis, 1960, 1961), and for chloride, chemical oxygen demand (C.O.D.) and phosphorus (as ortho-phosphate), according to the methods outlined in "Standard Methods for the Examination of Water and Wastewater" (1965). The methods used were mercuric nitrate titration for chloride, oxidation by potassium dichromate for C.O.D., and colorimetry using ammonium molybdate as a complexing agent for phosphate.

RESULTS & DISCUSSION

Fractionation of whey

The batchwise fractionation of whey was studied using a Havens 215 ultrafiltration module in order to investigate the effect of feed flow rate, temperature and hydrostatic operating pressure on the performance of the system. The effect of feed flow rate and hydrostatic operating pressure is shown in Figure 1. The hydrostatic operating pressure referred to here is the module average pressure; i.e., the arithmetic mean of the inlet and outlet pressures. The permeate rate increases nearly linearly with feed flow rate up to some limiting point after which any further increase in feed flow rate has little effect on the permeate rate. This observation indicates that the membrane transport process is not controlled by the characteristics of the membrane alone. At low flow rates, the primary resistance to membrane transport is that offered by a hydrodynamic boundary layer arising from protein concentration polarization. At higher flow rates mass transfer is goverened by the resistance of the membrane itself and of any gel precipitate which has been deposited thereon. An increase in feed flow rate increases turbulence, thereby decreasing the thickness of the hydrodynamic boundary layer and thus its effective resistance to mass transfer. This decrease continues until the resistance of the membrane and gel become comparable to that of the boundary-layer. Beyond this point the permeate rate stays constant in spite of increasing feed flow rate because the membrane and gel now provide the major resistance to water transport. Boundary-layer control of ultrafiltration has been previously suggested by Ginnette and Merson (1968) and by de Filippi and Goldsmith (1970). Figure 1 also indicates that the permeate flux increases slightly with increasing feed pressure up to about 150 psig, but that above this pressure there is no further increase in permeate flux.

Just as an increase in feed flow rate decreases the resistance of the system to water transport, so too does it decrease the resistance of the system to the transfer of other species to which the membrane is permeable. Thus the rates of lactose and ash transport through the membrane both increase with increasing feed flow rate and the concentrations of these species in the permeate show a corresponding increase. This effect is shown in Figure 2 where the total solids rejection ratio is shown to decrease with increasing flow rate, eventually approaching an asymptotic limit at high flow rates. The fact that an increase in feed pressure markedly increases the total solids rejection may be explained by an increase in the resistance to mass transfer arising from a reversible compaction of the membrane and/or of the gel layer. This pressure effect is reversible up to about 425 psig with the Havens 215 system, but above this pressure the membrane undergoes irreversible compaction resulting in a marked decline in permeate flux and an increase in total solids rejection.

In the limit where the rate of mass transfer is membrane, rather than boundary layer, controlled, the permeate flux may be expressed by a relation of the form:

$$J = \frac{(\Delta P - \beta \Delta \pi)}{R}$$

where
- J is the permeate flux;
- R is a characteristic resistance of the system to membrane transport;
- ΔP is the hydrostatic operating pressure difference across the membrane;
- $\Delta \pi$ is the osmotic pressure

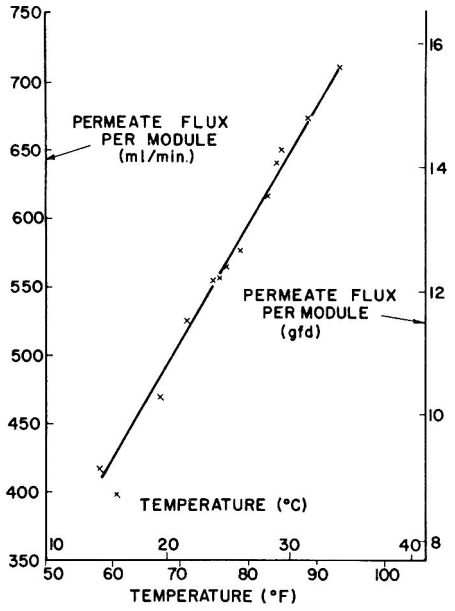


Fig. 4—Temperature variation of permeate flux. Havens module 215, 185 psig pressure, 2.0 gpm feed flow rate. Feed: Cottage cheese whey.

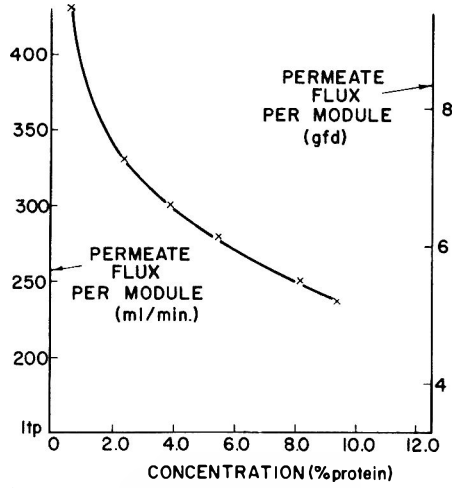


Fig. 5—Variation in permeate flux with feed protein concentration. Havens modules type 215 VDR, 200 psig inlet pressure, 1 gpm feed flow rate, 80°F temperature. Initial feed: Cottage cheese whey (6.0% T.S., 0.64% protein, 4.2% lactose, 0.8% ash). The permeate flux indicated is that measured from the first module in a bank of three.

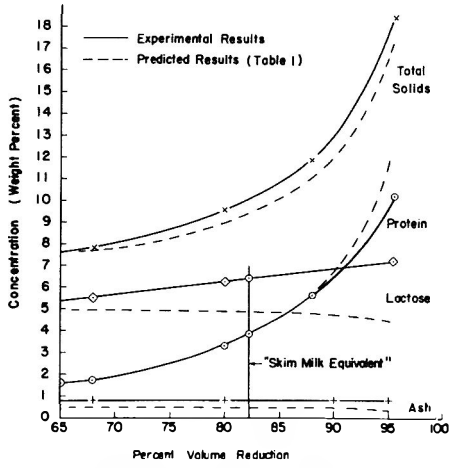


Fig. 6—Variation in concentration of whey components with increasing volume reduction during ultrafiltration. Feed and operating conditions as in Figure 5.

difference across the membrane; and

β is a factor to account for incomplete rejection at the membrane surface

However, it can be seen from Figure 1 that the asymptotic permeate flux does not increase with increasing pressure at pressures much beyond 150 psig. Moreover, from Figures 2 and 3 it can be seen that the total solids rejection, and thus the effective osmotic pressure difference across the membrane, continues to increase with increasing pressure. This increase in the effective osmotic pressure results from an increased rejection of both lactose and mineral salts. In one experiment using a Havens 215 module (feed flow rate 6.5 ℓ/min, temperature 68°F), as the average module pressure was raised from 105 to 250 psig, the chloride percent rejection increased from 0 to 8, calcium from 17 to 27, phosphate from 20 to 31, and lactose from 23 to 40.

Even though it is a contributing factor, the change in the osmotic pressure differential is not sufficient by itself to explain the fact that the asymptotic permeate flux is pressure independent above 150 psig. This fact requires that the resistance R in the above relation increase with increasing pressure. In many ultrafiltration applications R is a combination of the resistance of the membrane proper and that of any gel formed on its surface. From pure water permeability data it is evident that the membrane resistance remains unchanged at these pressures. The increased pressure will, however, cause compression of the gel, resulting in an increase in its resistance and thus in the value of R.

The strong temperature dependence of permeate flux is shown in Figure 4. It corresponds to an Arrhenius-type activation energy of about 5 kilocalories/mole. This value is consistent with that determined by Amerlaan and Wiley (1969). Consequently an ultrafiltration system should be operated at the maximum temperature which is compatible with microbiological and functional stability of the feed and the stability of the membrane. There was no measureable temperature variation in the membrane rejection characteristics. This fact indicates that in the temperature regime investigated, the temperature coefficients for the processes by which water and low molecular weight solids are transported through the membrane are nearly the same. Thus the concentration of the permeate remains constant, even though the actual flux rates of both water and dissolved solids in the permeate increase with increasing temperature.

When the effects of feed flow rate and pressure on the permeate flux at a fixed concentration had been determined, the system was used to carry out a batchwise fractionation of whey in order to determine the effects of increasing total solids and protein levels on the permeate flux. As the protein concentration of the feed is increased, the permeate flux declines as a result of an increase in the mass transfer resistance of the boundary layer. This decline is shown in Figure 5. In addition to causing a flux decline, the increasing protein level in the feed also increases the feed viscosity and the resultant pressure drop through the system. From Figure 5 it can be seen that the permeate flux decreases by about 40 per cent between the initial protein level and a concentration of 9% protein.

Whey fractionation using modules equipped with volume displacement rods

The Havens 215 VDR system was tested to determine the highest concentration of protein which could be obtained in practice. The variations of protein, lactose and ash levels at various stages of volume reduction in these experiments are shown in Figure 6. It was possible to obtain a product with a 10:8 protein:lactose ratio and a 15:1 protein:ash ratio. For operation at a constant feed rate, when the protein concentration had reached 10.5%, the original pressure drop had more than doubled. It would therefore not seem possible to achieve higher than this level in commercial operation with a system which has so large an inherent pressure drop across each set of modules. Using optimum conditions in a parallel-series staged Havens system it seems possible to obtain a membrane flux rate of 6–7 gfd in fractionating raw whey to obtain a product containing about 10%

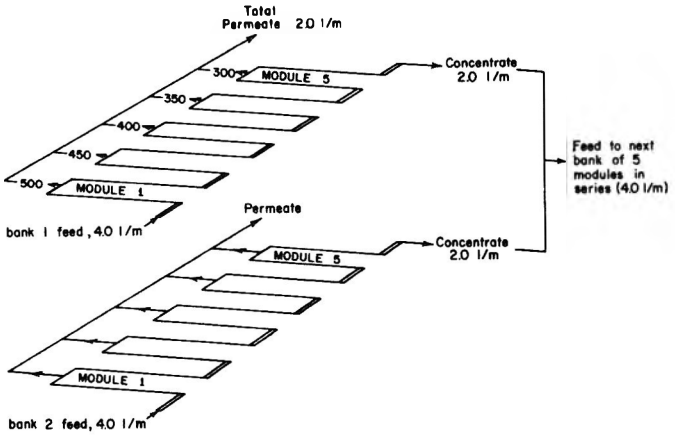


Fig. 7—Parallel-series configuration.

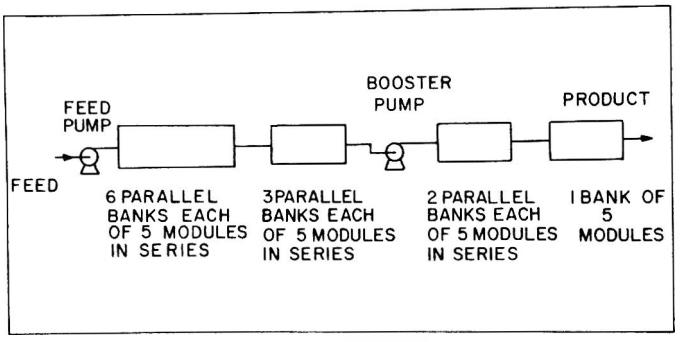


Fig. 8—Parallel-series configuration using an intermediate booster pump.

protein and 8% lactose. This product is equivalent to a volume reduction of 94-½% based on the raw whey. This corresponds to about a 5% lactose rejection at the membrane.

The permeate from the Havens system contained a small amount of TCA-coagulable nitrogen. The rejection of TCA-coagulable nitrogen was between 95 and 96%, indicating a slight loss of low molecular weight proteins and TCA-precipitated polypeptides. This loss was confirmed by subjecting the permeate to a polyacrylamide gel electrophoresis in which there was a faint band corresponding to the α-lactalbumin band in the control whey. The above data bear out the earlier predictions of the wide range of products available from ultrafiltration. The theoretical products outlined in Table 1 are shown as dotted lines in Figure 6 for comparison with the products obtained experimentally. The differences between the experimental products and those listed in Table 1 arise from three factors: (1) The membrane shows a 5% rejection of lactose thereby increasing the percentage of lactose in the concentrate during ultrafiltration; (2) The membrane does not yield the 100% rejection of protein assumed in Table 1; and (3) The whey used experimentally did not have an initial composition corresponding exactly to that used as the basis for Table 1.

Summary of recommended operating conditions based on above results

Based on the above discussion the following considerations relative to recommended operating conditions are pertinent to the type of system used in this work.

Feed flow rate. The strong dependence of permeate flux on feed flow rate suggests that ultrafiltration systems should be operated at the highest possible feed flow rate consistent with the considerations discussed below. In the Havens system the tubes are connected with tight U-bends which cause a large parasitic pressure drop through the membrane configuration. The feed flow rate can therefore be increased only to the point where the pressure drop through the system is at its highest acceptable value. In practice, this means operating the system below the asymptotic flux indicated in Figure 1. A second factor which may limit the feed flow rate is the bond between the membrane and the backing. This bond may become progressively weakened by the hydrodynamic shear stresses caused by a high linear velocity at the membrane surface. With improved casting techniques, however, this second factor should become unimportant in future systems. The exact level of the tolerable pressure drop is based on an optimum trade-off between the pumping costs associated with the pressure-drop and the processing capacity of the system.

If one has two or more modules or tubes in series, the liquid feed rate to a downstream module is less than that fed to the upstream modules by the amount of permeate removed in the upstream modules. In terms of the results presented in Figure 1, this fact implies that the permeate fluxes from successive modules in a series arrangement will decline as one proceeds downstream because of the decrease in the effective feed flow rates to the downstream modules. If one uses a number of parallel banks, each containing modules in series, it is highly desirable to reduce the number of banks in the downstream part of the overall system in order to maintain a satisfactorily high flow rate through each module. Such a parallel-series arrangement is shown schematically in Figure 7 for a hypothetical two-stage system.

Pressure. From Figures 1 and 2 it is evident that lower pressures are desirable for whey fractionation using the Havens system so as to minimize the lactose rejection ratio. Figure 3 indicates that acceptable permeate rates are achieved above about 35 psig downstream pressure (module average pressure = 50 psig). Thus the input pressure should be 35 psig plus the pressure drop through the system. In systems through which a large volumetric reduction is required the pressure drop at satisfactory feed flow rates may be as high as 250 psig. Consequently it may be necessary to introduce an intermediate booster pump to raise the pressure of the feed prior to downstream banks of modules. Such a system is depicted in Figure 8, using the bank structure of Figure 7 as a building block. Such a parallel-series system has previously been used for the concentration of sulfite pulp liquor by reverse osmosis (Amerlaan and Wiley, 1969).

Temperature. From the standpoint of flux considerations it is desirable to operate the system at the highest possible temperature (compatible with membrane stability) since every 20°F rise in feed temperature is accompanied by an increase in permeate flux of approximately 40%. For whey, microbiological activity will increase with increasing temperature up to about 125°F and then will fall off. At 125°F, and higher temperatures, long fluid residence times in the system will impair some of the functional properties of the whey proteins via thermal denaturation of proteins. Depending on the exact functional properties required in the high-protein product, it may therefore be necessary to operate at lower temperatures (65–75°F) where microbiological activity will be significantly decreased and where the functional properties of the feed will be unimpaired. Other economic considerations such as the availability and cost of heat exchange equipment may also play an important role in determining the temperature at which the system operates.

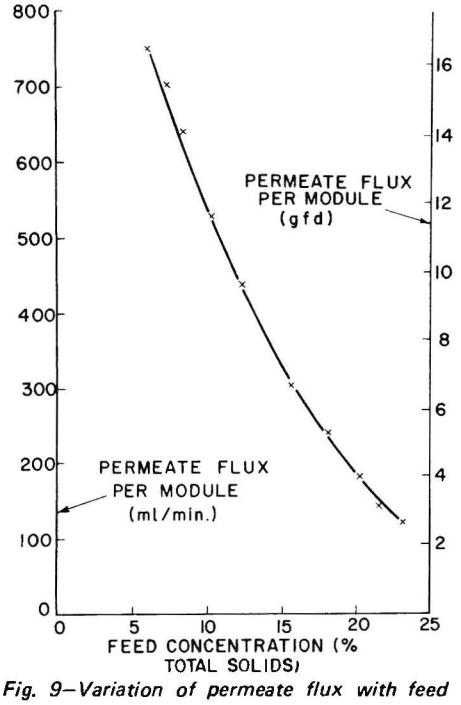


Fig. 9—Variation of permeate flux with feed concentration. Feed: permeate from whey fractionation (6.0% total solids, 0.03% protein, 5.2% lactose, 0.18% N.P.N. compounds, 0.42% ash). Havens type 300 module, 600 psig inlet pressure, 2 gpm feed flow rate, 92°F temperature.

Concentration of lactose permeate from whey fractionation operations

The permeate from the ultrafiltration operations described above contains 4–7% lactose with the original percentage of ash in the whey and some non-protein-nitrogen compounds. The volume of the permeate will be from 80–95% of the original volume of whey, depending on the percentage water removal in the ultrafiltration operation. This permeate stream will present much the same pollution problem as the initial whey unless it is treated in some manner. Reverse osmosis can conveniently be used to concentrate this permeate stream up to about 24% total solids, prior to final evaporation or crystallization for lactose recovery. The following paragraphs describe experiments in this area.

Using Havens 300 modules at 600 psig inlet pressure with a feed flow rate of 2.0 gal/min (feed temperature 90°F), it is possible to obtain a mean permeate flux of 12 gfd through the membrane. During this process the total solids content of the ultrafiltration permeate was increased from 6-½% to 24%. The variation in permeate rate with feed concentration is shown in Figure 9. The permeate rates are higher than those for raw whey since there is very little protein in the permeate (only 3–5% of the TCA-coagulable material) to enhance the boundary-layer resistance to mass transfer through the membrane.

The permeate collected from modules fed with dilute solutions contained only 0.3% total solids (0.04% lactose) while that obtained from modules whose feed contained 23% total solids was found to contain 1.7% total solids (0.97% lactose). The average C.O.D. of the mixed permeate from this concentration operation was 1800 ppm. This value can be reduced to 800 ppm using tighter membranes such as those in the Havens 102 modules. Similar runs at 825 psig with Havens 102 modules, with a feed flow rate of 1.5 gal/min and temperature of 90°F, yielded a mean permeate flux of 8.7 gfd in concentrating the ultrafiltration permeate from 6-½% to 25% total solids. A permeate containing 500–2,000 ppm C.O.D. (depending on the exact type of membrane used) would be typical of the effluent from a two-stage process utilizing ultrafiltration and reverse osmosis for the fractionation and concentration of whey into commercially useful liquid concentrates. The low level of solids and C.O.D. in the effluent underscores the potential usefulness of these processes for whey utilization and pollution control.

It should be pointed out that although there is currently a relatively high demand for lactose in the U.S. domestic market, the widespread adoption of ultrafiltration techniques for producing whey protein concentrates will probably be accompanied by the production of a large surplus of lactose as a by-product. Disposal of this lactose (except as waste) will require the development of new markets and uses for this material. One such use which is currently under study involves the utilization of lactose as the polyol monomer in the production of polyurethane (Hustad et al., 1970).

Production of a concentrated "skim milk equivalent"

The results depicted in Figure 8 show that it is possible to achieve the 3:5 protein:lactose ratio of skim milk as a whey fractionation product from ultrafiltration. Experiments were carried out to see if method A, suggested in Table 2, is a feasible means of achieving a concentrated "skim milk equivalent."

McDonough and Mattingly (1970) have shown that it is possible to concentrate whey fourfold. In this work whey was concentrated 2-½ times, and this concentrated product was fractionated by ultrafiltration. The results were very encouraging in that the lactose rejection properties of the Havens ultrafiltration membranes were found to be identical for feeds containing 5% and 18% lactose. At a feed flow rate of 1.14 gal/min to Havens 215 VDR modules with an input pressure of 200 psig the average flux rate through the modules was 4.5 gfd at 90°F for 81% water removal. In a typical experiment, defatted Cheddar whey (6.7% total solids, 0.67% protein, 4.9% lactose) was first concentrated using Havens 102 and 103 modules to 15.7% total solids (1.88% protein, 12% lactose). This concentrated product was then fractionated to give a "skim milk equivalent" (23.2% total solids, 8.65% protein, 13.3% lactose) and a concentrated lactose stream (13.1% total solids, 0.09% protein, 11% lactose).

Earlier it was shown that one can obtain products with protein:lactose ratios as high as 2:1. One could achieve higher ratios by mixing the 2:1 product with water and repeating the ultrafiltration operation to remove the added water along with more lactose. This technique of dialysing out an undesirable component by multistage ultrafiltration has been used for enzyme and virus concentration (Sinskey et al., 1969). In this way a product containing a 20:1 protein:lactose ratio could be achieved, but only at the expense of the proportionately higher capital outlay which would be required for the multistage ultrafiltration process.

Sanitary properties of available equipment

Most commercially available ultrafiltration equipment for the fractionation of whey utilizes the previously described tubular-membrane configuration with swollen cellulose acetate as a membrane. To meet sanitary requirements, these tubes should be connected together in such a manner that there are no dead zones or crevices in which microbiological action could occur. In order to provide the possibility of at least a partial visual inspection of the equipment, the tubes should be connected in such a manner that they can be inspected individually on a random basis. This inspection would be facilitated by use of transparent polymeric materials of construction wherever possible. One must also ensure that no microbiological action can occur in any of the dead zones which may exist at the membrane-backing interface, or within the membrane and backing themselves. It seems doubtful that any of the proprietary techniques for membrane casting can prevent there being regions within the membrane and backing in which the permeating solution might have a very long residence time. For feeds like whey which are good media for bacteriological and mycological growth, sanitation problems involving the membrane-backing interface may prevent the use of a number of polymers as construction materials in ultrafiltration and reverse osmosis equipment. An additional problem arises in that a number of polymers may themselves be nutrients for certain types of bacteria and fungi. These factors indicate that before any materials (such as cellulose acetate, PVC, fiberglass, polyolefins, etc., which are currently used) can be

utilized in ultrafiltration equipment for the production of human-grade foods, extensive studies will have to be undertaken on the microbiological characteristics of the complete system. One such study has been published in connection with the use of cellulose acetate reverse osmosis membranes for the concentration of maple sap (Kissinger and Willits, 1970). This study indicated that there was no evidence of degradation of cellulose acetate by *cryptococcus albidus* yeast or *penicillium* sp. mold, but that severe sliming of the membrane occurred.

It is probably possible to design a tubular configuration without dead zones or crevices. Any large membrane separation system will, however, require hundreds of tubes. Thus the use of random visual inspection (and cleaning of individual tubes) will not be practical in a plant-scale operation as the sole means of ensuring cleanliness. In addition, it should be pointed out that although the average velocity in the tube may ensure highly turbulent conditions in the bulk flow, there will nevertheless be a laminar-flow layer adjacent to the membrane. This condition may allow suspended casein, fat or proteinaceous materials to be deposited on the membrane. Such a deposit may or may not impair membrane performance, but it will certainly serve as a nutrient for microorganisms if not removed periodically. Before sanitizing the equipment any such deposits will have to be removed. This removal may require the use of detergents and/or proteolytic enzymes. Materials which are effective in removal of the deposits but which do not degrade the membrane will have to be selected on the basis of experimental results.

Once the deposit has been removed, the equipment could be sanitized by means of high temperatures (135°F for 10 min to eliminate some molds and yeasts, or 145°F for 30 min to eliminate milk-borne pathogens), or by means of the usual high- and low-pH washes (i.e., at pH 12 and pH 2) with intermediate water washes. A third possibility for sanitizing the equipment might be the use of commercially available chlorine- or iodine-based cleansers or quaternary ammonium halides.

The use of cellulose acetate, however, limits both the temperature and pH range of operation of the system. A pH outside the range 3–8 will markedly accelerate the rate of hydrolysis of cellulose acetate, thereby impairing its semipermeability characteristics. It is probable that the tight reverse osmosis membranes can withstand short periods of operation at 145°F. For the looser ultrafiltration membranes, however, this high-temperature operation may not be possible since the lifetime of the membrane may be drastically reduced by membrane compaction. It is also highly probable that the permeability and selectivity characteristics of these membranes will also be affected by this heat treatment. In order to determine the short-term, high-temperature limits for particular types of membranes, it will be necessary to carry out programs involving destructive tests of the membranes. If chemical cleansers are to be used, their compatability with the membrane must be determined. In the case of chlorine, maximum membrane tolerances of from 10–50 ppm and higher have been suggested. Kissinger and Willits (1970) reported that 50 ppm of chlorine effectively sanitized their equipment (for idle periods of up to three days) without causing any apparent damage to the membranes.

In systems where recycle is used, it may be possible to use a small centrifuge in the recycle stream in order to remove microorganisms. This operation would not remove the requirement of presanitizing the system, but rather would ensure that there was no build-up of microorganism concentration in the system during operation. Suitable bacterial centrifuges are commercially available. Their use for whey systems would lead to the loss of some of the higher molecular weight proteins which could later be recovered from the sludge with appropriate pasteurization.

The ideal membrane separation unit for food processing applications would therefore seem to be a tubular configuration using a membrane which will withstand temperatures up to 150°F and extremes of pH without long-term deterioration. The tubular system is suggested because its hydrodynamic flow characteristics correspond most closely to plug flow, thus giving the narrowest spread of feed residence times for all the potential configurations proposed to date.

Some non-cellulose acetate systems have been investigated for reverse osmosis and ultrafiltration. One such system uses the fine-fiber approach with a polyamide membrane (Robinson and Mattson, 1968). Other systems utilize polyelectrolyte membranes (Dorr-Oliver Bul. IO-1, 1969). There is now available a polyelectrolyte membrane which has a pH range of 1–13 and a molecular weight cut-off of 16,000. If the pH range of cellulose acetate proves to be insufficient for prolonged sanitary operation, the polyelectrolyte membranes should meet the sanitary requirements of high- and low-pH washes.

Any large scale reverse osmosis or ultrafiltration system will require a large amount of supporting equipment such as tanks, piping, instrumentation, pumps, etc. For the low pressure equipment, most of the commonly used dairy equipment will suffice. For higher pressure operations, manufacturers will need to provide suitable sanitary equipment such as valves, centrifugal booster pumps (with a high pressure on the suction side), pressure accumulators and a whole range of externally threaded high pressure pipe fittings. One manufacturer currently markets a range of centrifugal pumps specifically for reverse osmosis equipment. It is hoped that with the projected demand for this type of equipment, other manufacturers will see fit to produce the required supporting equipment in a sanitary design.

Economics

The main areas influencing the economics of reverse osmosis as applied to whey processing were outlined by McDonough and Mattingly (1970). The same considerations and uncertainties apply to whey processing by ultrafiltration. The major difference here, however, is that for whey fractionation, conventional thermal evaporation is not a competitive process as it cannot effect separation of the whey components. For fractionation, competing processes would be electrodialysis or ion-exchange (for demineralization), followed by concentration and lactose crystallization, or gel permeation chromatography, followed by concentration of the products thus obtained. The actual cost of the membrane requirements in ultrafiltration will, however, be higher than those indicated by McDonough and Mattingly (1970) for two reasons. First, most products from fractionation processes will require a significantly higher degree of volume reduction (usually greater than 90%) than that attainable with reverse osmosis (up to 75% volume reduction). Second, the permeate flux of Havens ultrafiltration membranes under normal operating conditions (less than 250 psig) is only two-thirds of that which would be obtained from a reverse osmosis membrane working at 800 psig and the same temperature. These two factors imply that, for a given volumetric feed rate, a larger area of membrane will be required for ultrafiltration than for reverse osmosis.

Cautionary note

It must be pointed out that the field of membrane technology is rapidly advancing. Much of the work presented in this paper was carried out with membrane equipment purchased prior to January 1969. While none of the discussion about the optimum values of operating parameters is outdated, the actual numerical values quoted in connection with whey fractionation may not be representative of the performance of equipment which is now commercially available.

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D. J. SCHRODER[1] and H. JACKSON
Department of Food Science, University of Alberta, Edmonton, Alberta, Canada

PREPARATION OF SOYBEAN CHEESE USING LACTIC STARTER ORGANISMS. 3. Effects of Mold Ripening and Increasing Concentrations of Skim Milk Solids

SUMMARY—Soybean cheeses were prepared from blends of skim milk powder and soybean milk in which the skim milk powder contributed 0, 25, 50 and 75% of total dry weight. The amount of skim milk had little effect on the flavor of the finished cheese, due to the dominating effect of the beany flavor of the soybeans. Similarly, the skim milk had little effect on the texture of the finished cheese, indicating that only a small amount of fibrous matter from the soybeans is necessary to impart a mealy texture to the product. Mold ripening resulted in desirable changes in texture, but these were offset by the development of bitter flavors.

INTRODUCTION

PREVIOUS studies showed that a satisfactory soybean cheese could be prepared by means of a lactic acid fermentation of soybean milk (Hang and Jackson, 1967 a; 1967 b). Although the product had good keeping quality, there was still a beany flavor and the texture was a little coarse. Therefore, further experiments were undertaken to assess the value of increasing the concentration of skim milk in the cheese and to study mold ripening of the finished product.

EXPERIMENTAL

Preparation of soybean milk

Dry mature soybeans (Grade No. 1, W. G. Thompson & Sons Ltd., Blendheim, Ontario, Canada) were steamed for periods ranging from 0–30 min. Steaming beans for 20 min under atmospheric conditions resulted in bland-flavored milk, but a considerable time was required for drying the beans. A 3-min steam treatment was sufficient to loosen the hulls, with the advantage of a short drying time. The hulls were then cracked in a cereal grinder (Model 4-E Quaker City Mill, The Straub Co., Philadelphia, Penn.) and blown off with air. The dehulled beans were blended for 5 min in a Waring Blendor with water at 80–100°C. 540 g of beans were added to 5 liters of water. The resulting milk was steamed for 30 min to destroy any remaining anti-digestive factors in accordance with the findings of Wilkens et al. (1967). This method is simple, fast and produces a bland product without caramelization or loss of protein solubility.

Preparation of soybean cheeses

Skim milk was blended with soybean milk so that the skim milk contributed 0, 25, 50 and 75% of the total dry weight. 5 kg of the soybean milk-skim milk mixture was brought to 41°C and 5% of starter culture added. When fine lines of whey appeared where a knife had cut the jelly-like curd, the curd was cut. After cutting, the curd was cooked by slowly raising the temperature to 48°C (1°C per min) and holding at that temperature for 30 min. The resulting curd was placed into hoops and pressed at 1 psi for 24 hr.

Streptococcus thermophilus 101 (Klenzade Products, Division of Economics Laboratory Inc., Beloit, Wisconsin) was used as the starter organism. Rennet, in the amount of 0.3% by volume, was added 1 hr after the starter to assist in coagulation of the milk. The cheeses were waxed and stored at 20°C.

Samples were collected during manufacture and storage for determinations of: pH, bacterial numbers, protein, water-soluble nitrogen, moisture content, texture and product acceptability. 4 separate cheese replicates were produced. The medium employed to enumerate the numbers of viable organisms was a modification of that developed by Donovan and Vincent (1955) using pH-sensitive indicators. Total nitrogen was determined by the standard micro-Kjeldahl procedure (A.O.A.C., 1965). Water-soluble nitrogen was determined by an adaptation of the method of Smith et al. (1952).

Mold ripening

To evaluate flavor and texture changes associated with mold ripening, 3 trials were carried out on soybean cheeses made from a blend of skim milk and soybean milk, each contributing 50% of the solids. The first trial consisted of inoculating soybean cheese surfaces with *Rhizopus oligosporus* and incubating the cheese to obtain a solid mat of surface growth on the cheese. In the second trial, *Penicillium camemberti* was grown on the cheese surface in the same manner. The third trial consisted of inoculating whole dehulled soybeans with *R. oligosporus* to produce an Indonesian food called "tempeh," in which a cake is formed by mycelial growth over the beans (Steinkraus et al., 1965). The cake was then ground in water at 90°C to produce a milk. Cheese was made by the normal method. Controls were made to compare the effects of the different organisms on the characteristics of the finished products.

RESULTS

Bacterial numbers and pH

The viable count of bacteria in the cheese is shown in Figure 1. In general, the numbers increased greatly during the manufacturing period, dropped off during pressing and then remained at that level. There was little difference between the cheeses made from the soybean milk-skim milk mixtures in which 25, 50 and 75% of the dry weight were contributed by skim milk. All organisms found in the cheese were acid-producing cocci and an occasional mold spore.

Figure 2 shows that the pH decreased quickly during the manufacturing process, then gradually dropped during storage. The cheeses containing the larger amounts of soybean were more acidic.

Water-soluble nitrogen

The water-soluble nitrogen, an indication of protein breakdown, increased during storage, as seen in Figure 3. The greatest breakdown occurred in the cheeses containing the highest percentages of skim milk solids. The increase in water-soluble nitrogen is probably due to the action of rennet and the enzymes and lactic acid excreted by the bacteria. According to Sumner and Myrback (1951)

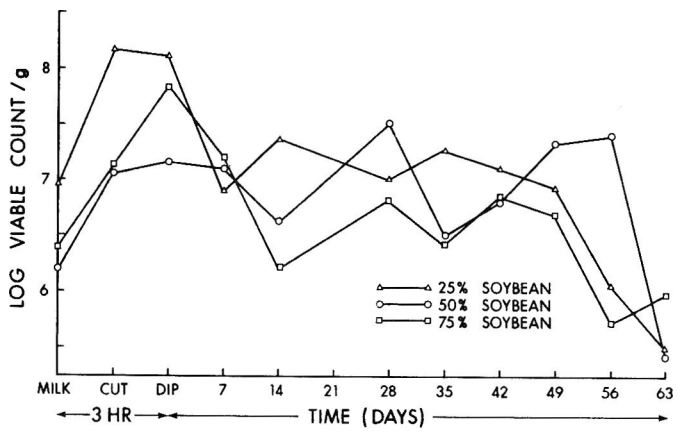

Fig. 1—Changes in bacterial numbers during manufacture and ripening of soybean-skim milk cheeses.

[1]Present address: Department of Food Science & Industries, University of Minnesota, St. Paul, Minnesota 55101.

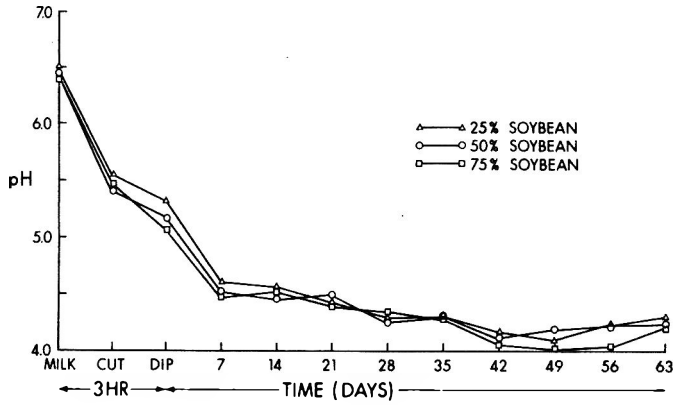

Fig. 2—Changes in pH during manufacture and ripening of soybean-skim milk cheeses.

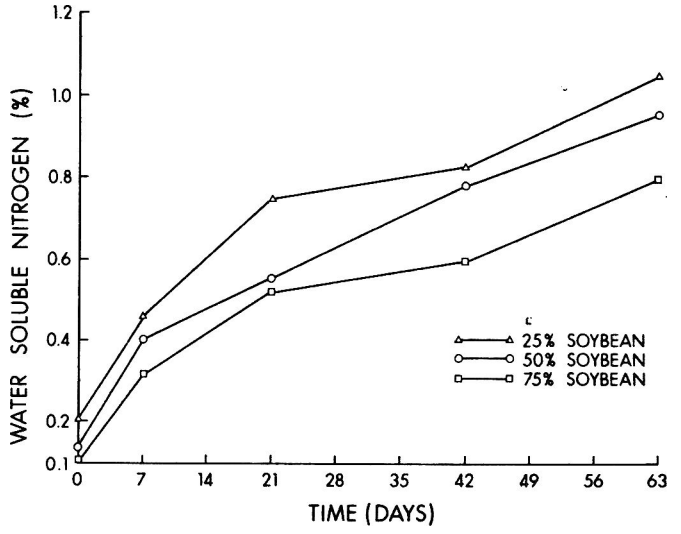

Fig. 3—Changes in water-soluble nitrogen during ripening of soybean-skim milk cheeses.

the optimum pH for the proteolytic activity of rennin is 3.7. Rennin may also digest some plant globulins. The water-soluble nitrogen increased with an increase in acidity. As the optimum pH for rennin activity was approached, one would suspect that it played an important role in the protein hydrolysis in the cheeses.

Moisture content

The moisture content remained relatively stable during storage. The cheeses with a higher amount of soybean had a higher moisture content than the skim milk samples. The skim milk curd probably facilitated whey drainage to a greater extent than did the soybean curd.

Mold ripening

Excellent surface growth was obtained in all the surface-ripening experiments. The samples soon became very bitter and astringent in flavor. The texture, however, was greatly improved, i.e., similar to that of creamed cheese. The cheese prepared from tempeh tasted slightly sweet at the milk stage, but on storage the cheese slowly developed an astringent flavor. The texture was similar to that of the surface-ripened samples. The cheeses in all 3 experiments had a higher moisture content than the controls. The mold-ripened cheese contained much higher soluble nitrogen than control samples after 3 weeks of storage. The highest protein breakdown occurred in the cheese inoculated with *P. camemberti*, with a water-soluble nitrogen at 3 weeks of 1.43% as compared to 0.28% of the control. The improved texture was the most desirable change produced in the cheese by the molds. The undesirable flavors produced were probably due to the mild enzyme hydrolysis of the protein giving the formation of bitter peptides.

DISCUSSION

PREVIOUS work by Hang and Jackson (1967 a; 1967 b) showed that addition of skimmilk (15% dry weight of soybean milk-skim milk mixture) and rennet could bring about improvements in flavor and texture of soybean cheese. Skim milk considerably reduced the time required for the manufacture of the curd. It is also a reasonable assumption that the skim milk improved the nutritional value of the product. In this work considerably more skim milk was incorporated into the cheese (25–75% dry weight of soybean milk-skim milk mixture) to bring about further improvements in the finished product.

The amount of skim milk had little effect on flavor, due to the dominating beany flavor of the soybeans. Similarly, skim milk had little effect on the cheese's texture. This would indicate that only a small amount of the fibrous material associated with soybeans is necesary to impart a mealy texture to the product. Skim milk did, however, considerably reduce the manufacturing period, due to the more rapid coagulation of the curd. The effect of the skim milk in this respect is probably 3-fold: increasing the percentage of skim milk solids would provide a more suitable medium for the starter culture; rennet has a degree of specificity for casein and, hence, its activity would be more apparent in the presence of increasing concentrations of skim milk; the isoelectric point of casein is higher than that of the major proteins of soybean and, hence, less time is required to reach the optimum pH for precipitation.

The bitter flavors in the ripened cheeses were probably due to the formation of peptides, resulting from protein degradation. This corresponds to the increase in the water-soluble nitrogen. The breakdown is probably from the action of the rennet extract. This process would be accelerated by the low pH from the production of lactic acid by the starter bacteria. According to Sumner and Myrback (1951), the optimum pH for the proteolytic activity of rennin is 3.7. Rennin may also digest some plant globulins. Pepsin, present in lesser amounts in rennet, acts on all native protein, although the optimum pH is quite low (pH 1.5–2). The starter organisms themselves are nonproteolytic.

The advantages in the fermentation of soybeans for the production of an edible food material have been discussed by Murata et al. (1967) and Hesseltine and Wang (1967). Fermentation may result in reduction or masking of the beany flavor and enhancement of the nutritional value of the product. However, the new flavors resulting from fermentation may be acceptable only to some palates.

Work by Fujimaki et al. (1968), employing 3 well-known enzymes, papain, bromelin and pepsin, and 9 proteolytic enzyme preparations of microbial origins, showed that the beany flavor could be reduced by enzymatic action as well as by fermentation. The major difficulty in either fermentation or enzymatic modification is the control of proteolysis to prevent formation of bitter peptides. This is confirmed by the results of the experiments on the mold ripening of soybean cheeses. Significant improvements were apparent in the texture of the product, but the flavor soon became very bitter and unacceptable.

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C. PERI[1] and W. L. DUNKLEY
Department of Food Science and Technology, University of California, Davis, California 95616

REVERSE OSMOSIS OF COTTAGE CHEESE WHEY. 1. Influence of Composition of the Feed

SUMMARY—Permeation rate, retention, and solute flux during reverse osmosis of whey and whey fractions were compared using two types of cellulose acetate membranes. When the feed solutions contained no molecules larger than lactose, concentration polarization had little influence on performance except at the highest available driving force (applied pressure minus difference between osmotic pressures of the feed and permeate = 37.8 atm). With the more complex feeds (whey and deproteinized whey), both concentration polarization and fouling of the membrane occurred. Concentration polarization decreased both permeation rate and retention. Fouling decreased permeation rate, but its influence on retention was variable and depended principally on the feed, the solute, and the available driving force. Proteins and other macromolecules in whey had a greater influence on performance during reverse osmosis than smaller solute molecules. With whey as feed, maximum permeation rates were achieved at low available driving forces (10–12 atm), and were similar for the two types of membranes (about 1 ml/cm^2·sec). Increasing the available driving force increased retention and therefore reduced solute flux. Choice between the two membranes requires a compromise between extent of desalting and loss of lactose in the permeate.

INTRODUCTION

PROCESSING WHEY by reverse osmosis is a promising method for concentrating and fractionating whey solids. A previous study (Marshall et al., 1968) indicated two problems that limit the permeation rate achieved during processing whey by this method – concentration polarization and gradual fouling of the membrane during operation.

Reverse osmosis is a membrane-separation process in which hydraulic pressure in excess of the osmotic pressure is used to force water (and in some applications selected solutes) through a semipermeable membrane. By this process, solutes of low molecular weight can be separated from their solvents. The term ultrafiltration designates a similar process in which solutes of relatively high molecular weight and colloidal particles are separated from their solvent and solutes of low molecular weight. In ultrafiltration, as a determinant of permeation rate, the osmotic pressure of the solute that is retained is negligible in comparison with resistance to hydraulic flow. Whey is such a complex mixture of solutes that, when it is processed by membrane separation, both reverse osmosis and ultrafiltration are involved.

The selectivity of reverse osmosis membranes is commonly explained on the basis of a solution-diffusion or diffusive flow mechanism, viscous flow through a microporous structure which achieves a sieving effect, charge effects, or combinations of these mechanisms (Merten, 1966). The performance of very "tight" reverse osmosis membranes used for water desalination conforms well with predictions from the diffusive flow mechanism, whereas the viscous flow mechanism appears to be more appropriate for explaining properties of open reverse osmosis membranes and ultrafiltration membranes.

When hydraulic pressure forces a solvent through a semipermeable membrane, solutes retained by the membrane become more concentrated at the upstream surface of the membrane. This is called concentration polarization. Rejection of microsolutes results in an increase in osmotic pressure of the solution at the membrane surface and a corresponding decrease in the driving force for reverse osmosis, $\Delta P - \Delta\pi$ (ΔP = hydrostatic pressure difference; $\Delta\pi$ = difference between osmotic pressure of upstream and downstream solutions). The increase in solute concentration at the membrane also causes a decrease in retention (the fraction of the solute present in the feed solution that is retained by the membrane, commonly expressed as percentage).

Concentration of macrosolutes (e.g., proteins) at the membrane surface also adversely affects performance of the membrane (Michaels, 1968a, b). Macrosolute concentration may foul the membrane in a number of ways, including plugging of pores, accumulation of a viscous gel, cake formation, and dynamic accumulation of solute within the membrane.

The study reported herein entailed an investigation of the effect of composition of the feed on the performance of cellulose-acetate reverse osmosis membranes. The results are discussed in relation to possible causes of changes in permeation rates, retentions and solute fluxes.

EXPERIMENTAL

Apparatus

The reverse osmosis assembly (Fig. 1) circulated the feed solution at controlled pressure, temperature, and flow rates through reverse osmosis tubes, and returned both the concentrate (retentate) and the permeate to the feed reservoir. The tubes (Havens Industries, San

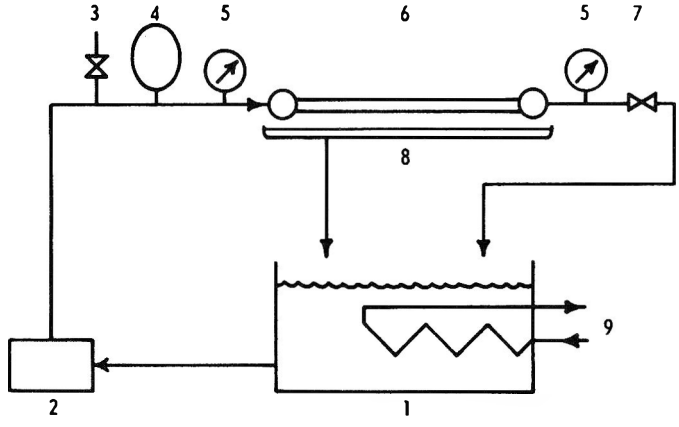

Fig. 1—Flow diagram of reverse osmosis assembly. Feed material in tank 1 was pumped by triplex pump 2 through the feed line equipped with a safety valve 3, pneumatic accumulator 4 and pressure gage 5, to three reverse osmosis tubes connected in parallel 6. The pressure in the tubes was regulated by back pressure valve 7, and the product was returned to the feed tank. Collecting troughs 8 permitted collecting permeate from each tube separately or returning the permeate to the feed tank. Heat exchanger 9 maintained constant feed temperature.

[1] On leave from Instituto di Industrie Agrarie, Universita di Perugia, Perugia, Italy.

Table 1–Composition and properties of feed solutions.

	Whey	Deproteinized whey	Simulated ultrafiltrate	Lactose solution	Salts solution	Proteins
Total solids, %	6.4	6.0				
Protein, %[a]	0.43	0.01				0.49
Lactose, %	4.11	4.13	3.87	4.00		
Lactic acid, mg/ml	0.47	0.35	0.10			
Citric acid, mg/ml	2.0		1.90		2.00	
Phosphorus, mg/ml	0.66	0.65	0.36		0.36	
Calcium, mg/ml	1.09	1.10	0.39		0.29	
Potassium, mg/ml	1.54	1.59	1.24		1.43	
Osmotic pressure, atm, 0°C	7.61	7.36	5.01	2.78	3.12	0.00
Density, g/ml	1.024		1.010			

[a](% Total N – % nonprotein N) × 6.38.

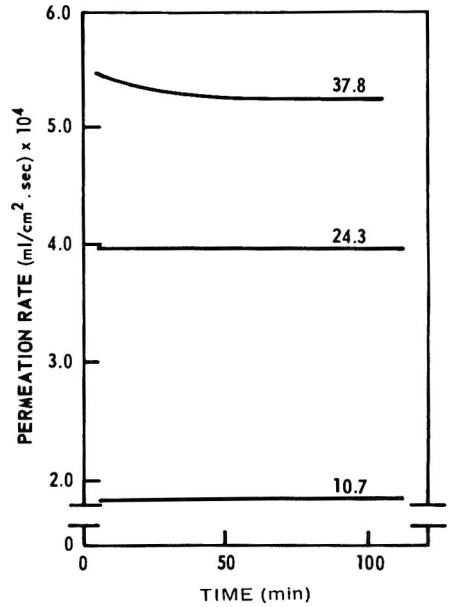

Fig. 2–Permeation rates during reverse osmosis of simulated ultrafiltrate. (Membrane A, available driving force, 10.7, 24.3, and 37.8 atm, as indicated on curves.)

Diego, Calif.) were porous fiberglass cylinders, 1.25 cm internal diameter and 57 cm long, lined with cellulose acetate membranes. Two membrane types (Havens type 2A and 3A, designated A and B, respectively) were used.

Feed solutions

Experiments were done with cottage cheese whey and with simplified solutions that permitted study of effects of individual whey components. The cottage cheese whey (obtained from Crystal Cream and Butter Company, Sacramento, Calif.) was centrifugally clarified and pasteurized and then stored at 3–5°C. For preparation of "deproteinized" whey, cottage cheese whey was boiled 10 min, cooled to 5°C, and stored overnight. The supernatant fluid was then removed and stored frozen. Before use it was thawed and clarified by centrifuging (continuous-flow Servall centrifuge, Model RC2, Ivan Sorvall, Inc., Norwalk, Conn.) at 17,500 ×g at a 6 ml/min flow rate. A "whey protein" solution was prepared by concentrating whey to 12% total solids by reverse osmosis, using an open membrane, and dialyzing the concentrate. Simulated milk ultrafiltrate and milk salts solutions were prepared as described by Jenness and Koops (1962) and adjusted to pH 4.6 with lactic acid.

Procedures

The feed was maintained at 15–16°C. In most experiments, the flow rate of the feed was maintained constant at 73 ml per second. This provided an average linear velocity of 20 cm/sec when three tubes were operated in parallel.

During runs, samples of permeate were collected at selected intervals from each tube separately, and permeation rates were recorded. Water permeation rates were measured periodically to determine constancy of the permeation characteristics of the membranes.

Analytical methods applied to the feed and permeate solutions included colorimetric determination of lactose (Seliwanoff reaction using Hycel reagent, Anon., 1964), lactic acid (Barker and Summerson, 1941), citric acid (Marier and Boulet, 1958), and phosphorus (Harris and Popat, 1954). Other procedures included fluorimetric determination of riboflavin (Hand, 1939), atomic absorption analysis for potassium, sodium, and calcium, a Kjeldahl procedure (Rowland, 1938) for protein and nitrogen distribution, determination of osmotic pressure by freezing point depression (freezing point osmometer, Model 31L, Advanced Instruments, Inc., Newton Highlands, Mass.) and determination of viscosity using a rotating cylinder viscometer (Brookfield Model LVT with UL adapter, Brookfield Engineering Laboratories, Stoughton, Mass.). Protein fractions in the feed were characterized by polyacrylamide gel electrophoresis (Grindrod and Nickerson, 1967).

RESULTS & DISCUSSION

Feed solutions

Typical analyses of feed solutions are summarized in Table 1. The feeds were selected to permit evaluation of the effects of individual whey constituents or fractions on results obtained by reverse osmosis processing. The whey was removed from the cheese vat by a steam injector, which caused a slight dilution by the condensing steam. Distribution of nitrogen (Rowland, 1938) in the whey proteins was, in mg N/ml whey, total protein 0.068, casein 0.013, soluble whey protein 0.055, lactalbumin plus lactoglobulin 0.050, and proteose-peptones 0.005. The presence in whey of α_S and β-casein α-lactalbumin, and β-lactoglobulin was demonstrated with polyacrylamide gel electrophoresis. The deproteinized whey did not give a precipitate with trichloroacetic acid, but still contained some relatively large molecules, such as partially hydrolyzed protein and riboflavin. The electrophoretogram of deproteinized whey that had been concentrated 10-fold by evaporation and then examined by polyacrylamide gel electrophoresis did not have bands characteristic of casein components or lactalbumin or lactoglobulin. However, it did have a faint band indicating the presence of a component with mobility greater than that of components of a reference sample of milk. The simulated ultrafiltrate differed from deproteinized whey principally in that it did not contain molecules larger than lactose. The salts solution was similar to the simulated ultrafiltrate except that the former contained no lactose.

The experiments were limited to concentration of whey constituents typical of those in commercial cottage cheese whey. The composition of the feed was kept essentially constant during runs by returning both the permeate and retentate to the feed tank. Maintaining constant composition of the feed eliminated one variable that influences performance of reverse osmosis membranes.

Permeation rates

Permeation rates obtained with simulated ultrafiltrate during runs at three operating pressures are plotted against time in Figure 2. Similar results were obtained with the less complex solutions (salts, lactose) and water. The permeation rate increased with increase in the available driving force, $\Delta P - \Delta\pi$, and remained constant during the runs. The curve for simulated ultrafiltrate at the highest available driving force (37.8 atm) is an exception in that it indicates a small decrease at the start of the run. This decrease is attributed to concentration polarization. After equilibrium was established between the rates of permeation through the membrane and of transport of solutes by diffusion from and convection to the membrane, a steady-state condition was established.

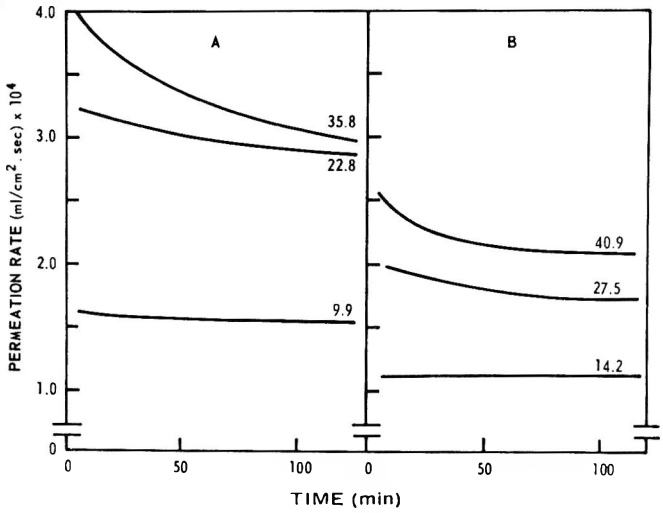

Fig. 3—Permeation rates during reverse osmosis of deproteinized whey (Membranes A and B, ΔP−Δπ given on curves).

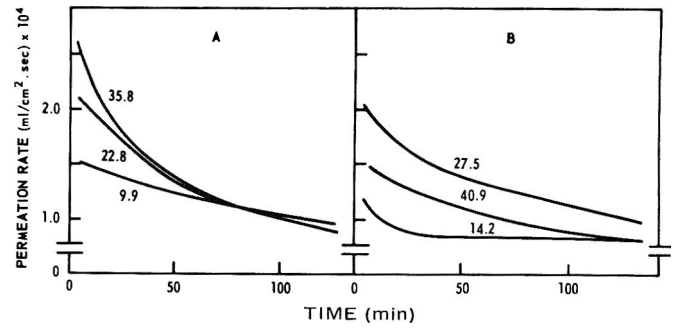

Fig. 4—Permeation rates during reverse osmosis of whey. (Membranes A and B, ΔP−Δπ given on curves.)

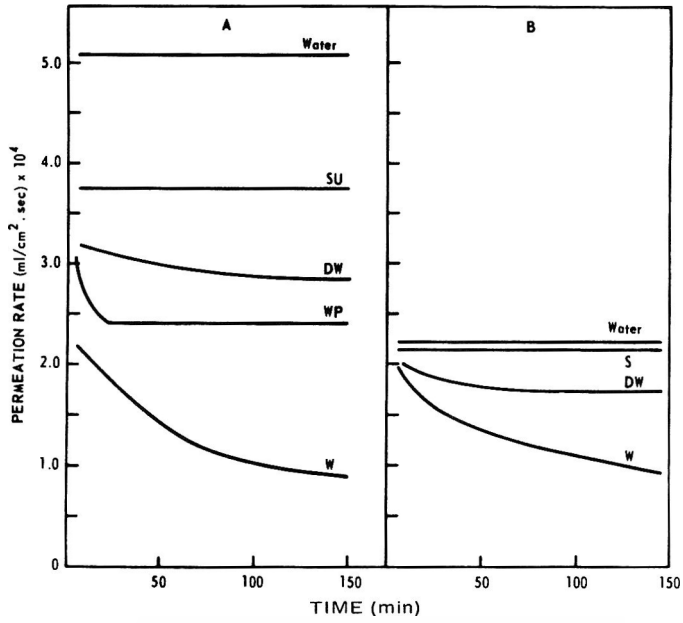

Fig. 5—Influence of feed composition on permeation rates (ΔP−Δπ 22.8 and 27.5 atm, for memnranes A and B, respectively. Abbreviations: SU, simulated ultrafiltrate; DW, deproteinized whey; WP, whey proteins; W, whey; S, salts. Curves for simulated ultrafiltrate, salts, and water were calculated by interpolation from data obtained at similar ΔP−Δπ).

Permeation rates with deproteinized whey are depicted in Figure 3. At the lowest available driving force, the permeation rate remained almost constant during the run with membrane A, and constant with membrane B. At the intermediate and highest available driving forces, decreases occurred. With membrane A the permeation rates at the higher available driving forces (22.8 and 35.8 atm) decreased to similar values.

The permeation rates with whey are shown in Figure 4. With membrane B, the intermediate available driving force gave the highest permeation rate, but differences among the three pressures decreased as the run progressed. The available driving force had less effect on permeation rates through membrane A than through membrane B.

The decreases in permeation rates in Figure 4 were caused by fouling. When the data in Figure 4 were plotted as permeation rate against cumulative permeate volume, curves similar to those in Figure 4 were obtained. The decreases in permeation rates indicated that the total amount of permeate that passed through the membrane was an important factor influencing the extent of fouling, but the curvature is evidence that other factors also had a marked influence on permeation rate.

We visualize that the fouling at the start of runs was principally by pore plugging. With microporous membranes, the majority of the fluid flows through the larger pores (Michaels, 1968a). Fouling by pore plugging occurs more rapidly than fouling resulting from the formation of a gel-like layer or cake. This is so because of the early predominance of pore flow and the limited opportunity for convective flow to remove macrosolutes or colloidal particles that become lodged in pores. After the initial rapid decrease in permeation rate, the gradual accumulation of a gel-like layer or cake causes increasing resistance to hydraulic flow and aggravates microsolute concentration polarization. Therefore the decrease in permeation rate caused by fouling is attributable to both an increase in resistance and a decrease in driving force.

Although fouling occurred with both deproteinized whey and whey, differences between Figures 3 and 4 indicate that whey protein is the principal fouling material in whey. The differences also show that the permeation rate is an important factor in determining the rate of fouling. Fouling took place much more rapidly at the higher permeation rates, whether they were achieved by use of high operating pressure or by using the more open membrane. During the runs with whey using membrane B (Fig. 4), the highest operating pressure caused such rapid fouling that the permeation rate was lower than at the intermediate pressure.

With whey as feed (Fig. 4), the permeation rates decreased to similar values for the two membranes at the three operating pressures. At the end of these runs, permeation rates were being determined more by the extent of fouling and concentration polarization than by the characteristics of the membranes.

The effects of feed composition on permeation rate at constant available driving force (membrane A, 22.8 atm, and membrane B, 27.5 atm) are indicated in Figure 5. When the data are considered progressively from the simple to the

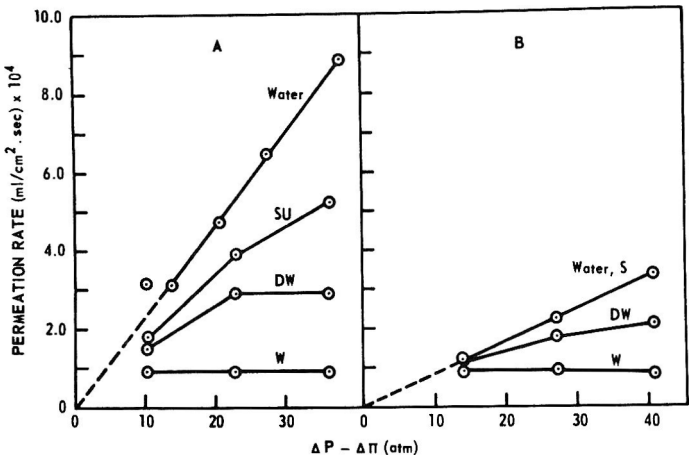

Fig. 6–Relation between available driving force, ΔP–Δπ, and permeation rate (at end of runs) for membranes A and B with selected feed solutions. (Abbreviations as for Fig. 5.)

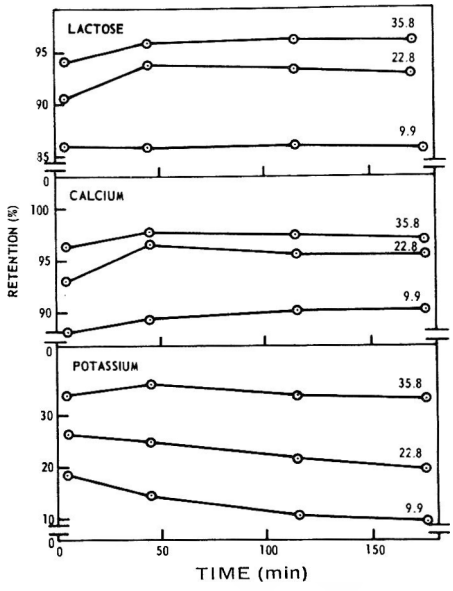

Fig. 7–Changes in retention of potassium, calcium and lactose during runs (membrane A, with whey as feed, at ΔP–Δπ given on curves).

complex feed solutions, there is a reduction in permeation rate, a greater decrease in permeation during the runs, and smaller differences between the two membranes in the rates obtained.

The results obtained with the whey protein solution are particularly helpful in clarifying the influence of fouling on permeation rate. The osmotic pressure of the solution was negligible. Even the osmotic pressure of a thin layer of protein gel that may have deposited on the membrane would be very small compared with the applied pressure difference. Therefore, the decrease in permeation rate must have been caused by an increase in resistance to hydraulic flow or diffusion of water. The resistances to flux of water attributable to the membrane and to the fouling material may be expressed numerically as reciprocals of flux (Markley et al., 1967):

$$1/J_1 = 1/J_1^\circ + 1/J_1^1$$

where $1/J_1$ is the total resistance, $1/J_1^\circ$ is the resistance due to the membrane (i.e., J_1° is the water permeation rate, g/cm² · sec), and $1/J_1^1$ is the resistance due to fouling. For the results in Figure 5, the resistances due to the membrane and to fouling by the whey protein are 0.20 and 0.24 cm² · sec/g, respectively. Therefore, fouling by the whey protein caused resistance to permeation similar to that attributable to the membrane itself.

The decrease in permeation rate obtained with simulated ultrafiltrate, as compared with that with water, is attributable mainly to concentration polarization without fouling, whereas the decrease with whey protein solution is attributable to fouling without concentration polarization of microsolutes. If the effects are assumed to be additive, a permeation rate curve for whey calculated by adding the decreases attributable to concentration polarization in simulated ultrafiltrate and to fouling from whey protein, would decrease more rapidly at the beginning than does the experimental curve for whey, and would level off at a higher permeation rate. Interactions of concentration polarization and fouling, however, are responsible for the differences between the calculated and experimental curves. With the whey protein solution as feed, the absence of microsolute concentration polarization would result in higher initial permeation rates than that with whey, and therefore in more rapid pore plugging. After the initial pore plugging, the mixture of solutes in whey contributes to continuous fouling, as compared with establishment of steady-state conditions with simulated ultrafiltrate and the whey protein solution. As noted by Michaels (1968a), anomalously low permeation rates may be encountered with feeds containing a broad spectrum of solute sizes.

In runs with simple solutions (water and salts) using conditions that caused little or no concentration polarization, a plot of available driving force against permeation rates gave a straight line that passed through the origin (Fig. 6). The curvature of the line obtained with simulated ultrafiltrate indicates that some

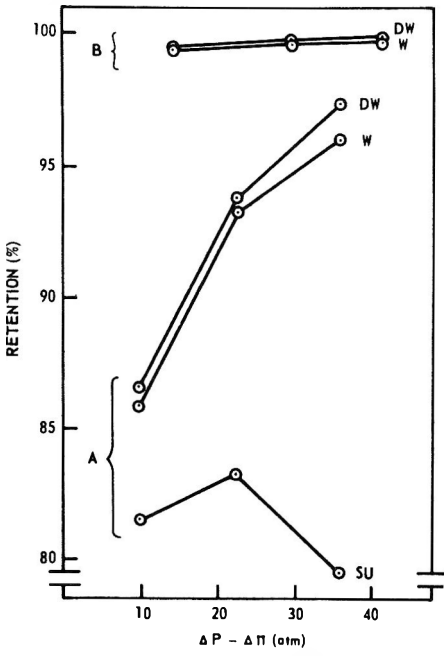

Fig. 8–Relation between available driving force and retention of lactose for membranes A and B with selected feeds. (Abbreviations as for Fig. 5.)

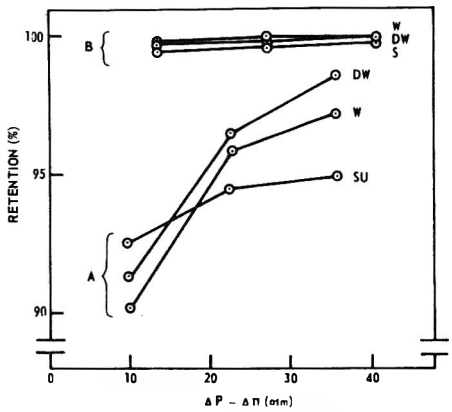

Fig. 9–Relation between available driving force and retention of calcium for membranes A and B with selected feeds. (Abbreviations as for Fig. 5.)

concentration polarization occurred at the higher available driving forces. The fouling that occurred with whey and deproteinized whey resulted in a marked reduction in permeation rate at the higher available driving forces. The protein in whey had such a marked effect in decreasing permeation that the maximum permeation rate was reached at low available driving force.

During the experiments the runs were continued until the permeation rate was essentially constant (usually 1 hr) or until it decreased at a relatively constant rate (usually 3 hr). At the end of a run the membranes were washed with water. This treatment restored the permeation rate to a value characteristic of the membrane. Water permeation rate was determined periodically throughout the study. The results remained approximately constant at rates shown in Fig. 6. The direct relationship between permeation rate and available driving force for water and the salts solution is evidence that the performance of the membrane was not changed by compaction within the range of operating pressures used in these experiments.

Retention

In selected experiments, comparisons were made of retentions and solute fluxes for nonprotein nitrogen, riboflavin, lactose, chloride, calcium, sodium, and potassium, and phosphoric, citric, and lactic acids. Table 2 contains examples of retentions of these solutes. To reduce the number of analyses, however, three solutes (lactose, calcium, and potassium) were selected for detailed study. Potassium was selected to represent monovalent ions, and calcium and lactose were selected to represent solutes with lower permeabilities through reverse osmosis membranes. Except for the data reported in Figure 7, the analyses were made on samples collected near the ends of runs when permeation characteristics were relatively stable.

Table 2—Rejection of selected whey constituents during reverse osmosis of whey and deproteinized whey.

	Retention, %			
	Whey		Deproteinized whey	
Constituent	Membrane A[a]	Membrane B[a]	Membrane A[a]	Membrane B[a]
Sodium	17.2	69.8		
Potassium	21.2	63.8	29.0	72.3
Chloride	70.2	75.0		
Calcium	95.7	99.9	96.6	99.9
Phosphorus	72.0	98.6		
Citric acid	93.1	99.3		
Lactic acid	27.9	46.2		
Lactose	93.5	99.6	93.7	99.7
Riboflavin	67.2	98.6	59.7	97.2
Nonprotein nitrogen			57.6	69.3

[a]Available driving force 22.8 and 27.5 atm for membranes A and B, respectively.

Changes in retention during runs with whey are indicated in Figure 7. For lactose and calcium, at the higher available driving forces, retention increased at the start of the run and then remained constant. Except for an initial increase at the highest available driving force, retention of potassium decreased during the runs, particularly at the lower available driving forces.

We attribute increases in retention at the higher available driving forces at the start of the runs to pore plugging. Pore plugging decreases leakage of solutes with water that flows through pores. This increases the proportion of the permeation that transfers by the diffusive mechanism, with a corresponding increase in retention.

The influence of available driving force on retention of lactose is indicated in Figure 8. With whey and deproteinized whey, increasing the available driving force had a marked effect on retention by membrane A but a much smaller effect on retention by membrane B, which had a higher retention of lactose initially. With membrane A, retention of lactose from simulated ultrafiltrate was much lower than that from whey or deproteinized whey, and did not increase with available driving force. These differences in retention in relation to the feed indicate that fouling by macrosolutes present in the whey and deproteinized whey increased the retention for lactose. The similarity of the results with whey and deproteinized whey indicates that macrosolutes other than protein had an important influence on retention.

The data for retention of calcium (Fig. 9) are similar to those for lactose. Retention increased with available driving force, particularly with membrane A, and the presence of macrosolutes increased retention.

As expected, retention for potassium (Fig. 10) was much lower than for lactose and calcium. With either membrane, retention increased with increase in available driving force, particularly with feeds that contained macrosolutes. A notable difference, however, was that with membrane B, retention of potassium was higher with the salts solution as feed than with whey or deproteinized whey.

Fouling causes a decrease in permeation rate, and usually a decrease in retention. Accumulation of a gel-like layer on the surface of the membrane increases resistance to flow, which decreases permeation rate. In most circumstances the presence of a gel-like layer would be expected to aggravate microsolute concentration polarization by impeding diffusion and convection of solutes from the membrane. However, the reduction in permeation rate attributable to the increased resistance to flow might counter-balance the effects of impeded diffusion and convection. If microsolute concentration polarization increases, it would decrease retention.

Several explanations may be postulated for the increase in retention of lactose and calcium in the presence of the macrosolutes in whey and deproteinized whey. As noted above, pore plugging increases the proportion of the permeation that occurs by a diffusive mechanism, which increases retention. A fouling layer of gel or cake could be selective with respect to passage of different solutes through it. The selectivity could result from steric or charge effects, or interactions of solutes with components of the fouling layer. Another possible influence is internal fouling of the microporous structure. As noted by Michaels (1968b), feeds that contain a wide range

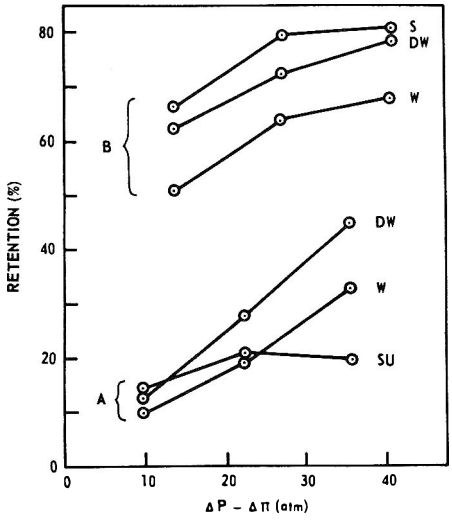

Fig. 10—Relation between available driving force and retention of potassium for membranes A and B with selected feeds. (Abbreviations as for Fig. 5.)

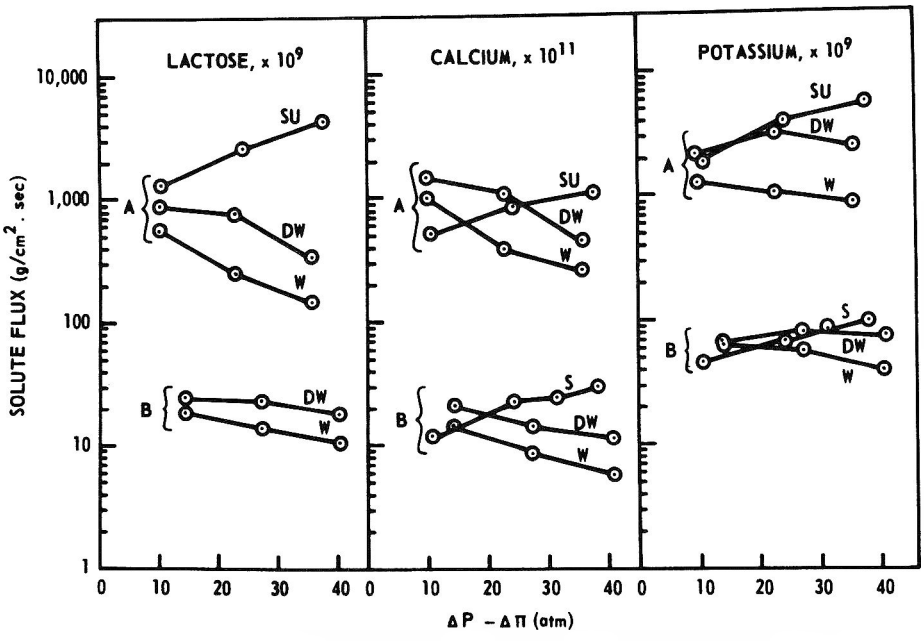

Fig. 11—Relation between available driving force and solute flux of lactose, calcium and potassium for membranes A and B with selected feeds. (Abbreviations as for Fig. 5. Note that for the solute flux the scale is logarithmic, and the units for lactose and potassium are × 10^9, and for calcium are × 10^{11}.)

of solute sizes cause serious fouling of the microporous structure. This reduces solvent permeability but may increase solute retentivity.

Solute flux

The solute flux data for lactose, calcium, and potassium are plotted in Figure 11. The influence of available driving force on solute flux resulted from the combined effects on permeation rate (Fig. 6) and retention (Fig. 8–10). For lactose and calcium, flux from whey and deproteinized whey decreased with increase in available driving force, but flux from simulated ultrafiltrate and salts solution increased. Increasing the available driving force had more influence on flux of lactose and calcium than on that of potassium.

The solute flux data emphasize the influence of composition of the feed on results obtained with processing by reverse osmosis. Fouling of the membrane by macromolecules in whey and deproteinized whey decreased permeation rates but increased retention of lactose and calcium. The greater effect on retention at the higher available driving force resulted in the decrease in solute flux of lactose and calcium with increasing available driving force. Thus, in processing whey, an advantage in using a higher operating pressure than the lowest that gives the maximum permeation rate, would be a reduction in loss of lactose in the permeate.

An objective of processing whey by reverse osmosis is to concentrate lactose and proteins while eliminating the monovalent salts and lactic acid. When concentration polarization or fouling took place, the two membranes tested gave similar permeation rates. The more open membrane, because of its low retention of salts, yielded a product with a lower concentration of salts in the solids. However, a disadvantage of the more open membrane is a greater loss of lactose in the permeate. The choice between the two membrane types requires a compromise between extent of desalting and tolerance of loss of lactose with the permeate.

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E. LOWE and E. L. DURKEE
USDA Western Regional Research Laboratory ARS, Albany, California 94710

DYNAMIC TURBULENCE PROMOTION IN REVERSE OSMOSIS PROCESSING OF LIQUID FOODS

SUMMARY—The phenomenon of concentration polarization operates to reduce the efficiency of solvent-solute separation in the processing of foods by reverse osmosis. The viscous nature of most food concentrates aggravates the problem. Disrupting the boundary layer by mechanically inducing turbulence in the fluid stream improves the permeation efficiency. Flux improvement of up to 3-fold is possible with dynamic turbulence promotion involving plastic spheres moving randomly with the orange juice concentrate. Concentrate (and sphere) movement is pulsed alternately from one end of the membrane flow channel to the other. The spheres are confined within the flow channel to avoid the problem of moving them through the recycling pumps. A net movement of concentrate results when the pump volume is greater in one direction than the other. The permeation rate with dynamic turbulence promotion does not drop off significantly with time. The long-term effects of sphere movement on the membrane surface are not known but are believed not to be serious.

INTRODUCTION

EGG WHITE, maple sap, fruit juices and cheese whey are liquid foods or food by-products that have been concentrated and processed by reverse osmosis in the past few years. Most of the work has been done on an experimental basis, but at least 1 commercial installation is reported to be in operation at the present time.

The selective permeability of reverse osmosis membranes to solutes provides a simple and effective method of fractionating liquid foods and food by-products into components that are each more useful and valuable than the composite. A case in point is cheese whey which, because of its high biochemical oxygen demand, usually represents a serious disposal problem. By using membranes permeable to water and small solute molecules, whey can be processed into 2 useful fractions, both with reduced lactic acid and salt concentrations. The protein-rich fraction is useful as a whipping aid or as a nutritious food supplement; the lactose fraction, as a flavor carrier or as a binder in pharmaceutical products.

Processing of foods by reverse osmosis is not without problems. From a practical or commercial standpoint, the most important problem is the rate at which water and selected solutes can be removed from the feed material. The parameters controlling the transport of water and of solute through the membrane itself are well understood, Merten (1966). The phenomenon of concentration polarization, which operates to reduce the efficiency of solvent-solute separation, has been analyzed by Brian (1965), Dresner (1964) and Sherwood et al. (1965). Blatt et al. (1969) and Merson and Ginnette (1970) have extended the analyses to the separation of moderate-to-high molecular weight solutes and colloids.

Blatt et al. (1969) concluded that in a laminar flow regime, concentration polarization can be decreased (and the efficiency of solute separation increased) by 1) decreasing the channel height, 2) increasing the inlet velocity and 3) decreasing the channel length. For macrosolute solutions and colloidal dispersions, the permeation rate is also affected by the anomalous rheological properties of the layer of concentrated material deposited on the upstream surface (concentrate side) of the membrane. For many liquid food products, viscosity is roughly an exponential function of the solids-to-water ratio. Because of its high viscosity, the concentrated layer next to the membrane surfaces moves very sluggishly, or does not move at all. In effect, the adherent layer becomes an in situ membrane through which solutes and solvent must pass to reach the working membrane.

Merson et al. (1970) investigated the possibility of destroying the boundary layer by introducing periodic mixing events. Under conditions of the experiment, the improvement in both predicted and observed permeation rate was small.

EXPERIMENTAL

CONCENTRATED orange juice exhibits rheological properties typical of many liquid food products containing macromolecules, in this case pectin. As might be expected, the permeation rate for such a solution is much lower than the intrinsic pure water rate for the membrane. The current studies were undertaken to study the effect of dynamic turbulence promotion on the efficiency of solute-solvent separation of the concentrated juice.

Simple logic points to turbulence as a potentially effective way of improving the permeation efficiency. To be effective, the turbulence must be great enough to shear the boundary layer at the membrane interface. The shear rate required to produce stresses in excess of the yield point of the boundary layer material is a function of the rheological properties of the concentrated solute. For very viscous materials, the rate of shear that must be developed for effective boundary layer control is very high. Simple adjustments of fluid dynamic parameters, such as increasing the fluid flow velocity, are unlikely to create enough turbulence to have a marked influence on permeation efficiency.

Theoretically, there is no upper limit to the shear rates that can be generated by mechanically induced turbulence. In reverse osmosis, the difficulty with dynamic turbulence promotion is one of mechanics—how to introduce the work energy into a narrow flow channel under hyperatmospheric pressures without damaging the semipermeable membrane surface. A promising approach involves the use of spheres moving randomly with and within the bulk liquid. The scouring action of the moving

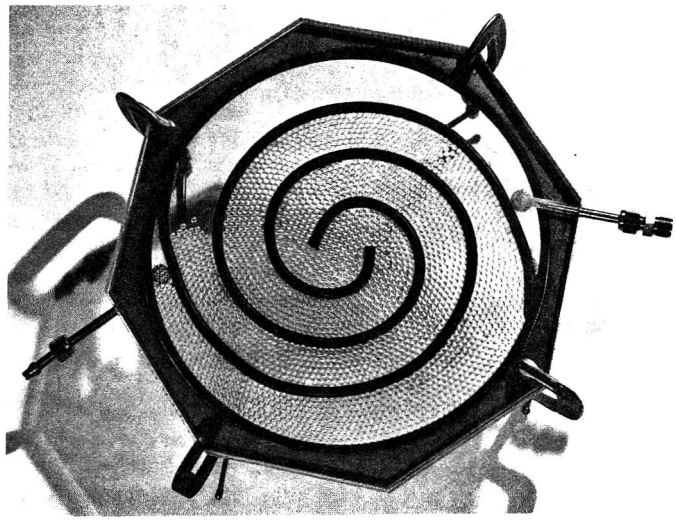

Fig. 1—Spheres in flow channel of transparent plastic mock-up (10.75-in.-diameter membrane area).

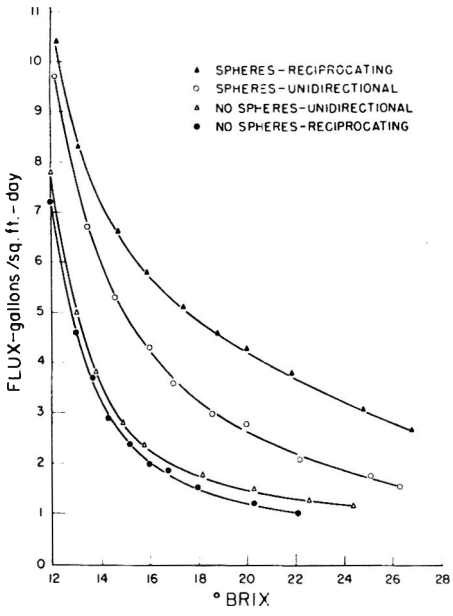

Fig. 2—Permeation rate—orange juice concentrate—20 pulse cycles/min.

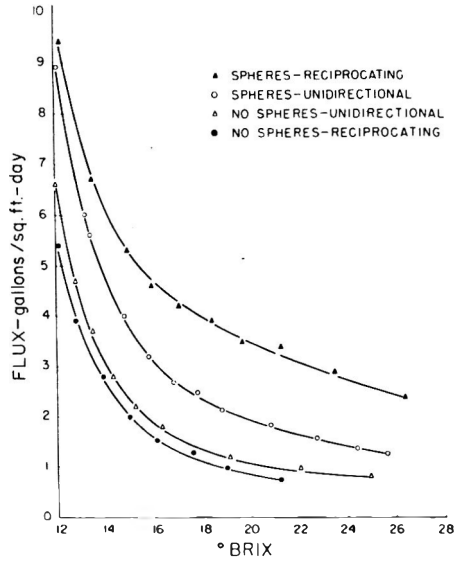

Fig. 3—Permeation rate—orange juice concentrate—10 pulse cycles/min.

spheres would repeatedly disrupt the layer of material deposited on the membrane surface. Additionally, the sphere movements produce an effect equivalent to a continuous series of mixing events.

The spheres used in this study are of acrylic resin, with a true density of about 1.2 g/cc. Diameter was $5/32 \pm 0.005$ in. The spheres were circulated with orange juice concentrate in a channel 0.174 in. deep by about 1.25 in. wide. Channel configuration is in the form of a spiral, with a mean effective length of approximately 54 in. (see Fig. 1). The plate-and-frame reverse osmosis apparatus used for these experiments has been described elsewhere (Lowe et al., 1968; 1969).

Ideally, the spheres would enter and leave the flow channel along with the concentrate. As a practical matter, however, the spheres were confined within the flow channel to avoid the problem of moving the spheres through the recycling pumps.

The ratio of bulk sphere volume to total channel volume was about 0.8. The spheres were kept in motion by pulsing the concentrate flow, alternately from one end of the channel to the other. A net movement of concentrate results when the pump volume is greater in one direction than the other.

Experiments were conducted at 2 different pulse rates, 10 cycles per min and 20 cycles per min, corresponding to a fluid velocity of about 0.3 and 0.6 fps for the smaller pump volume and 0.45 and 0.9 fps for the larger pump volume.

Experiments were also conducted with the flow unidirectional but pulsing and with unequal pump discharge. All experiments were duplicated with and without spheres in the flow channel.

The membranes used in the experiments were Eastman Chemical Products, Inc. HT-00 heat treated for 10 min at 85°C to achieve a pure water rate of approximately 18 gal/sq ft-day at 1,200 psig.

The feed material was a commercial frozen orange concentrate diluted back to 12% solids, the approximate solute level for a fresh juice. The diluted feed was strained through 2 layers of cheesecloth to remove coarse fibrous fragments and then pooled.

All experiments were conducted with an upstream hydraulic pressure of 1,200 psig and a downstream pressure of 0 psig.

Permeate flow rate and concentrate solids readings (by refractometer) were recorded periodically during the course of a run, beginning with the most dilute condition, 12% solids. Figure 2 shows the initial permeation rate at various solids levels for several conditions of operation at 20 pulse cycles per minute. Figure 3 shows comparable data for a pulse rate of 10 cycles per minute.

RESULTS

IT IS clear from the data that there is a beneficial effect when the flow channel is filled with spheres, and an even greater improvement when the spheres are kept in motion. At 24% solids, for example, the permeation rate with unidirectional flow and 20 pulse cycles per minute was 58% greater with spheres than without. When the spheres were made to flow back and forth across the membrane surface, the flux was improved by another 110%.

Flux improvement is even greater at lower flow velocities. At 10 pulse cycles per minute, stationary spheres improved the flux by 60%, moving spheres by an additional 150%, for an over-all flux improvement of approximately 3-fold.

The relationship between permeation rate and fluid velocity

$$J = f(U^1/3)$$

where J = water flux through the membrane and U = average fluid velocity in the channel, is largely confirmed by the data for unidirectional flow without spheres. On this basis, the fluid velocity without spheres would have to be 20 to 30 times greater than the velocity with moving spheres for the same flux.

The permeation rate with dynamic turbulence promotion does not drop off significantly with time. After a full week of continuous operation at 24% solids, the flux dropped from an initial value of 3.5 gal/sq ft-day to a final value of 3.3 gal/sq ft-day. The 7-day test period is believed to be adequate for food applications because of the unavoidable need for periodical cleanup of the equipment. At the end of the test period, the membrane surface was completely free of any accumulation of solids, a situation that did not exist under any other operating condition. Without spheres, the permeation rate dropped from 1.5 gal/sq ft-day to 0.23 gal/sq ft-day.

The experimental data indicate a lower flux for reciprocating flow without spheres than for unidirectional flow without spheres. This implies less effective mixing of the fluid in the flow channel with reciprocating flow than with unidirectional flow.

The long-term effects of sphere movement on the membrane surface are not fully known. The spheres have a density very close to that of the bulk liquid and a surface that is extremely smooth and free of imperfections such as flashings. These are factors that would lessen any tendency to damage the membrane surface. After a week of continuous operation, there was no detectable change in either the selectivity (as measured by the refractive index of the permeate) or the permeation rate coefficient for the test membrane.

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

JOHN D. HATCHER[1], DONALD W. LYONS[2] and J. EDWARD SUNDERLAND[1]
North Carolina State University and Clemson University

AN EXPERIMENTAL STUDY OF MOISTURE AND TEMPERATURE DISTRIBUTIONS DURING FREEZE-DRYING

SUMMARY—Measurements of the moisture and temperature distributions were made during freeze-drying beef at a pressure of 1 torr. The transient moisture distributions measured with gamma ray techniques indicate that the phase change region would have a thickness less than 3/16 in., that no drying takes place until the phase change region reaches a given position, and that no additional drying occurs after the phase change passes a given position. A careful study of the data showed that the drying rate was influenced by heat transfer through the frozen region to the phase change position.

INTRODUCTION

THERE HAVE BEEN a number of papers written concerned with predicting the rate of freeze-drying. Excellent reviews of these papers have been presented by Harper and Tappel (1957) and Burke and Decareau (1964). Due to the extremely complicated nature of the problem, a number of simplifying assumptions must be made before an analytical solution can be obtained. Dyer and Sunderland (1968) studied the influence on drying time caused by convective heat transfer between the vapor and the dried region. More recently, Dyer and Sunderland (1969) studied the magnitude of errors caused by assuming that the interface temperature remained constant during the drying process. One assumption made in nearly all analytical studies is that the phase change takes place at one temperature and that the region between the frozen and dried zones of the product is infinitesimal. This assumption neglects any secondary drying that may occur in the dried region and any drying that might occur in the frozen region before the arrival of the phase front.

Fusi (1965) studied the moisture distribution in fish during freeze-drying. He periodically stopped the process and cut the fish samples into sections to determine the moisture content. The data obtained by this technique is dependent on the thickness of the size of the sections cut from the samples and inherent difficulties seem to limit the extent of the conclusions that can be made.

Bralsford (1967) obtained good agreement between experimental results and an analytical study which assumes the existence of a plane phase front with zero thickness. Although the correlation between analytical and experimental results is good, this study, by itself, does not provide the basis for complete confidence in the plane phase front assumption.

Clark and King (1968) used a model similar to Bralsford and assumed a plane receding ice front. In order to substantiate this assumption they partially freeze-dried samples of turkey and then completed the drying process at atmospheric pressure and high temperatures. The dark discoloration of wet meat when dried at high temperatures was used to determine which section of the meat contained moisture. The demarcation between the light and dark section of the meat was relatively sharp and thus was assumed to justify the existence of a plane receding ice front. The time required for high temperature drying of the meat was long enough to allow the ice to melt and migrate to dry sections. Also there is no indication that partially dried meat would not discolor to the same extent as undried meat. Although the method seems to justify the plane phase interface assumption, the results are not conclusive.

Sandall et al. (1967) used a model which assumed a plane receding ice front in conjunction with experimental data to calculate thermal conductivity of the dried region of freeze-dried turkey. The conductivity was also determined independently of the freeze-drying model with a guarded hot plate technique. The resulting conductivities obtained with the two methods are compared with a maximum deviation of 10.4% between the two values. From this observation they indicate that there is a strong evidence of the existence of a plane receding ice front.

It is the purpose of the current paper to determine experimentally the validity of the assumption of a plane phase front with zero thickness and to present data for the transient temperature distribution during freeze-drying. This information would be useful in obtaining a better understanding of the physical nature of freeze-drying.

EXPERIMENTAL

Local moisture measurements

It was important to determine a technique for measuring the moisture distribution during actual drying conditions. The technique to be used must be sensitive to chemically bound water as well as water in either the solid or liquid state. The measurement technique must not influence the drying process and in order to determine the local moisture content, the area over which the measurements are taken must be comparatively small.

Several methods were considered for the moisture measurements. Hardracker and Rawcliffe (1952) presented a method which measures the electrical resistance between two probes. Since the resistance depends on moisture content, the system can be calibrated to give moisture measurements. A capacitance method was developed by Vitins (1960). A probe coated with a conducting hydroscopic salt was developed by McLeod and Yallup (1961). X-ray techniques could be used, but changes in the sample thickness would alter the results measured. Higher energy rays such as neutrons, alphas, betas, or gamma rays offer distinct advantages over x-rays. Gamma rays offer certain inherent advantages for the type of measurements involved with freeze-drying, therefore the experimental studies used gamma ray techniques for measuring the moisture distribution. Gamma rays are particularly well suited for measuring the moisture content during freeze drying because the measurement does not affect the normal drying process; there is no major restriction on the sample thickness; it is equally sensitive to chemically bound and unbound water; the gamma ray beam can be collimated into a small area so that the moisture measurement approximates a point determination; and the gamma ray source is inexpensive.

The moisture distribution is measured by passing a beam of photons (gamma rays) through the sample during the actual drying process. The attenuation of the beam is measured and related to moisture. Photon absorption in the energy range used in the current investigation is exponential, and the equation of absorption can be expressed by

$$I_X = I_O e^{-u_o x}$$

where I_x is the intensity at position x, I_o is the initial photon beam intensity before absorption occurs and u_o is the total attenuation coefficient. If moisture is removed from the region of the sample exposed to the gamma rays, u_o can be related directly to this moisture change. Therefore, the calibration of the photon beam to determine moisture directly during the drying process is obtained from

$$u = \frac{1}{k} \ln \frac{I_d}{I_w}$$

where u is the moisture content, k is a constant that is determined experimentally, I_w is the intensity of the photon beam after passing through the wet sample, and I_d is the intensity

[1]Department of Mechanical and Aerospace Engineering, North Carolina State University, Raleigh, North Carolina 27607.

[2]School of Industrial Management and Textile Science, Clemson University, Clemson, South Carolina 29631.

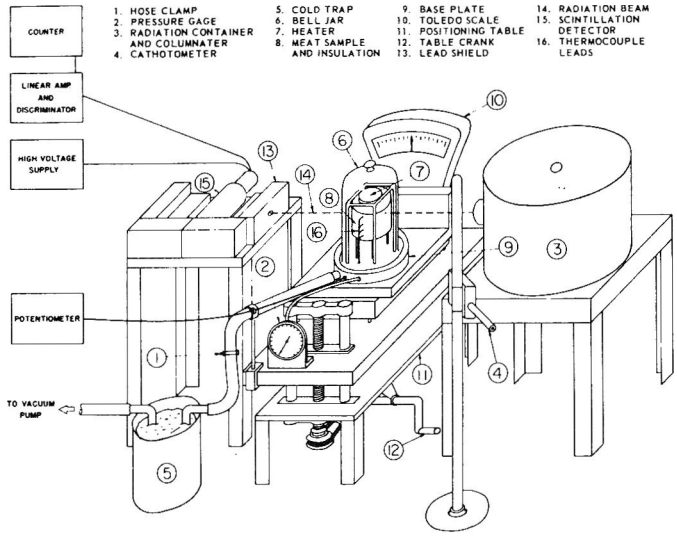

Fig. 1—Experimental set-up.

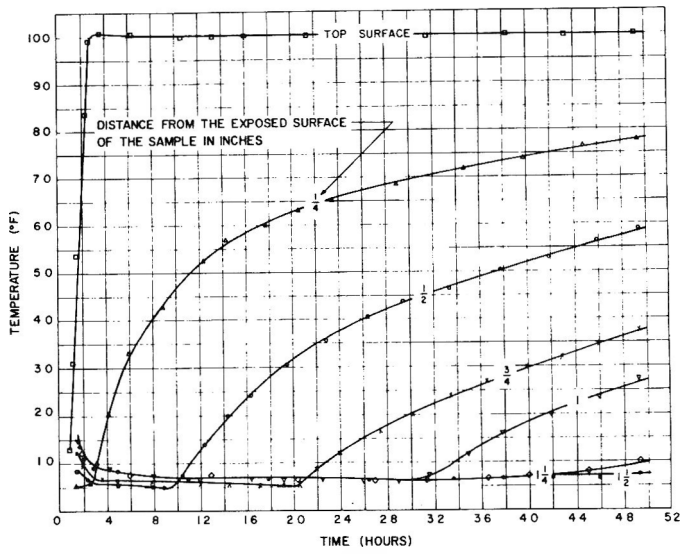

Fig. 2—Temperature as a function of time at various distances from the exposed sample surface with a chamber pressure of 1 torr.

after passing through the sample during the drying process.

The experimental equipment is shown in Figure 1. The photon beam was generated by one Currie of radioisotope Co^{60} which was placed in the lead radiation container labelled (3) in Figure 1. A 1/16-in. dia hole, 8.5 in. long was drilled through the lead to collimate the radiation emitted by the cobalt.

The lead container was placed as near to the meat sample as possible so that the radiation beam would be very small when it passes through the sample. The length of the hole through the lead container also influenced the degree of collimation obtained since radiation is emitted in a diffused manner from the cobalt. The area of the beam at the meat sample was calculated and also determined experimentally (with photographic paper). Both methods showed that the beam was no greater than 3/16 in. dia area of the meat sample.

The intensity of the radiation beam after passing through the meat sample was determined by use of a scintillation counting technique. This technique consisted of placing a crystal in the radiation beam and using a photomultiplier tube to measure the amount of light that was produced by the ionizing radiation as it was absorbed by the crystal. The pulses from the photomultiplier tube were then counted and the number of counts was proportional to the intensity of the gamma ray beam. The scintillation detector used in this work was a Harshaw Nal (TI) Scintillation Detector, Type 12512, with a 3 in. dia crystal 3 in. long.

A Franklin Electronics Linear Amplifier and Discriminator was used to eliminate background radiation effects; the output of the discriminator was counted by a Systron Counter (Model 1032).

Temperature and pressure measurements

The temperature measurements were taken at different positions in the meat sample during the drying process. These temperatures were measured with copper-constantan thermocouples which were made from 30 gage copper-constantan wire and calibrated with standard mercury-in-glass thermometers. The thermocouples were inserted into small holes that were drilled in frozen meat samples. It was observed that drying did not occur prematurely around the thermocouples. The thermocouple emf was measured with a potentiometer.

The measurement of absolute pressure in the vacuum chamber was accomplished by a Wallace-Tiernan absolute pressure gage, calibrated from 0.2 to 20.0 torr.

Freeze-drying chamber

The freeze-drier consisted of a bell jar, an acetone-dry ice cold trap, and a mechanical vacuum pump as shown in Figure 1. The bell jar was placed on a counter-balanced Toledo scale which could measure the weight within 0.01 lb. The scale was located on a positioning table that could be raised and lowered by a pully and crank that operated two screw jacks. This arrangement permitted the sample to be moved into the gamma ray beam.

A cathotometer was used to determine the position where the photon beam passed through the meat sample. This was accomplished by alternately sighting the top of the meat sample and the hole in the lead shield from which the photon beam originated.

The heater used in the vacuum chamber was the same diamater (three inches) as the meat sample in order to minimize the addition of heat to the side of the sample. The heater was controlled by a powerstat. The top surface tem-

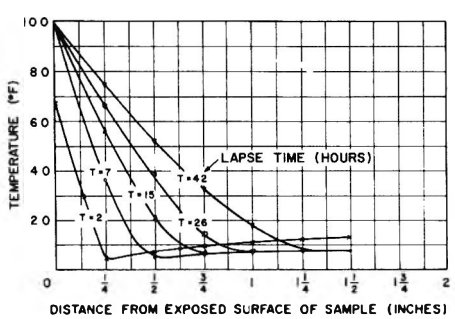

Fig. 3—Temperature as a function of position at various times with a chamber pressure of 1 torr.

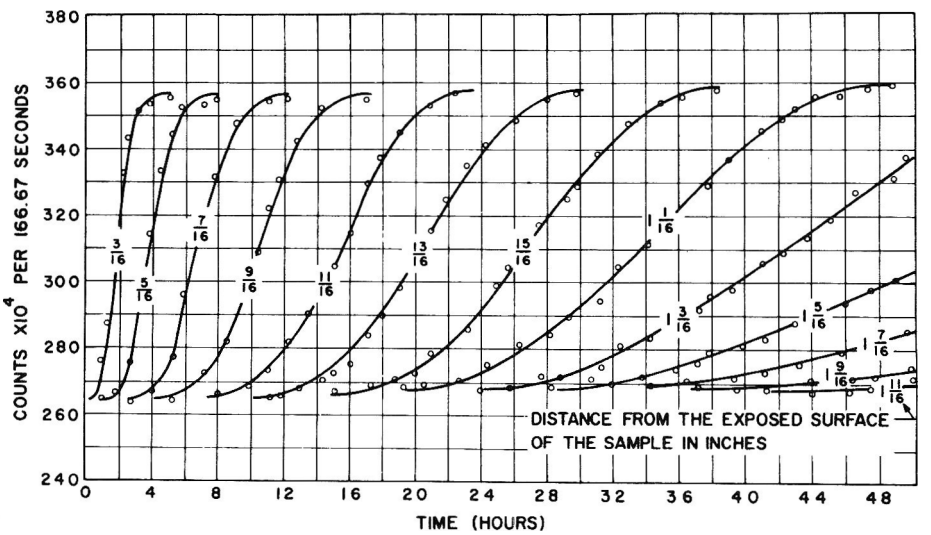

Fig. 4—Count rate as a function of time at various positions and at a chamber pressure of 1 torr.

perature was brought up to 100°F as quickly as possible and maintained at that temperature throughout the test.

Sample preparation

The meat sample used in this work was obtained from a "top of the round" cut of utility beef. The samples were carefully cut with a band saw from frozen meat to insure a uniform grain direction from top to bottom. Using this cut of beef it was possible to obtain a sample 2-in. thick and 3 in. dia that contained very little fatty inclusions and also an essentially straight grain direction.

It was found that by wrapping the sample in plastic wrap and tape and using fiber glass insulation there was no side or back face drying of the sample and the drying process was one-dimensional.

After the sample was wrapped, the thermocouple holes in the sample were drilled and the thermocouples inserted. The holes drilled in the sample were no larger than required to accept the thermocouple (size 60 drill). It was found that the thermocouples had no effect on the drying of the sample when installed in this manner. Thermocouples were placed in the center of the sample and equally spaced from the exposed surface. Tape was placed over the thermocouple holes to prevent any vapor from escaping through the holes in the plastic. The sample was surrounded (sides and back face) by 2 in. of fiber glass insulation with aluminum foil on the outside of the insulation to reduce radiant heat transfer to the outside of the insulation from the surroundings. After the sample was placed in the vacuum chamber, the heater was located one quarter of an inch from the exposed surface of the sample.

The above procedure was followed to measure the transient phase front position and temperature distribution of the sample at a chamber pressure of one torr. The tests were completed when the residual beam intensity indicated that the sample was completely dry at 1 inch from the top surface.

RESULTS & DISCUSSION

MEASUREMENTS were obtained for the transient temperature and moisture distributions and the weight of the sample as a function of time. Although some of the experiments lasted as long as 66 hr, the data for moisture distribution and weight loss are significantly influenced by side and back drying after about 20 hr. Conclusions that would be influenced by side drying should be made from data taken at early times.

Temperature distribution

The transient temperature distributions are presented in Figure 2 for a pressure of 1 torr. Temperature distributions for 2 and 3 torr are presented by Hatcher (1964). The temperature at any given position appears to reach a minimum value just before it begins to rise. This is probably caused by heat transfer to the phase front that occurs through the frozen region as well as the dry region. Although a serious effort was made to obviate heat transfer through the frozen region, we were not entirely successful. This can be observed somewhat easier in Figure 3 which presents the temperature distributions at various times. It can be seen that the initial temperature distribution still influenced the temperatures measured in the frozen region at 2 hr. After 7 hr the temperature distribution in the frozen region appears to have reached a quasi-steady state. The rate of heat transfer to the interface from the frozen region may be more important than one would expect by observing the data presented in Figure 3 because the thermal conductivity of the frozen region is between 15 and 20 times as large as the conductivity in the dried region.

At 1 torr (Fig. 3), the temperature of the interface is about 5°F at 2 hr. By comparing this to the data of Dyer et al. (1966), it can be seen that the pressure drop in the dried region is very small. As time becomes larger, the temperature of the interface increases slightly which indicates that a larger pressure difference occurs between the interface and the free surface. By comparing this temperature to the data of Dyer et al. (1966), it can be seen that the pressure difference is negligible.

Moisture distribution

The experimental data for the transient moisture distribution for a chamber pressure of 1 torr is presented in Figure 4. The vertical scale of this graph is the residual count rate of the photons after passing through the sample. The relative change in moisture of the sample can be obtained from the count rate. Measurements that are placed on these figures represent the count rate during the drying process at a given location. The data obtained before drying started or after it was completed at any given position are not presented on these graphs.

The diameter of the beam was 3/16 in.; therefore, if the phase change region is a plane (rather than a volume region), even if this plane is aligned parallel to the beam, it is possible for part of the beam to be in the completely frozen region while another part passes through a completely dried region of the product. Due to the nonuniformities in the meat, and the difficulty of making sure that the beam is parallel to the plane of the phase front (if such a plane exists), it is difficult to make objective decisions about the nature of the phase change region. By observing the data in Figure 4 at 4 hr it can be seen that the measurements at 3/16 in. show that essentially complete drying has occurred. Drying has just started at 7/16 in. Therefore the phase change region would be less than about 1/4 in. at this time of the drying process. Since the beam diameter is 3/16 in., it is concluded that the phase change region could be as small or even smaller than the beam diameter. Although this observation is not verified by considering the other curves in this figure, this particular curve was chosen for several reasons. First, it is important to consider the data in the early stages in the drying process before appreciable drying from the sides of the sample takes place. Second, experimental inaccuracies caused by nonuniform heating of the sample surface, by not having the grain direction of the sample perpendicular to the free surface, by not having the free surface of the sample perfectly flat, or by not having the surface of the sample in a plane parallel to the axis of the beam, all cause the measured drying regions shown in the graphs to be larger than would actually take place. Thus all of the expected experimental errors would show up by increasing the size of the drying region. For these reasons, we consider the data that give the minimum drying region size to be the most accurate.

There are two other important observations that can be seen from Figure 4: no drying occurs at a given position until the phase change region comes close to that position; and furthermore, after the phase change region moves beyond a given position, no additional drying occurs.

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THOMAS S. STEPHENS, GUADALUPE SALDANA, HAROLD E. BROWN and FRANCIS P. GRIFFITHS
USDA Southern Utilization R&D Div., ARS, Food Crops Utilization Research Laboratory, Weslaco, Texas

STABILIZATION OF CARROT JUICE BY DILUTE ACID TREATMENT

SUMMARY—A problem arises if juice extracted from raw carrots is heated to about 180°F before or during conventional canning practices. An unsightly, unappetizing coagulum forms and the color of the juice precipitates with the coagulum. The coagulum is not as evident if the carrots are heated 5 min in water prior to juice extraction, but the juice loses some of its bright-orange color. The canned juice extracted from carrots heated 5 min in a .05 N acetic acid solution does not coagulate and the amount of juice which can be extracted from the carrots is 3.3% greater than that from carrots heated 5 min in water. The centrifuged juice from acid-treated carrots had a brighter orange color than juice from the water-treated carrots. The color notation was Rd, 23.7, a, 30.4 and b, 34.8 for juice from acid-treated carrots compared with a notation of Rd, 19.2, a, 24.4 and b, 31.4 for the juice from water-treated carrots. The canned juice extracted from raw carrots had less pectic substances and starch and about the same amount of protein as the canned juice from the carrots heated in water or .05 N acetic acid, but the canned juice from raw carrots coagulated. The juice from carrots heated in acid does not form a coagulum and maintains its orange color, probably because of the action of the heat and acid on the protoplasmic liquid of the cell before or immediately after the cell is ruptured.

INTRODUCTION

IT IS difficult to extract carrot juice from the vegetable tissue and heat it to a sterilizing temperature without undesirable changes taking place in the juice. The high temperature required for sterilizing raw carrot juice causes a coagulum to form in the juice. A smaller amount of coagulum will form if the raw carrots are heated in water before juice extraction. Tressler and Joslyn (1961) reported that in one operation whole carrots were blanched at 200°F for 5 min, ground and the extracted juice acidified with citric acid. One of the products was adjusted to about pH 4.2 and another with a pH of 5.28 was not adjusted. Both juices had a characteristic sweet, clean, carrot flavor.

Several investigators have recommended other methods of preparing canned carrot juice. Lachele (1938) prepared a carrot juice resumbling orange juice in color and consistency by passing carrots through a Schwarz comminution machine. The machine is capable of extracting 60–80% of the juice from raw carrots. The juice is heated at 180°F to coagulate unstable material, then passed through the comminution machine for homogenization, which prevents coagulation during further heat treatment. Further treatment consists of filling the juice hot into 8-oz cans, exhausting to 160°F and processing for 30 min at 240°F.

Beattie and Pederson (1943) ground carrots and pressed them in a hydraulic press, but the product as well as the yield was unsatisfactory. However, a satisfactory yield was obtained from a R.Y.P. extractor or from a Chisholm-Ryder continuous press. They found that carrots were most difficult to extract with a satisfactory yield of juice, and very fine maceration before pressing was necessary.

Another method of preparing carrot juice is described by Turner (1939). The carrots are blanched 15 min in boiling water, crushed and the juice extracted in a hydraulic press. From the press, the juice flows through 2 screens to a storage tank, then to the filling machines. Filled cans are exhausted to 185°F, sealed, cooked 22 min at 240°F and cooled as quickly as possible to 100°F.

Marsh (1942), Cruess et al. (1937) and Beattie and Pederson (1943) added acid or juice from acid-type vegetables to low-acid vegetable juice after extracting the juices from the vegetable tissue; they reported the acidified juices to be more palatable and easier to process than unaltered juices.

The previous investigators did not mention treating the vegetables with acid prior to grinding and juice extraction. It was the purpose of this investigation to study the effect on the canned juice and presscake of carrots heated in weak acid solutions before the carrots were ground and the juice extracted.

EXPERIMENTAL

IMPERATOR variety carrots in lots of approximately 200 lb each were obtained from the reject belt of a local packing-shed over a 2.5-month period. The lots of carrots were obtained March 1, 8 and 14, April 26 and May 1, 6, 13, 15 and 20. The carrots were washed and all insect-damaged and severely discolored ones removed. Duplicate 25-lb samples of the cleaned carrots were taken from the original 200-lb lot for juice extraction. Juice was extracted from raw carrots, carrots heated in water and those heated in a weak acid solution. The carrots were heated in 30 liters of distilled water or 30 liters of the weak acid solution, thoroughly drained and ground through a Fitzpatrick comminuting machine fitted with a .064-in. screen. The juice was extracted from the weighed ground carrots in a Palmer rack and cloth-type press. The pressure on the cake was increased slowly to 6000 psig and held at this pressure for 15 min. The juice was heated to 180°F in a steam kettle, filled hot into 303 × 406 enameled cans, sealed and processed 30 min at 240°F. The processed cans were cooled rapidly in tap water. Approximately 4-lb samples of the presscake were packaged in polyethylene bags and frozen in still air at −10°F.

25-lb samples of carrots were heated at several time intervals, using various concentrations of hydrochloric, citric and acetic acid. The extracted juice was tasted and a concentration of .05 N acetic acid arbitrarily selected as the heating media for the remainder of the tests. Carrots were heated in water for 0, 5, 15 and 25 min and in .05 N acetic acid for 0, 3, 5 and 15 min to determine heating time prior to juice extraction to obtain a desirable quality juice.

The percentage yield of juice, suspended solids, percent light transmission, °Brix, pH, titratable acidity and color were determined on juice from the 9 lots of carrots. The pectic substances, starch and protein were determined on the juice and presscake of the last 4 lots of carrots.

Table 1—Yield and analysis of canned juice from raw carrots and carrots heated in water and in acetic acid for increasing lengths of time.[a]

Minutes heated	Juice yield (%)	Light trans. (%)	Brix (°)	pH	Titratable acidity (%)	Color		
						Rd	a	b
Juice from carrots heated in water								
0	74.0	95	7.2	6.1	.07	3.3	-3.0	7.5
5	71.3	59	7.2	5.7	.07	18.3	17.8	29.5
15	52.4	67	7.5	5.4	.09	16.2	12.5	28.9
25	48.9	74	7.0	5.3	.09	14.6	10.3	27.8
Juice from carrots heated in 0.5 N acetic acid								
0	74.0	95	7.6	6.0	.09	1.1	-2.1	5.4
3	75.4	32	8.6	5.5	.12	21.1	28.3	33.0
5	71.0	31	8.5	5.3	.13	25.1	33.7	35.2
15	54.7	36	8.2	5.1	.17	25.6	24.0	36.4

[a]Data represent the mean of duplicate determinations for each treatment, except that the 25-min cook in water and the 15-min cook in .05 N acetic acid represent 1 determination.

Suspended solids were determined by centrifuging 50 ml of juice 10 min at 1250 rpm according to the procedure recommended in the United States Standards for Grades of Grapefruit Juice (1968). The liquid was decanted from the top of the centrifuge tube and analyzed. The percent light transmission through the juices was determined by diluting 10 ml of the decanted liquid to 100 ml and reading on a Lumetron Colorimeter fitted with filter No. 580. Total titratable acidity was determined by titrating with standardized sodium hydroxide to an end point of pH 8.2. °Brix was measured on a Zeiss refractometer, pH measured with a Beckman Zeromatic pH meter and color determined with a Gardner Color Difference meter standardized with color plate MY-1: Rd, 27.3, a, −3.3 and b, 34.7.

Each can of juice was thoroughly mixed and a sample removed for determination of pectic substances, starch and protein. The frozen presscake was cut into pieces and samples weighed for pectic substances, starch and protein while the cake remained frozen. The pectic substance of the juice and presscake was extracted by the method of McColloch (1952) and pectic substances determined by the colorimetric method of Dietz and Rouse (1953). Starch was extracted from the samples of carrots according to the procedure of McCready et al. (1950) and percent starch determined by the method of Roberts and Friloux (1965). Protein was determined according to the method in A.O.A.C. Official Methods of Analysis (1965). (Kjeldahl N × 6.25.) Data were subjected to an analysis of variance as described by Steel and Torrie (1960).

RESULTS & DISCUSSION

THE CANNED juice from carrots heated in a weak acetic acid solution prior to juice extraction was compared with the canned juice from carrots heated in boiling water and with canned juice from raw (unheated) carrots.

A .05 N acetic acid solution was decided upon as the heating media because a coagulum did not form and the canned juice retained its typical carrot flavor after processing. Neither did juice prepared from carrots heated in a .05 N hydrochloric acid solution coagulate during canning, but it was felt that use of a food-grade acid would be more acceptable, should the process be commercialized. Juice from carrots heated in a .05 N citric acid coagulated during canning. However, juice from carrots heated in a .1 N citric acid solution did not coagulate and could have been used. Those who tasted the juices considered the juice from carrots heated in the acetic acid solution to have the most typical carrot flavor.

The carrots were heated 5 min in water or acid because the combination of the highest yield and best quality of juice from the heated samples was obtained at this time interval (Table 1). As the heating interval in water or acid increased, the yield of juice decreased and the orange color of the juice faded. The juice from carrots heated 3 or 5 min in .05 N acetic acid was about the same in quality but, in order to compare the juice from carrots heated in water with the juice from carrots heated in acid, the 5-min heating interval was chosen.

The amount of juice extracted from the raw carrots was not significantly different from the juice extracted from the heated carrots (Table 2). There was an increase of 3.3% yield in juice from carrots heated in acid compared with juice from carrots heated in water, and the increase was highly significant. The amount of juice which could be extracted from each lot of carrots with the methods employed was approximately the same, whether the carrots were obtained from the packing-shed on March 1 or May 20. These data show that the maturity and condition of the cull carrots are rather uniform throughout the harvesting period. Early in the harvest period there may be a slightly higher percentage of small immature carrots delivered to the packing-shed than during the latter part of the harvest period, but the differences are not large enough to greatly influence juice yield from the cull carrots. A processor in South Texas who wishes to can carrot juice from cull Imperator carrots could expect the yield of juice to remain rather constant throughout the harvest period.

There is a decrease in suspended solids and percent light transmission and an increase in orange color intensity of juice prepared from heated carrots as compared with juice prepared from raw carrots (Table 2). The same relationship exists if juice prepared from carrots heated in acid is compared with juice from carrots heated in water. A heavy orange-colored coagulum formed in the canned juice prepared from raw carrots. The light transmitted through the transparent juice was 97% and the color was light-green with a color notation of Rd, 1.7, a, −3.6 and b, 4.5. This juice would not be acceptable as a canned carrot juice.

The 1.5% suspended solids in the juice from the water-treated carrots were not statistically different from the .9% suspended solids in the juice from acid-treated carrots, but the 2 juices were different in percent light transmitted through the juices and the color of the juices was different. The juice from water-treated carrots allowed 50.2% of the light to pass through the centrifuged juice, whereas the juice from the acid-treated carrots allowed only 35.1% of the light to pass through the centrifuged juice. The centrifuged juice from acid-treated carrots had a brighter orange color than the juice from water-treated carrots. The color notation was Rd, 23.7, a, 30.4 and b, 34.8 for juice from the acid-treated carrots compared with a notation of Rd, 19.2, a, 24.0 and b, 31.4 for the juice from water-treated carrots. These differences are due to differences in the composition of the material comprising the suspended solids of the 2 juices. Small particles of the ground carrots were squeezed through the press-cloth during the extraction of both juices and were washed into the container as the juices were collected from the press. The suspended solids of the juice from the acid-treated carrots was made up mostly of these small carrot pieces, whereas the small carrot pieces plus the coagulum comprised the suspended solids of the juice from carrots heated in water. The coagulum which formed and settled from the juice from water-treated carrots removed the suspended solids responsible for color in the juice.

The increase in titratable acidity and decrease in pH between juices of the 3 treatments is probably due to heat decomposition of the pectic substances to pectinic acid and the absorption of the acetic acid by the carrots during the acid treatment of the carrots.

The amounts of the pectic substances, starch and protein in the canned juice and presscake are presented in Table 3. The juice from raw carrots had less pectic substances and starch and about the same amount of protein as the 2 other juices but formed the most coagulum during the canning operation. The heavy coagulum

Table 2–Yield and analysis of canned juice from raw carrots and carrots heated in boiling water and in acetic acid.[a]

Sample treatment	Juice yield (%)	Suspended solids (%)	Light trans. (%)	Brix (°)	pH	Titratable acidity (%)	Color Rd	Color a	Color b
Raw	71.1	9.9	97.0	8.1	6.1	.08	1.7	-3.6	4.5
Heated in boiling water	68.4	1.5	50.2	8.5	5.7	.11	19.2	24.0	31.4
Heated in .05 N acetic acid	71.7	.9	35.1	8.9	5.4	.15	23.7	30.4	34.8
Raw vs. heated	NS	**	**	NS	**	**	**	**	**
Water vs. acid	**	NS	**	NS	**	**	**	**	**

**Significant at the 1% level.

[a]Data represent the mean of duplicate determinations on 9 replications.

Table 3–Analysis of canned juice and presscake from raw carrots and carrots heated in water and in acetic acid.[a,b]

Sample treatment	Canned juice						Press cake					
	Pectic substances						Pectic substances					
	Water (%)	Oxalate (%)	Acid (%)	Total (%)	Starch (%)	Protein (%)	Water (%)	Oxalate (%)	Acid (%)	Total (%)	Starch (%)	Protein (%)
Raw	.05	.09	.01	.15	.25	9.0	.77	7.39	9.45	17.61	8.96	6.2
Heated in boiling water	.40	.35	.02	.77	.40	7.6	1.19	6.67	8.61	16.47	8.23	8.5
Heated in .05 N acetic acid	.28	.15	.02	.45	.33	7.8	.87	7.02	9.73	17.62	7.67	8.1
Raw vs. heated	**	**	NS	**	**	NS	NS	*	NS	*	**	**
Water vs. acid	**	**	NS	**	NS	NS	**	NS	**	**	NS	NS

*Significant at the 5% level; **Significant at the 1% level.

[a]Data represent the mean of duplicate determinations on 4 replications.

[b]Expressed as % dry wt.

which occurred in the canned juice from raw carrots was thought to have been caused by the heat denaturation of the protein. The coagulated protein precipitated from the juice and in the process took the .15% total pectic substances and the .25% starch down with it.

The coagulum which occurred in the juice extracted from carrots heated in water was also believed to be due to the denaturing of the protein. However, the heating process caused more of the pectic substances and starch to be extracted into this juice than was extracted into the juice from raw carrots. The additional heating during the canning process caused partial coagulation as it did in the juice from raw carrots, but the increase in pectic substances and starch acted as a stabilizer, to hold most of the coagulum in suspension. Kertesz (1951) pointed out that the pectic substances play an important part in the stabilization of the colloidal systems in fruit juices. It is reasonable to believe the same system would help stabilize carrot juice.

Another possible explanation for the partial stabilization of the juice from heated carrots is that the easily denatured proteins coagulated and remained in the presscake, whereas different and less heat-sensitive proteins were extracted into the juice. Additional heating at a higher processing temperature was either insufficient to cause the less heat-labile proteins to coagulate or the pectic substances and starch held most of them in suspension; consequently, only a small precipitate occurred in the juice.

The data in Table 3 would seem to indicate that the juice from carrots heated in acid should contain more coagulum than the juice from carrots heated in water, because there are less pectic substances, less starch and the same amount of protein in the juice from acid-treated carrots compared with juice from water-treated carrots. However, in this juice there is a 2-fold simultaneous treatment of heat and acid on the juice. The amount of protein is the same in this juice as in the juice from carrots heated in water, but the action of the acid has changed the proteins so that although there is less pectic substances and starch in the juice, there is a sufficient amount to keep any coagulated protein in suspension. Tressler and Joslyn (1961), in their discussion of juice from nonacid vegetables, state that when the vegetable is macerated, the enzymes released act upon the released protoplasmic liquid causing rapid metabolic changes unnatural to the undamaged tissues. The juice from carrots heated in acid does not form a coagulum and maintains its orange color because of the action of the heat and acid on the protoplasmic liquid of the cell before the cell is ruptured or immediately thereafter. Those materials responsible for changes in the juice, whether they are enzymes or denatured protein, or a combination of the two, are altered by the action of the heat and acid.

After the juice was extracted from carrots heated in acid, the remaining 28.3% presscake contained 8.1% protein. This by-product could be valuable as a food or feed supplement.

There is an estimated 20–24 thousand tons of carrots discarded each year in the South Texas area as cull or reject carrots, because the carrots are misshapen, too small, too large or have small blemishes as a result of growing and harvesting. A large percentage of these carrots are good food and could be manufactured into a very good juice product. Manufacturing these reject carrots into an edible product would greatly reduce the disposal problem often confronting the packer of fresh carrots.

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W. A. SISTRUNK
University of Arkansas, Agricultural Experiment Station, Fayetteville, Arkansas 72701

CARBOHYDRATE TRANSFORMATIONS, COLOR AND FIRMNESS OF CANNED SWEET POTATOES AS INFLUENCED BY VARIETY, STORAGE, pH and TREATMENT

SUMMARY- Two varieties of sweet potatoes were canned at three storage intervals, six buffered pH levels, and two holding times to determine the influence on color, firmness, carbohydrates and other constituents. Color and firmness were improved when the pH was decreased from 8 to 3. There was an increase in brightness of color and firmness by holding peeled sweet potatoes 24 hr in buffers before canning. The sugars and phenolic substances were leached out during holding. Total polyphenols decreased with an increase in pH although tannic and chlorogenic acids were not changed appreciably. There was a decrease in starch and hemicellulose as a result of storage; whereas, water- and Calgon-soluble pectin were not affected. Starch decreased with an increase in pH regardless of other variables. Water-soluble pectin increased when pH was altered up or down from the normal pH of canned sweet potatoes of approximately 6.0. In comparison, Calgon-soluble pectin and hemicellulose reacted inversely to pH. It appeared that pH had a far greater effect on color and firmness than length of post-harvest storage of raw product after curing and other variables because of the direct effect on carbohydrate transformations and discoloration.

INTRODUCTION

THERE ARE MANY important factors connected with the canning of good quality sweet potatoes. Since sweet potatoes have a high carbohydrate content in the storage roots in addition to active amylase and polyphenoloxidase enzyme systems, there is a rapid breakdown in firmness and development of discoloration unless rigid conditions are met during handling and processing. Factors related to softening are difficult to assess, and therefore, carbohydrate transformations and other chemical changes responsible for loss of firmness have not been defined sufficiently. Discoloration of sweet potatoes during peeling, preparation, processing and storage of the canned product has jeopardized the acceptance of this item, as well as presenting innumerable problems to the processor.

Most of the sweet potatoes for canning are processed in late summer and early fall, within a short time after harvest, in order to avoid softening and discoloration of the final product. It has been shown that post-harvest handling had the greatest effect on firmness of canned sweet potatoes (Kattan and Littrell, 1963). Firmness was highly correlated with alcohol-insoluble-solids and starch. There was a progressive decrease in firmness of the product. However, Baumgardner and Scott (1963) mentioned that starch played a lesser role in firmness, while pectic substances were more closely associated with degree of firmness and decreased during storage. Earlier studies by Heinze and Appleman (1943) indicated that there was an increase in soluble pectin during the curing of sweet potatoes, but a decline during the storage period. Pectin substances ranged from 0.78 to 1.09%. Later Ahmed et al. (1958) recorded 3 to 5% pectin in sweet potatoes expressed as anhydrogalacturonic acid. Intrinsic viscosity decreased markedly after curing, and to a lesser degree during 6 mo of storage.

More serious discoloration was encountered when sweet potatoes were stored beyond 6 wk for the manufacture of dried flakes (Hoover and Kushman, 1966). Hoover (1963) demonstrated that sodium acid pyrophosphate (SAPP) at the rate of 0.3–0.4% on a dry basis was sufficient to control discoloration in dried flakes. A mixture of tetrasodium pyrophosphate (TSPP) and SAPP (1:3) gave good results in addition to holding the pH at 6 to prevent an acid taste. Other studies on frozen sweet potatoes (Hoover, 1963) showed that 0.4% SAPP was sufficient to control discoloration. Higher percentages produced off-flavor and sloughing of the surface of whole and sliced frozen product.

The present study was designed to determine the influence of variety, storage, pH, and treatment on color and firmness. Carbohydrate transformations and other chemical changes taking place as a result of these variables are presented.

EXPERIMENTAL

Raw material

Five bushels each of two varieties of sweet potatoes (Unit I Porto Rico and Goldrush) were obtained at harvest. The canning size (1–2 in.) was further separated to increase the uniformity. After curing for 1 wk at 85°F, one lot of each variety was canned, while the remaining lots were stored at 60°F for 3 and 6 mo prior to canning.

Preparation and processing

The roots were peeled in 10% boiling lye solution for 5 min and rinsed under a cold water spray. After trimming and cutting into approximately 1 oz pieces, they were filled into 303 × 406 'C' enamel cans to a fill of 300g. Buffer solutions at pH 3, 4, 5, 6, 7 and 8 were prepared by adjusting .025M sodium citrate to the appropriate pH levels with 50% citric acid. Half the cans for processing were promptly filled with hot buffer at 160°F and vacuumized 5 min at 15 in. of vacuum; the rest were filled with cold buffer and held 24 hr at 35°F prior to processing. In both instances, the buffer was drained off and replaced with water. The cans were exhausted to 180°F in a steam exhaust box before closing and retorting for 35 min at 240°F. Cans were cooled in cold water and stored at room temperature until analyzed.

Analytical

The canned sweet potatoes were drained, weighed and rated by a three member panel for color and firmness. Resistance to shear was determined by the Allo Qualitometer using a 200g sample and a standard cell. Readings were recorded when the instrument reached a steady state and at peak force. The shear press sample was recovered and mixed with the remainder of the sample. After measuring the liquid from the can, distilled water was added to make a 2:1 blend of liquid to sweet potatoes in order to obtain a mixture thin enough for blending.

Relative viscosity was measured on the blend by the time in seconds necessary for 100 rev on a Stormer viscosimeter using a 100g falling weight. 6g of the blend was transferred to polypropylene centrifuge tubes with 4 ml of distilled water. Starch was determined by adding 1 ml of 1% Taka-diastase to each tube and digesting for 1 hr in a water bath at 55°C with agitation. Duplicate tubes without enzyme received the same treatment in order to determine total sugars. After 1 hr, tubes were filled to 40 ml with 95% ethanol. ½ teaspoon of Celite was added to each tube before stirring and heating at 80°C for 10 min in a water bath. Following the heating period, the tubes were centrifuged for 10 min and decanted into volumetric flasks. After repeating the extractions by heating with 70% ethanol, the enzyme-digested samples were extracted twice each with water, 0.5% calgon, and 1N NaOH at 25°C. Total sugars, starch and hemicellulose were determined by the Phenol method (Dubois et al., 1956). Water- and Calgon-soluble fractions were determined as percent anhydrogalacturonic acid by the Carbazole method (Dietz and Rouse, 1953). Modifications of these methods have been previously described (Sistrunk, 1965). Calculations were made on the fresh weight basis.

The Hunter Color and Color Difference Meter (CDM) was used to record color of the blend by standardizing the instrument with a sweet potato standard plaque ('L' = 58.4; 'a' = 29.5; and 'b' = 32.1).

Soluble solids were determined on the liquid in the cans by a refractometer. Total solids were obtained by weighing 20g samples in tared aluminum dishes and drying for 24 hr at 70°C.

Tannic and chlorogenic acid were determined by diluting the acidified 80% ethanol

extract used for total sugars and reading the absorption at 280 and 330 mμ respectively. The concentration was calculated from standard curves of the two acids.

Total polyphenols were determined by the method of AOAC (1965). Data were analyzed as a factorial experiment by conventional statistical procedures. Since there was no significant interactions except in minor instances, most of which are shown in the figures, only the means of main effects are recorded in the tables.

RESULTS & DISCUSSION

NORMALLY, sweet potatoes are canned at harvest or as soon after harvest as possible to retain wholeness and a bright color. In this experiment, the primary interest was in obtaining an acceptable canned product from cured and stored raw material. Therefore, the sweet potatoes were not canned at the time of harvest.

Effect of variety

The Goldrush variety was darker yellow-orange than Unit I as indicated by the lower CDM 'L' and higher 'a' values (Table 1). There was no difference in color by panel scores since color intensity was not rated, only brightness and attractiveness. Firmness between varieties was not distinguished by the panel, but because of the greater amount of fiber in Unit I, it was firmer by the shear press. Soluble and total solids and total sugars were higher in Unit I regardless of other variables (Table 2).

Kattan and Littrell (1963) showed that the starch content in canned sweet potatoes was 5–7% when canned at harvest, but declined rapidly during curing and subsequent storage. The canned product was objectionably soft when the raw product was held 14 days or longer. In this experiment, when Unit I was canned at pH 3.0 after curing, the starch content was approximately 6%, although the mean for all variables was 1.81%, and the mean for sweet potatoes canned after cure was 4.0% (Figure 1a). Starch and water-soluble pectin were higher in Unit I, but other polysaccharides were similar between the two varieties. There was no difference between varieties in phenolic substances (Table 2). Viscosity was greater in Unit I. This coincides with the firmness and part of the polysaccharides.

Effect of storage

The canned product was slightly darker in color after 6 mo of storage, although there was a loss of yellow color as demonstrated by the lower 'a' value and visual color scores (Table 1). The firmness, as rated by the panel, and as measured with the shear press, was lower at 3 mo storage (Table 1). This indicates that length of storage of raw material was not as important as other factors in determining firmness of canned sweet potatoes. There was a decrease in soluble and total solids, but not in total sugars during storage (Table 2). Among the polysaccharides, only starch and hemicellulose decreased during storage (Fig. 1a–d). The decrease in total solids probably resulted from the change in these two constituents. Since there was less starch to degrade to sugar in roots stored 6 mo, soluble solids of the liquor would be expected to decrease during storage. Viscosity was not affected by the length of storage, which indicates that viscosity was more related to pectins than other chemical constituents (Table 2 and Figure 1b, 1c). Ahmed et al. (1958) found a decrease in viscosity in Maryland Golden sweet potatoes during storage.

Total polyphenols, tannic acid and chlorogenic acid increased during storage (Table 2) although data for the two latter constituents is not shown. These substances might have been responsible for the darker and duller color of the canned product at 6 mo storage. Similar increases in phenolic substances and discoloration

Table 1—Main effects of variety, storage, and other factors on quality of canned sweet potatoes.

Main effects	CDM color 'L'	CDM color 'a'	Visual[a] color	Firmness[a]	Shear press (lbs) (1st peak)
Variety					
Unit I	56.03	7.16	6.43	4.97	20.2
Goldrush	49.98	14.98	6.57	4.69	16.5
F value	483.323**	1076.849**	.811 N.S.	2.205 N.S.	32.636**
Storage					
After cure	53.15	11.49	6.56	4.98	19.5
3 months–60°F	53.07	12.20	7.06	4.50	16.8
6 months–60°F	52.81	9.52	5.88	5.02	18.7
F value	.535	45.116**	19.951**	3.192	5.770**
L.S.D. @ 5% level	N.S.	.59	.38	N.S.	1.6
L.S.D. @ 1% level	N.S.	.79	.51	N.S.	2.2
pH					
3	56.61	11.86	7.58	7.17	21.0
4	55.74	11.32	6.96	6.38	18.4
5	53.52	11.03	6.71	5.08	17.6
6	52.72	10.93	6.67	4.54	18.0
7	49.47	10.99	5.71	2.79	16.1
8	50.00	10.29	5.38	3.04	18.9
F value	74.457**	3.086*	18.786**	58.474**	4.144**
L.S.D. @ 5% level	.97	.84	.54	.66	2.3
L.S.D. @ 1% level	1.30	N.S.	.73	.88	3.1
Treatments					
Vacuum	52.25	10.86	6.40	5.21	17.4
24 hour hold	53.77	11.28	6.60	,4.46	19.3
F value	30.544**	2.975 N.S.	1.593 N.S.	16.073**	7.918**

[a]Rated by a three member panel on a scale of 10–best and 1–poorest.
*Significant at 5% level.
**Significant at 1% level.

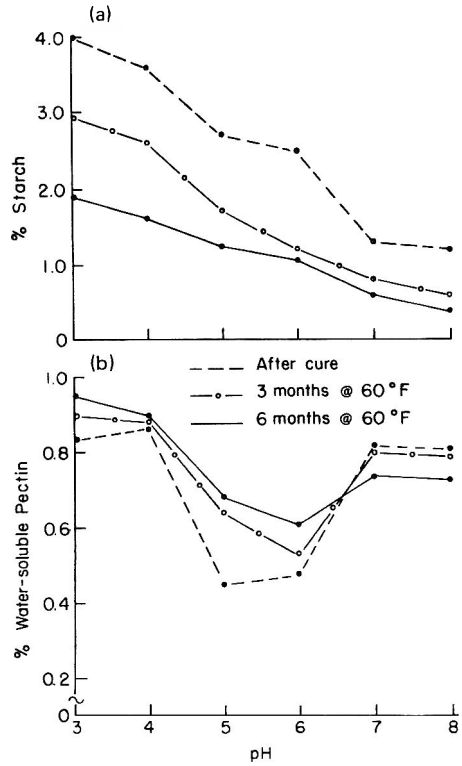

Fig. 1 a–b—Interaction of time of storage and pH on starch and water soluble pectin in canned sweet potatoes.

have been found in Irish potatoes (Mondy et al., 1960).

Effect of pH

This variable had a greater influence on attractiveness than either storage or treatment as exemplified by the 'F' values for 'L' (Table 1). The 'a' value decreased also when pH was increased but the change was less pronounced. Color differences were quite noticeable when rated by the panel. There was a highly significant effect of pH on firmness as rated by the panel, and when measured by the shear press (Table 1). The pH would not be expected to affect the peak force to shear which was attributed to fiber. Soluble and total solids were not affected by pH (Table 2). However, total sugars were lower at pH 7 and 8, which resulted from more solubilization and leaching during the holding treatment.

The degradation of starch was inhibited by reducing the pH (Fig. 1a). There was almost a straight line negative relationship between the decrease in starch and increase in pH. An inverse relationship was exhibited between water-soluble pectin and Calgon-soluble pectin (Fig. 1b, 1c). An increase in acidity or alkalinity from the normal pH of 6 increased the water-soluble pectin and decreased Calgon-soluble pectin. Hemicellulose followed a pattern of solubility similar to Calgon-soluble pectin, except at pH 8 the values were higher (Fig. 1c, 1d).

Total polyphenols were influenced as much by pH as by storage. The values dropped sharply with an increase in pH, although tannic and chlorogenic acid were affected only slightly, the values being lower at pH 6 and 7 (Table 2).

Viscosity corresponded rather closely with the Calgon-soluble pectin and hemicellulose (Table 2 and Fig. 1c, 1d).

Effect of treatment

The 5 min vacuum treatment removed most of the air remaining in the intercellular spaces and infiltrated the various buffer solutions into the pieces of sweet potato. Commercially, this process would be expensive to apply, but the 24 hr holding at 35°F, or a shorter time should be practical. Under some conditions this method has been effective in increasing firmness.

Color was slightly better when the cut product was held in buffer 24 hr, although significant differences were not obtained in all instances (Table 1). The poor color resulting from the pH 8 treatment influenced greatly these data for main effect (data not shown separately for 24 hr hold at different pH levels).

Firmness ratings by the panel were lower for 24 hr holding, but the shear press showed more firmness (Table 1). Since so much leaching occurred in the outer cells as demonstrated by the soluble solids, total solids and total sugars (Table 2), the panel probably detected this softer exterior while the shear press values were the total measurement of firmness.

There was an increase in Calgon-soluble pectin and hemicellulose by holding. This phenomenon has been demonstrated in this laboratory in other experiments and has been attributed to enzymatic conversion in injured tissues.

Total polyphenols, tannic acid and chlorogenic acid decreased during holding in buffers for 24 hr (Table 2). The change was expected since these substances are water soluble. Loss of phenols during holding might have been responsible for the better color in this treatment. Viscosity decreased markedly as a result of holding as compared to vacuum treatment.

In conclusion, firmness of canned sweet potatoes was influenced to some extent by variety, storage after curing and holding but the greatest difference was produced by pH. There was some varia-

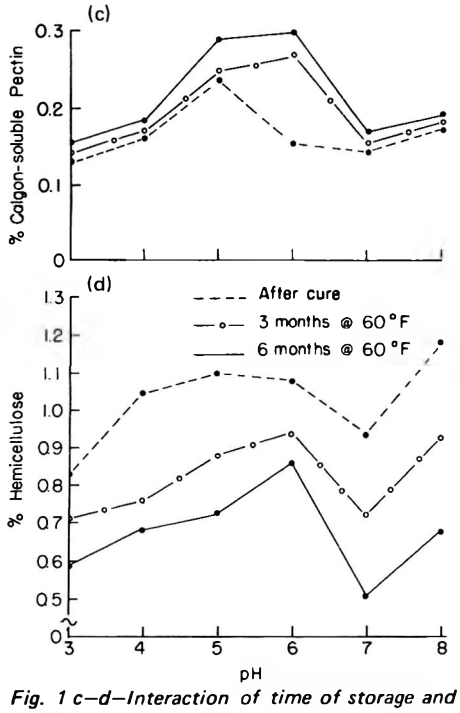

Fig. 1 c–d–Interaction of time of storage and pH on Calgon-soluble pectin and hemicellulose in canned sweet potatoes.

Table 2–Main effects of variety, storage, and other factors on quality of canned sweet potatoes.

Main effects	% Soluble solids (liquor)	% Total solids[a]	% Total sugars	% Total poly-phenols[b]	Viscosity (sec/100 rev)
Variety					
Unit I	14.80	21.43	15.62	.324	52.6
Goldrush	14.24	20.46	15.10	.317	37.9
F value	16.251**	28.469**	18.147**	1.114 N.S.	35.181**
Storage					
After cure	15.12	21.35	15.36	.276	45.4
3 months–60°F	14.51	21.16	15.47	.326	43.7
6 months–60°F	13.93	20.40	15.25	.362	46.7
F value	23.792**	9.683**	1.063	48.511**	.476
L.S.D. @ 5% level	.35	.45	N.S.	.018	N.S.
L.S.D. @ 1% level	.47	.61	N.S.	.024	N.S.
pH					
3	14.72	20.85	15.72	.385	41.6
4	14.61	20.66	16.18	.366	32.5
5	14.29	20.94	15.48	.316	57.2
6	14.27	21.03	15.89	.315	58.8
7	14.63	21.19	14.43	.238	39.1
8	14.61	20.99	14.44	.286	42.4
F value	1.226	.605	25.425**	43.222**	11.868**
L.S.D. @ 5% level	N.S.	N.S.	.43	.025	8.7
L.S.D. @ 1% level	N.S.	N.S.	.57	.034	11.7
Treatments					
Vacuum	15.81	22.72	16.99	.334	55.6
24 hour hold	13.23	19.18	13.73	.317	35.0
F value	337.742**	375.644**	720.507**	14.492**	68.851**

[a]Entire contents of can, liquid and roots.
[b]Calculated as quercitannic acid.
*Significant at 5% level.
**Significant at 1% level.

tion in the definition of firmness when measured by the panel, shear press and viscosity. Color was influenced by all variables when measured by the CDM, yet pH and storage had the largest effect on attractiveness or appearance. It appeared that sweet potatoes could be canned successfully after storage by applying the correct technology.

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G. FULLER, D. G. GUADAGNI, M. L. WEAVER, G. NOTTER and R. J. HORVAT
USDA Western Regional Research Lab., ARS, Albany, California 94710

EVALUATION OF OLEIC SAFFLOWER OIL IN FRYING OF POTATO CHIPS

SUMMARY—A recently introduced variety of safflower, UC-1, produces an oil in which oleic:linoleic acid ratios of ordinary safflower oil are reversed, giving an oil with iodine value 90. This oil was compared with cottonseed oil and hydrogenated vegetable oil (HVO) for frying potato chips. After accelerated storage at ambient temperature under fluorescent light, the quality of chips fried in oleic safflower oil was ranked equal to that of chips fried in hydrogenated oil. Chips fried in either oil were more stable than those fried in cottonseed oil. Effect of propyl gallate antioxidant was negligible.

INTRODUCTION

POLYUNSATURATED vegetable oils have not been satisfactory as frying oils because of high oxidative instability resulting from the chemical structure of linoleic acid and linolenic acid present in some oils. Polyunsaturated fatty acids have two or more double bonds separated by single methylene groups. It has been known for many years that these isolated methylene groups are extremely vulnerable to oxidative attack. Much of the early work on elucidation of oxidation mechanisms was performed using polyunsaturated fatty acid esters (Bateman, 1954). Oxidative attack on polyunsaturated glycerides is the initiation of the drying process of paints (Wexler, 1964), but the formation of varnishes and films, while desirable on the side of a house, is certainly unwanted on a potato chip. Moreover, rancid flavors and odors result from lipid oxidation during storage of food products.

Early kinetic studies on oxidation of fatty acids (Gunstone and Hilditch, 1946) noted the great difference in rates of oxidation of linoleic and oleic acid derivatives. The much slower rate of oxidation of oleic compounds and saturated acid derivatives has been the reason for hydrogenating vegetable oils. Elimination of at least one double bond in a fatty acid greatly increases its stability toward oxygen, but hydrogenation is expensive and winterizing and deodorization processes are required following hydrogenation. An inexpensive oil that can be used for frying without hydrogenation is desirable.

In 1963 a new variety of safflower, UC-1, was reported (Knowles and Mutwakil, 1963) in which the usual ratios of linoleic to oleic acid were reversed. While ordinary safflower oil contains ca. 75% linoleic acid with 12–15% oleic acid, the triglycerides of UC-1 have almost 80% oleic and 12–13% linoleic acid. Iodine values for ordinary safflower and UC-1 oils were respectively 144 and 90. Stability of oleic safflower oil at high temperatures was excellent (Fuller et al., 1966, 1967), and very good potato chips were prepared with oil extracted from UC-1 safflower seed (Purdy and Campbell, 1967). UC-1 safflower oil is now commercially available. The purpose of our experiments was to evaluate oleic safflower oil relative to other commercial oils used for frying potato chips, especially with regard to storage stability of the product. The effect of an antioxidant in the oils was determined and an attempt was made to develop an instrumental method which would show incipient rancidity in the chips as rapidly as would panel evaluation.

Table 1—Composition and characteristics of frying oils.

	Oleic safflower oil	Cottonseed oil	Hydrogenated vegetable oil
Iodine value	90	105	82
Melting point, °F	—	—	92
Stability, AOM (hr)[a]	38	23	41
Fatty acid content %			
16:0	5.4	23.7	24.8
18:0	1.7	3.0	3.8
18:1	80.7	19.4	50.8
18:2	12.2	53.9	20.6

[a]AOM values are reported for oils without antioxidant only.

EXPERIMENTAL

Materials

Conditioned Kennebec potatoes from the production line of a commercial chip producer were used for all the tests. Refined oleic safflower oil without antioxidant was purchased from the Pacific Vegetable Oil Corporation, Richmond, California. Refined, deodorized cottonseed oil was obtained from the Ranchers Cotton Oil Company. Hydrogenated vegetable oil was purchased from Durkees Fine Foods. Tenox S-1 antioxidant was added to a portion of each oil to provide a concentration of 0.01% propyl gallate and 0.005% citric acid. Composition and properties of the oils are listed in Table 1.

Preparation of potato chips

Potatoes were peeled and sliced on a Hobart slicer with a setting of 5 (to give the same thickness as that of commercial chips). The slices were washed free of starch and dried. Chips were fried in 1-lb batches for 4 min at an initial oil temperature of 190°C (374°F). The oil-to-slice weight ratio was 10:1. Chips were drained 1 min and cooled in air on laboratory towels. Eight batches of chips fried in the same oil were combined and 40g samples were placed in 1-pt Mason jars. Twelve samples of chips fried in each oil were packed under nitrogen and placed in the dark as controls. Six samples were packed in air. Deterioration of the chips was accelerated by constant exposure to fluorescent light (G.E. F 40SGN sign white bulbs) at two levels of intensity, 13 and 100 foot candles, measured with a Weston Master IV light meter.

Evaluation of chips

Samples of the potato chips were evaluated

Table 2—Odor comparison of chips fried in different oils.

Oil	Total rank sums[a] Light intensity = 13	Light intensity = 100
	13 days	12 days
HVO[b] (dark, N_2)	25*	24*
Oleic safflower	49	49
HVO	47	49
Cottonseed	80*	82*
Commercial Chips	99*	96*
HVO (dark, N_2)	22*	23*
Oleic safflower	62	62
Oleic safflower w/antioxidant	62	53
HVO w/antioxidant	60	63
Cottonseed w/antioxidant	96*	99*
	21 days	21 days
HVO (dark N_2)	22*	24*
HVO	80*	78*
HVO w/antioxidant	68	55
Oleic safflower	77*	85*
Oleic safflower w/antioxidant	51	63

[a]Lower numbers represent fresher odor.
[b]HVO = hydrogenated vegetable oil.
*Significant at $P \leqslant 0.01$.

by a trained panel of 20–26 individuals in the laboratory. Odor proved to be a much more sensitive measure of rancidity than taste, so evaluations presented are on the basis of chip odor. The chips were sniffed from the original pint jars which were coded and completely randomized before evaluation under subdued lighting (7.5-watt green bulbs) in individual booths. Three methods of sensory evaluation were used. The triangle test was employed to determine the time required to cause significant odor changes in samples held under light intensities of 13 and 100 foot candles. The controls for these tests were the nitrogen-packed samples held in the dark. A like-dislike hedonic rating scale was used to estimate the degree of acceptability of the different chips after various storage periods. The chips fried in different oils and held under different conditions were ranked together in sets of five according to fresh chip odor. In the triangle test the control and treated chips were presented as the odd sample an equal number of times.

An attempt was made to determine incipient rancidity by GLC analysis of headspace gases. Both pentane and hexanal have been used as indices of rancidity (Buttery et al., 1961; Scholz and Ptak, 1969; Evans et al., 1969) but it was found here that sensory evaluation was much more sensitive than was GLC. Results are reported only for the sensory evaluations.

RESULTS & DISCUSSION

ALL THE CHIPS initially contained ca. 35% oil and 3% moisture. Effects of storage and light intensity on hedonic rating are shown in Figures 1 and 2. Chips fried in all oils were acceptable at the beginning (Rating > 5). Chips fried in oleic oil with antioxidant and hydrogenated oil (HVO) with antioxidant were rated slightly above the same materials without antioxidant and significantly above the chips fried in cottonseed oil.

At zero time of course, there should be no difference between samples under air and under nitrogen, but after only 3 days (Fig. 3 and 4) the panel was able to differentiate between cottonseed oil samples at both light intensities and the same samples under nitrogen in the dark. The oleic oil chips with antioxidant at light intensities = 100 could also be detected, from their controls, but the hedonic rating had not dropped as it had with the cottonseed oil samples. After 12 days at light intensity = 13, the samples fried in HVO and oleic oil were still acceptable. By 21 days all of the oils under air were rated below five and presumably unacceptable. The nitrogen controls had not dropped significantly during this period. It should be emphasized that acceptability ratings of laboratory panels do not necessarily represent consumer acceptance. The rank data for different oils, storage time and light intensities are shown in Table 2. Obviously, the control HVO sample stored in the dark was always ranked significantly fresher than all exposed samples. Chips fried in cottonseed oil with or without antioxidant were ranked significantly less fresh (or more stale) than other samples at both light intensities. Oleic safflower and HVO chips were ranked similarly when held under the same conditions. After 12 and 13 days at light intensities = 100 and 13, oleic oil chips appeared to be similar in quality to those fried in the same oil containing antioxidant or HVO with antioxidant. After 21 days there was a slight but significant advantage for the oils with antioxidant.

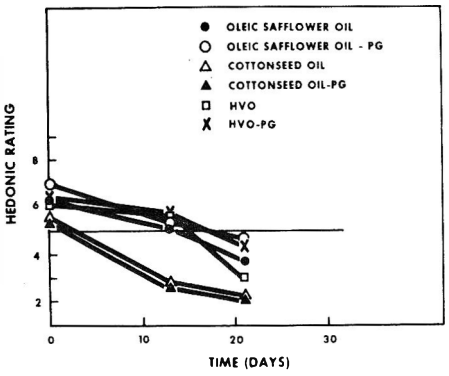

Fig. 1—Average hedonic ratings of potato chips (light intensity = 13): 9—like extremely; 8—like very much; 7—like moderately; 6—like slightly; 5—neither like nor dislike; 4—dislike slightly; 3—dislike moderately; 2—dislike very much; and 1—dislike extremely.

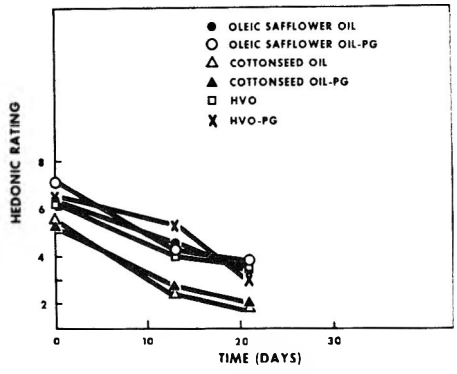

Fig. 2—Average hedonic ratings of potato chips (light intensity = 100).

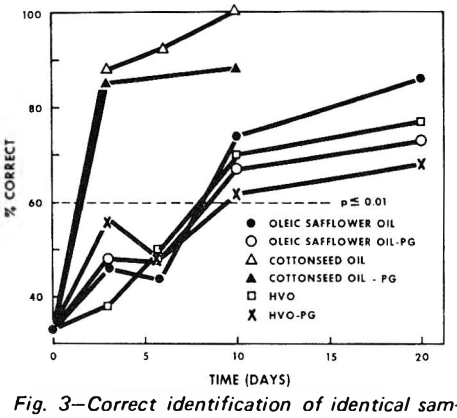

Fig. 3—Correct identification of identical samples (intensity = 13).

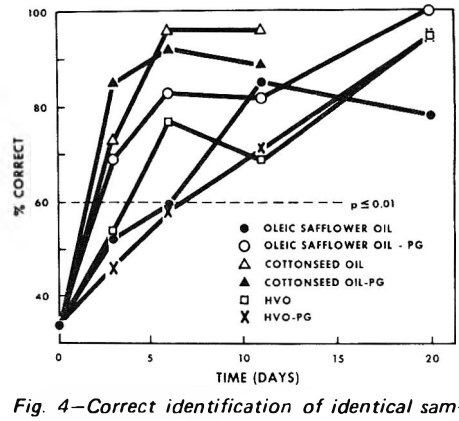

Fig. 4—Correct identification of identical samples (intensity = 100).

CONCLUSIONS

RESULTS show that oleic oil is approximately equivalent to hydrogenated vegetable oil when storage stability of fried potato chips is measured. Both oils are significantly better for frying than ordinary cottonseed oil. Effects of added antioxidant appeared to be small at the concentration employed. This lack of effect may be explained by loss of antioxidant through steam distillation when moisture from the chips was removed during frying. Fluorescent light is an effective accelerator of oxidation at ambient temperature. At an intensity of 13 foot candles the acceleration was at least three-fold over storage in air in the dark. The additional acceleration at higher light intensities was relatively small.

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L. E. JEREMIAH, G. C. SMITH and Z. L. CARPENTER
Department of Animal Science, Texas A&M University, College Station, Texas 77843

PALATABILITY OF INDIVIDUAL MUSCLES FROM OVINE LEG STEAKS AS RELATED TO CHRONOLOGICAL AGE AND MARBLING

SUMMARY—5 muscles from each of 243 leg steaks from sheep ranging in age from 74–665 days were evaluated mechanically for tenderness using a Warner-Bratzler shear and organoleptically for tenderness and juiciness by a trained 3-member panel. The rectus femoris, vastus lateralis, biceps femoris, semitendinosus and semimembranosus muscles were evaluated. The r. femoris and semitendinosus were given the highest ($P < .05$) organoleptic ratings for tenderness, whereas the semimembranosus was least tender ($P < .05$) among the 5 muscles compared. However, no significant difference was found between the shear force values of the b. femoris and semimembranosus. The b. femoris and r. femoris received the highest juiciness ratings, while the v. lateralis and semitendinosus were least desirable in juiciness ($P < .05$). Significant correlations ($P < .01$) were observed between cooking losses and juiciness ratings for the combined muscles and between marbling scores and juiciness ratings for the r. femoris, v. lateralis and semitendinosus. However, gross amount of marbling was of minor importance in explaining differences in juiciness between muscles since r. femoris had the least marbling ($P < .05$). Marbling appeared to be of little consequence in determining either tenderness or thaw drip loss, based on the extremely low correlation coefficients observed between marbling and each of these variables. Correlations were significant ($P < .01$) between chronological age and measures of tenderness and cooking loss. Cooking loss was also negatively correlated ($P < .01$) with taste panel tenderness ratings. Therefore, the detrimental effect of increasing age on organoleptic tenderness may be manifested through a drying effect during cookery. These results indicate that the muscles of the ovine leg vary in palatability attributes and that cooking loss appears to affect organoleptic evaluations of both tenderness and juiciness. Increasing chronological age has a detrimental effect on tenderness measured organoleptically or mechanically. The contribution of marbling to tenderness is questionable; however, increased marbling enhanced the juiciness of the r. femoris, v. lateralis and semitendinosus muscles.

INTRODUCTION

QUALITY grading standards for ovine carcasses include subjective evaluations of physiological maturity and fatness levels in attempts to predict the palatability of the subsequent meat products. Various researchers have reported small but significant differences in tenderness or juiciness, or both, due to differences in maturity (Batcher et al., 1962; Carpenter et al., 1965b; Kirton, cited by Pearson, 1966; Paul et al., 1964a; Smith et al., 1970a; 1970b; 1970c). However, neither Smith et al. (1964) nor Weller et al. (1962) observed significant relationships between tenderness and either USDA maturity or age at slaughter.

Numerous other workers have observed significant relationships between measures of fatness and tenderness or juiciness (Carpenter et al., 1965a; Forrest, 1962; Hammond, 1932; Lowe, 1932; Oldfield et al., 1966; Paul et al., 1964a; 1964b). Other reports fail to support a relationship between estimates of fatness level and tenderness or juiciness (Batcher et al., 1962; Carpenter et al., 1964; Cover et al., 1944; Hankins and Ellis, 1939; Murphey et al., 1942; Rupnow et al., 1961; Smith et al., 1964; Stouffer et al., 1958; Weber et al., 1931 and Wilcox et al., 1952). Zobrisky et al. (1961) pointed out that the interaction of maturity and fatness is of greater importance in determining palatability as maturity advances.

Several workers have observed significant antemortem treatment effects on maturity evaluations in the carcass. Lambs fed stilbestrol (Acker et al., 1959; Hartman et al., 1958) or subjected to preslaughter stress (Forrest et al., 1961; Hedrick et al., 1961) produced more mature carcasses than untreated or unstressed control animals. Bray (1963) concluded that the relative effect of changes in maturity upon palatability can not be established until maturity can be measured objectively.

Carpenter et al. (1968) and Ramsbottom et al. (1945) observed differences in tenderness within and between muscles of pork and beef. The present study enumerates differences in palatability and cooking loss between ovine muscles, and relates these differences to changes in chronological age and marbling.

EXPERIMENTAL

243 EWES and wethers of fine wool × medium wool parentage ranging in age from 74–665 days provided the leg roasts evaluated in this study. The roasts were frozen and stored in a −18°C freezer. Upon removal from the freezer, individual steaks (2.54 cm) were removed at an approximate angle of 25° from the longitudinal axis of the intact leg from a point opposite the proximal tip of the patella. Each steak was thawed in a 2°C cooler for 24 hr and cooked to an internal temperature of 75°C in a 177°C oven. The steaks were weighed to the nearest gram upon removal from frozen storage, after thawing and after cooking to determine weight losses resulting from thaw drip and from the cooking process. Individual core samples (1.27-cm) were obtained for shear force or organoleptic analyses and were removed in parallel orientation to the direction of the muscle fibers (Fig. 1). Core samples for shear force determinations were removed after the chops had reached room temperature.

5 muscles from each of the 243 leg steaks were evaluated mechanically for tenderness using a Warner-Bratzler shear and organoleptically for tenderness and juiciness by a 3-member trained panel. The rectus femoris, vastus lateralis, biceps femoris, semitendinosus and semimembranosus muscles were evaluated. Individual core samples from each of the 5 muscles were scored for tenderness and juiciness

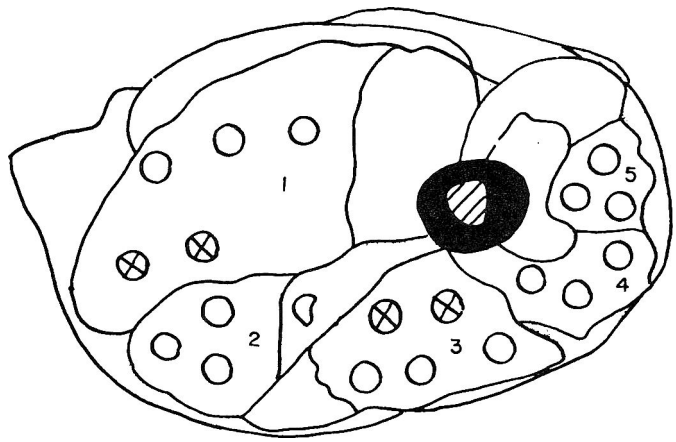

Fig. 1—Core positions for shear force and taste panel determinations. 1 = Semimembranosus. 2 = Semitendinosus. 3 = Biceps femoris. 4 = Vastus lateralis. 5 = Rectus femoris. O = Cores for taste panel determinations. ⊗ = Cores for shear force determinations.

Table 1—Mean separation analyses for palatability attributes and marbling scores between individual muscles from ovine leg roasts.

Muscle	Tenderness rating	Juiciness rating	Shear force (kg)	Marbling score[1]
Rectus femoris	6.52[a]	5.51[a,b]	—	4.65[d]
Vastus lateralis	5.45[b]	5.26[d]	—	5.13[b,c]
Biceps femoris	5.30[b]	5.57[a]	8.00[a]	5.02[c]
Semitendinosus	6.46[a]	5.31[c,d]	—	5.49[a]
Semimembranosus	4.50[c]	5.39[b,c]	7.73[a]	5.33[a,b]

[a,b,c,d]Means with different superscripts are significantly different ($P < .05$).

[1]Marbling scores were coded as follows: 4 = slight amount; 5 = small amount; 6 = modest amount; etc.

Table 2—Simple correlation coefficients between weight losses, palatability attributes and chronological ages for combined muscles from ovine leg steaks.

Trait	Tenderness rating	Juiciness rating	Shear force (kg)	Chronological age (days)
Thaw drip loss (%)	0.01	0.03	0.07	0.01
Cooking loss (%)	−.29**	−.49**	0.11	0.34**
Total weight loss (%)	−.13*	−.10	0.19**	0.11

*P < .05. **P < .01.

using a 9-point hedonic scale, ranging from 9 (like extremely) to 1 (dislike extremely). Shear force values were obtained on adjacent cores from the semitendinosus and b. femoris.

The data were analyzed using the mean separation technique of Duncan (1955) and linear regression analyses to obtain simple correlation coefficients.

RESULTS & DISCUSSION

SEMITENDINOSUS and r. femoris muscles were rated significantly higher in tenderness ($P < .05$). These findings are in partial disagreement with the results for beef reported by Ramsbottom et al. (1945), who found b. femoris to be most tender, followed by the r. femoris, and that the 3 other muscles included in the present study were very similar in tenderness.

Shear force values for the 2 muscles tested were negatively correlated ($P < .01$) to taste panel ratings for tenderness, but the coefficients were relatively low (r = −.20, b. femoris; r = −.36, semimembranosus). B. femoris and semimembranosus samples exhibited similar shear force values (Table 1), but differed significantly ($P < .05$) in tenderness ratings. The comparative shear force values are in contrast with the findings of Ramsbottom et al. (1945) for the same 2 muscles in beef carcasses. The highest juiciness ratings were given to b. femoris and r. femoris, followed by semimembranosus, semitendinosus and v. lateralis, respectively (Table 1). The negative correlation coefficients ($P < .01$, Table 2) for cooking loss percents with ratings for tenderness and juiciness may account for the differences observed in taste panel tenderness and juiciness scores (Table 1), especially if moisture and fat are lost at different rates by specific muscles.

The semitendinosus had significantly more marbling ($P < .05$) than b. femoris, v. lateralis or r. femoris; the r. femoris had significantly less marbling ($P < .05$) than any of the other muscles studied (Table 1). Both significant and nonsignificant relationships between marbling and palatability attributes have been reported by workers previously cited. Data from the present study (Table 3) enumerate the contributions of marbling to the palatability characteristics of individual muscles. Relationships between marbling and juiciness were significant ($P < .01$) for r. femoris, v. lateralis and semitendinosus, but the relationships for b. femoris and semimembranosus were not significant. The extremely low correlation coefficients observed with taste panel ratings for tenderness indicate that marbling is of little consequence in determining tenderness in any of the muscles, even though significant relationships ($P < .01$ and $P < .05$) were obtained for marbling score with shear force value (b. femoris) and tenderness rating (semitendinosus), respectively. These findings are in general disagreement with the reports of Forrest (1962) and Carpenter et al. (1965b), which suggested low but significant relationships between marbling and tenderness measures. Furthermore, marbling score did not appear to be significantly related to moisture losses in any of the muscles, with the exception of the semitendinosus during thawing (Table 3), which disagrees with the findings of Oldfield et al. (1966).

Table 3—Simple correlation coefficients between marbling scores and palatability ratings, shear force values and weight losses of individual muscles from ovine leg steaks.

Muscle	Tenderness rating	Juiciness rating	Shear force (kg)	Thaw drip loss (%)	Cooking loss (%)	Total moisture loss (%)
Rectus femoris	0.10	0.30**	—	−.02	−.10	−.02
Vastus lateralis	0.00	0.25**	—	−.03	−.11	−.10
Biceps femoris	0.07	0.01	0.18**	−.06	−.05	−.00
Semitendinosus	0.13*	0.27**	—	−.15*	−.06	−.10
Semimembranosus	0.06	−.03	0.08	−.06	−.07	−.02
Overall	0.09	0.29**	0.14*	−.08	−.09	−.07

*P < .05. **P < .01.

Table 4—Simple correlation coefficients between chronological age and quality attributes of individual muscles in ovine leg steaks.

Muscle	Tenderness rating	Juiciness rating	Marbling score	Shear force (kg)
Rectus femoris	−.28**	0.03	0.04	—
Vastus lateralis	−.48**	−.02	0.09	—
Biceps femoris	−.38**	−.08	0.02	0.25**
Semitendinosus	−.21**	−.01	0.07	—
Semimembranosus	−.31**	−.11	0.11	0.33**
Overall	−.46**	−.04	0.07	0.33**

**P < .01.

Chronological age had no significant effect on either marbling or juiciness scores (Table 4), in general disagreement with the relationships previously reported utilizing physiological maturity reported by Batcher et al. (1962), Cassard et al. (1965) and Smith et al. (1970a; 1970b). Chronological age was significantly related ($P < .01$) to measures of shear force for b. femoris and semimembranosus and to taste panel tenderness for all muscles (Table 4). The latter finding agrees with the reports of Batcher et al. (1962), Cassard et al. (1965) and Smith et al. (1970b), who used physiological maturity

as the independent variable. However, some of this effect may be due to the significant ($P < .01$) relationship observed between chronological age and percent cooking loss (Table 2).

The relationships in Table 4 for chronological age with taste panel tenderness and juiciness ratings are comparable to but slightly lower than the relationships ($r = -.52$ and $-.12$, respectively) reported by Smith et al. (1969).

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T. D. PATE, R. O. SHULER and R. W. MANDIGO
Animal Science Department, University of Nebraska, Lincoln

THE INFLUENCE OF GLUCONO DELTA LACTONE ON CURED HAM COLOR AND COLOR STABILITY

SUMMARY—Pairs of hams from 72 crossbred barrows slaughtered at three weight intervals (68.2, 90.9, and 113.6 kg) were assigned to three treatments of glucono delta lactone (GDL) (0, 30, or 60 g/l) and two post cure aging periods (1 or 14 days). Following post cure aging, samples from the boneless hams were exposed to light (200 ft-c) for various time intervals (0, 30, 60, 90, 120, 150, 180, and 1440 min) and nitric oxide- and total-pigments extracted with acetone/water and acetone/acid respectively. There were no significant differences in color formation or color stability of hams aged 1 or 14 days. The influence of GDL on nitric oxide- and total-pigment formation and stability was nonsignificant at most exposure intervals. Chemical analysis showed a significant decrease in moisture content of hams with increasing amounts of GDL added. Fat, chloride, and ash levels were not different for the three GDL treatments. Residual nitrite levels were found to be significantly lower for the 30 g/l of GDL in hams extracted one day post cure.

INTRODUCTION

IN COOKED CURED meats, particularly hams, color is an important quality factor. The pink color formed by the nitric oxide hemochrome pigments on heating must be desirable initially and also resistant to fading. Watts et al. (1955) and Erdman and Watts (1957) noted color fading is affected most markedly by light. Tarladgis (1962) states that fading is a light induced dissociation of nitric oxide from the hemoglobin complex, and can be delayed by adding excess nitrite and by strengthening the reducing conditions.

Many different reducing agents have been used in curing meats to stimulate color formation. Ascorbic acid and sodium ascorbate have been found to increase color and decrease fading in hams (Henrickson et al., 1956). Kelley and Watts (1957) found cysteine and glutathione to have the same effect as ascrobic acid in catalyzing color reactions; however, in the absence of nitrite it will degrade the heme, according to Siedler and Schweigert (1959) Mullins et al. (1958) found that the addition of reducing agents did not increase color development or stability, which is contrary to the findings of others (Henrickson et al., 1956; Kelley and Watts, 1957; and Siedler and Schweigert, 1959).

A new product, glucono delta lactone (GDL), has been used successfully in cooked comminuted products to give a more rapid and improved cure color development (Meester, 1965). Sair (1963 and 1965) discussed the commercial development and application of GDL. His work was an application of GDL to sausage emulsions. GDL reacts little or not at all when added to the meat at low temperatures (4.4°C), then decreases the pH when the meat is heated (66°C), by conversion to gluconic acid (Anon., 1964). A relatively high pH is favorable to water- and fat-binding properties, and a relatively low pH is favorable to cured color formation.

GDL aids in a rapid and more effective formation of the cured color pigment nitric oxide hemochrome without being harmful to other quality factors (Meester, 1965). Due to the success of GDL in sausage products, a study of possible application to cured hams was felt desirable. This experiment was designed to determine the effect of GDL on cured color development and color stability of hams.

MATERIALS & METHODS

HAMS WERE FROM 72 crossbred barrows slaughtered at three weight intervals (68.2, 90.9, and 113.6 kg). Following a 24 hr carcass chilling at 2.8°C, right and left hams were excised and assigned to three levels of glucono delta lactone (GDL), 0, 30, or 60 g/l, and 1 or 14 days of post cure storage.

Curing

The hams were skinned, defatted, weighed and stitch pumped to 110% of their green weight with brine. The brine contained 101.4 g/l of sodium chloride; 61.4 g/l of prepared cure (Griffith Co.–RC612A: salt, sugar, dextrose, sodium nitrate, sodium nitrite, sodium erythrobate, sodium bicarbonate); 32.8 g/l of polyphosphates (Griffith Co.–FOS); 10.5 g/l of dextrose; water; and 0, 30, or 60 g/l of GDL (Griffith Co.).

Brines were prepared fresh prior to use, kept cold to avoid hydrolysis and the evolution of nitric oxide which is associated with rapid loss of nitrites and a shift in pH. The hams were then boned and the seam fat was removed. The boneless hams were stuffed in fibrous casings and placed in stockinettes, pressed in steel molds, and placed immediately in the smokehouse.

Smoking and curing

A 10-hr smoking cycle, starting at 54°C followed by an increasing temperature to 93°C to fully cook the hams (65°C internally), was used. The smoked and fully cooked hams were removed from the smokehouse, showered, and chilled overnight at 2.8°C.

Color evaluation

The cured, smoked, and fully cooked hams were evaluated after 1 day or were stored for 14 days. All pigment extraction procedures were carried out in a darkened, refrigerated laboratory cooler (2.8°C) illuminated by a 60w red light. Samples were prepared by removing a 7.5 cm section from the center of each ham and trimming all outside edges. This section was then ground through a 32 mm grinder plate and mixed. Thirty-two 10 gm samples were weighed from each ground section and spread uniformly over the surface of petri dishes. The remaining ground ham was placed in a sample jar and frozen for chemical analysis.

The samples were placed in a light chamber for 0, 30, 60, 90, 120, 150, 180, and 1440 min and exposed to 200 ft-c of cool, white deluxe fluorescent light. After exposure, the nitric oxide- and total-pigments were extracted with acetone/water and acetone/acid solutions respectively, as described by Hornsey (1956). The nitric oxide-pigments were read at 540 mμ and the total-pigments at 512 and 640 mμ on a Spectronic 600 spectrophotometer.

Chemical analysis

The frozen samples were thawed in a 2.8°C cooler overnight. Chloride was determined by precipitation with silver nitrate as described by Kamm (1964).

The samples were analyzed for moisture, fat, and ash using the methods described by the A.O A.C. (1965).

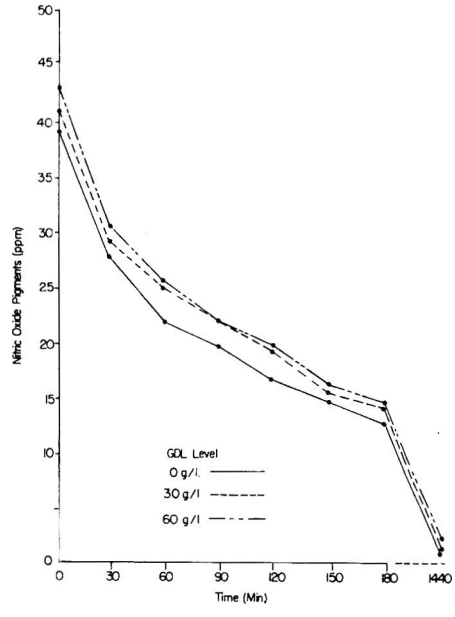

Fig. 1—Nitric oxide pigments (ppm) extracted following exposure to 200 ft-c of light – 1 day post cure.

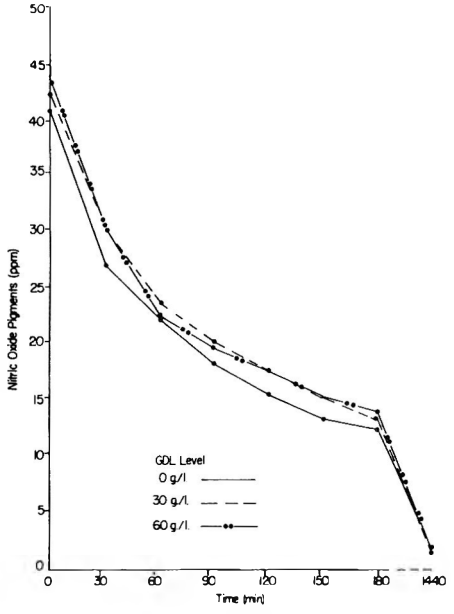

Fig. 2—Nitric oxide pigments (ppm) extracted following exposure to 200 ft-c of light — 14 days post cure.

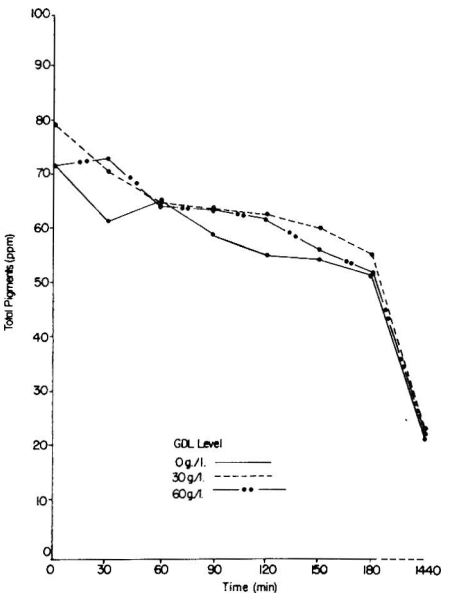

Fig. 3—Total pigments (ppm) extracted following exposure to 200 ft-c of light — 1 day cure.

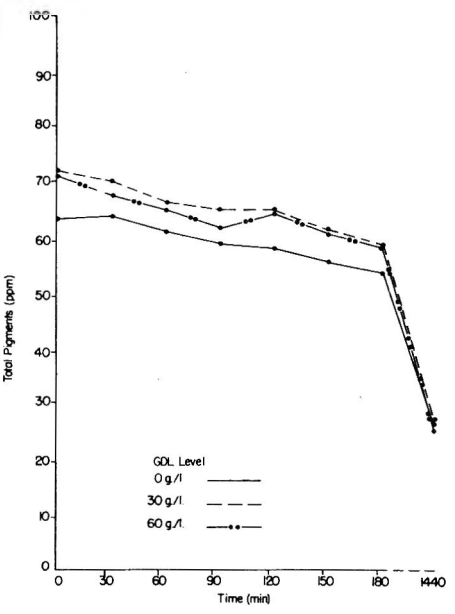

Fig. 4—Total pigments (ppm) extracted following exposure to 200 ft-c of light — 14 days post cure.

The amount of nitrite was determined for duplicate 5g samples using the spectrophotometric sulfanilic acid method described by the A.O.A.C. (1952).

RESULTS & DISCUSSION

INITIAL nitric oxide-pigment levels in the hams and those retained after exposure to light are shown in Figures 1 and 2. Color loss on exposure to light is in agreement with the findings of Erdman and Watts (1957). Both 1 day samples and those stored for 14 days prior to exposure show a very rapid initial decrease in nitric oxide-pigments. No significant differences ($P < .05$) were found between hams stored 1 and 14 days, indicating that no apparent increase in stability was gained in the 14 day period. This approximated the length of time between processing and retail marketing of hams.

In hams extracted 1 day post cure (Fig. 1), initial levels of nitric oxide-pigments were not significantly different between GDL treatments ($P < .05$). GDL influenced color stability only after 1440 min (24 hr) of exposure to light. After 1440 min of exposure to light, hams treated with 60 g/l of GDL had significantly ($P < .05$) greater nitric oxide-pigment levels than control hams or those treated with 30 g/l of GDL. Under these test conditions, hams lack sufficient color to be merchantable after 1440 min of exposure. In hams extracted 14 days post cure, no significant differences ($P < .05$) were found in nitric oxide-pigment levels, either initially or after exposure to light (Fig. 2).

The failure of the addition of GDL to increase initial nitric oxide-pigment is contrary to the findings of Meester (1965) who found that GDL improved cured color development in cooked comminuted meat. GDL has also been successful in the frankfurter industry (Anon., 1964). The addition of GDL to frankfurters produced a more pronounced color that lasted longer. Improvement in color stability was noted at only one exposure interval in this experiment and it is questionable as to how much emphasis can be placed on this improvement. Although GDL has been credited with improving cured color stability (Anon., 1964; Meester, 1965), no improvement in cured color stability with the addition of GDL was found in this study.

Total-pigments were compared between the GDL treatments. The pigments were extracted to measure the efficiency of nitric oxide-pigment extraction, as described by Hornsey (1956). All efficiency ratios (total-pigments 512/640 mμ) were within the acceptable range set by Hornsey (1956). In hams extracted 1 day post cure, no significant differences ($P < .05$) were found in total-pigment levels between the three GDL treatments, either initially or throughout the exposure interval (Fig. 3). In hams extracted 14 days post cure, the initial total-pigment levels of hams receiving the zero level of GDL were significantly ($P < .05$) lower than those of hams receiving the other two GDL treatments (Fig. 4). However, after exposure to light, no significant differences ($P < .05$) in total-pig-

Table 1—Chemical composition of hams subjected to different levels of GDL treatment.[1]

Analysis	Days	GDL Zero Level	GDL 30 g/l	GDL 60 g/l	Standard Deviation
Moisture %	1	67.56[a]	66.18[b]	64.65[c]	0.51
	14	66.66[a]	64.59[b]	63.01[c]	0.53
Fat (EE) %	1	7.16	7.80	8.23	0.44
	14	7.43	8.27	7.72	0.43
Chloride %	1	1.62	1.56	1.57	0.02
	14	1.70	1.54	1.65	0.02
Nitrite (ppm)	1	17.91[a]	10.95[b]	18.17[a]	2.21
	14	18.04	12.58	17.91	2.71
Ash %	1	2.19	2.18	2.05	0.06
	14	2.46	2.47	2.29	0.12

[1] Means within rows with different superscripts are significantly different ($P < .05$).

ments were noted between the GDL treatments.

Since GDL acts in nitric oxide-pigment formation to provide a more acid medium (Anon., 1964 and Meester, 1965), it was not expected to have an effect on total-pigment levels. It is not clear whether the difference in the initial levels in hams extracted 14 days post cure is due to the effect of GDL or whether a chance occurrence in sampling took place. Little work has been done concerning the action of GDL on total-pigment levels in cured meats.

Chemical analysis of the hams revealed that the moisture content significantly ($P < .05$) decreased with increasing levels on GDL (Table 1). This is contrary to earlier work cited (Anon., 1964 and Meester, 1965). Moisture levels in frankfurters and in hams were improved by GDL due to the delayed acid formation, thus retaining the water-binding properties of the meat. No explanation can be given for the reverse reaction that resulted in this experiment. Further studies involving other processing conditions and evaluation of possible pH shifts are needed.

Fat, chloride, and ash concentrations were not found to be significantly different ($P < .05$) for the different GDL treatments. If is somewhat surprising that the fat content did not increase as the moisture content decreased, as noted by Stant et al. (1968). They found that in all components of the carcass, moisture levels decreased as fat levels increased. Therefore, differences in moisture levels would be expected to be accompanied by differences in fat levels.

Residual nitrite levels were significantly ($P < .05$) lower for the 30 g/l level of GDL in hams extracted one day post cure, than for either the zero or 60 g/l levels of GDL. In hams extracted 14 days post cure, there were no significant differences ($P < .05$) in residual nitrite levels for the different levels of GDL. Meester (1965) found that with the addition of GDL, residual nitrite decreased as cured color increased. However, since no differences were found in nitric oxide-pigment levels, this does not explain the difference in residual nitrite. The added levels of nitrite being equal, more color formation should have occurred in the 30 g/l treated hams for less residual nitrite to have been retained.

The effect of the three slaughter weights used in this experiment (68.2, 90.0, 113.6 kg) on color development, color stability, and chemical composition of ham has been discussed by Mandigo et al. (1970).

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T. MOTOHIRO and N. INOUE
Laboratory of Marine Food Technology, Faculty of Fisheries, Hokkaido University, Hakodate, Japan

EFFECT OF SOME CHEMICAL COMPOUNDS ON THE BUFFERING CAPACITY OF HORSEHAIR CRAB (Erimacrus isenbeckii) MUSCLE DURING INTERMOLT STAGES

SUMMARY—Calcium, phosphoric acid, hexosamine and amino acids were determined in horsehair crab muscle during the intermolt stages, to study possible variations in the buffering capacity. The calcium contents varied from 1.1—2.2 mg/g throughout the intermolt cycle; phosphoric acid and hexosamine level varied in a similar way to the calcium content and the amino acid level was almost constant. The buffering capacity of horsehair crab muscle was also shown to vary throughout the intermolt stages. It was strong in papercrab muscle and weak in hardcrab muscle. The organic and inorganic reserves affected the buffering capacity, so that removal by washing with water resulted in a weakening of the buffering capacity.

INTRODUCTION

IN THE previous paper (Motohiro and Inoue, 1970), it was shown that a cause of abnormally high pH in canned crabmeat was the use of crab as raw material after ecdysis. The phenomenon did not occur in canned meat if the crab had a hard shell, but it was significant if the crab had paper shell, as occurs soon after ecdysis.

Organic reserves are an important feature of crustacean physiology. They are used to meet the special demands for materials and energy which occur during molting (Drach, 1939). Accumulation of organic reserves characterizes the pre-ecdysis stage (Hoet and Kerridge, 1926); intermolt mineral accumulation is of general occurrence in decapods.

The main portion of mineral reserves consists of Ca and Mg phosphate (Paul and Sharpe, 1919). The cations are utilized in post-ecdysial cuticular mineralization, and phosphates are necessary for chitin synthesis (Renaud, 1949). Therefore, it is assumed that there are many organic and inorganic reserves in crab muscle in stages pre- and post-ecdysis, and that there are differences in buffering capacity in crab muscle from stage to stage (Suyama, 1958).

If buffering capacity is strong in papercrab and weak in hardcrab, the former would be expected to maintain high pH value, the latter low. The aim of the present investigation was to find out whether such organic and inorganic reserves in horsehair crab at different intermolt stages affect the buffering capacity. This paper reports on the effect of calcium, phosphoric acid, hexosamine and amino acid on the buffering capacity of horsehair crab muscle.

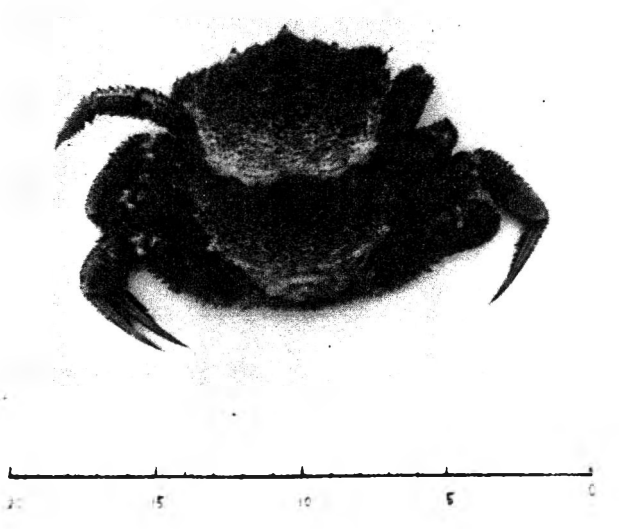

Fig. 1—Horsehair crab (♂) immediately after ecdysis. Photograph by T. Takahashi at the Wakkanai Fisheries Experimental Station.

Table 1—Stages in intermolt of the raw horsehair crab (Erimacrus isenbeckii).

Sample No.	Stage[1]	Name	Characteristics	Activity level	Feeding	Weight of body (g)	Length of carapace (cm)	Sex
1	A_1	Newly molted	Continued water absorption and initial mineralization	Slight	None	240	8.0	♂
2	$B_2 \sim C_1$	Paper shell	Active endocuticle formation, chelae hard;	Considerable	None	215	7.5	♂
		Hard	tissue growth begins	Full	Yes			
3	C_4	Hard	Completion of exoskeleton; membranous layer formed	Full	Yes	235	8.0	♂
4	$D_1 \sim D_2$	Pre-ecdysis	Exocuticle secretion begins	Full	Yes	240	8.0	♂
5	E	Molt		Slight	None	220	7.5	♂

[1]Classified according to Drach (Compt. rend. 202: 2103–2105, 1939).

EXPERIMENTAL

Materials

12 horsehair crabs *(Erimacrus isenbeckii)*, 5 male and 7 female, were caught October 9, 1967, at 45° 30′E by crabpot and placed in a rectangular glass aquarium (200 by 100 by 100 cm) in Wakkanai Fisheries Experimental Station, where successful cultivation of the crab was first achieved.

The tankwater, at 10–13°C, was filtered continuously through a sandbed and circulated at the rate of 150 liters/hr. The animals were given a mixture of Alaska pollack *(Theragra chalcogramma)*, Atka mackerel *(Pleurogrammus azonus)*, herring *(Clupea pallasii)*, pink shrimp *(Pandalus plavceros)* and flat fish *(Cleisthenes pinetorum herzensteini)* on alternate days, about 70 g per crab.

Ecdysis started suddenly for 3–4 hr at night-time and finished at early morning. The physiological observations on ecdysis will be reported in another communication.

The crab after ecdysis (stage E in Table 1), having been cultivated for 48 days, from the tank, was placed in a box with sufficient dry ice and sent to the laboratory by air. The appearance of the crab after molting is shown in Figure 1.

4 other crabs were kept in the tank until reaching the respective stages A_1, B_2–C_1, C_4 and D_1–D_2. They had been cultivated in the tank for 62, 68, 106 and 116 days, respectively. Although the intermolt period varied depending on food given and individual physical condition, it usually took 7 days to reach stage A_1 from ecdysis, 10 days to reach stage D_1–D_2.

The classification proposed by Drach (1939) was applied in this experiment to distinguish the crab at different intermolt stages. Table 1 indicates a detailed record of the materials with their physiological characteristics.

Methods

Items and procedure. Calcium content was determined by grinding the 1st leg muscle from each crab, heating it in an electric furnace at 600°C for 7 hr until organic material had disappeared, extracting with deionized water, filtering, adding the filtrate with 5% $(NH_4)_2C_2O_4$ to precipitation, washing the precipitate with sulfuric acid (1:1) and titrating the solution with 0.1 N $KMnO_4$.

Phosphoric acid content was determined by using the filtrate obtained by a procedure similar to the one just described, shaking the filtrate with 5 N sulfuric acid, 2.5% $(NH_4)_2MoO_4$ and reduction material (mixture of 1-amino-2-naphthol-sulfuric acid, $NaHSO_4$ and Na_2SO_4), leaving for 10 min exactly and esti-

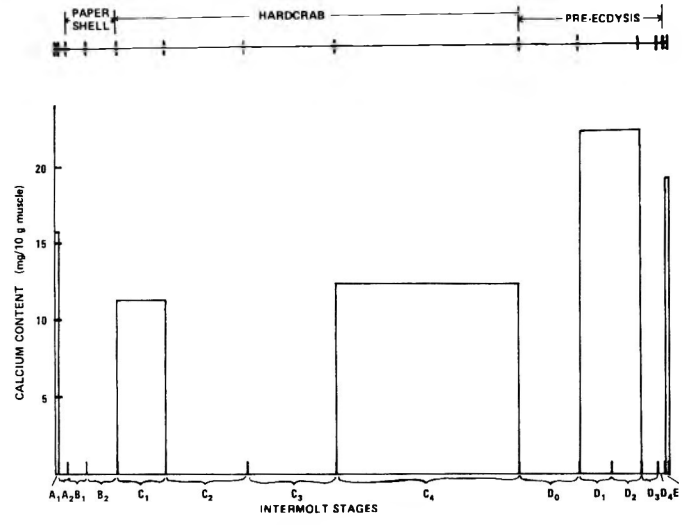

Fig. 2–Change in calcium content of horsehair crab throughout intermolt stages.

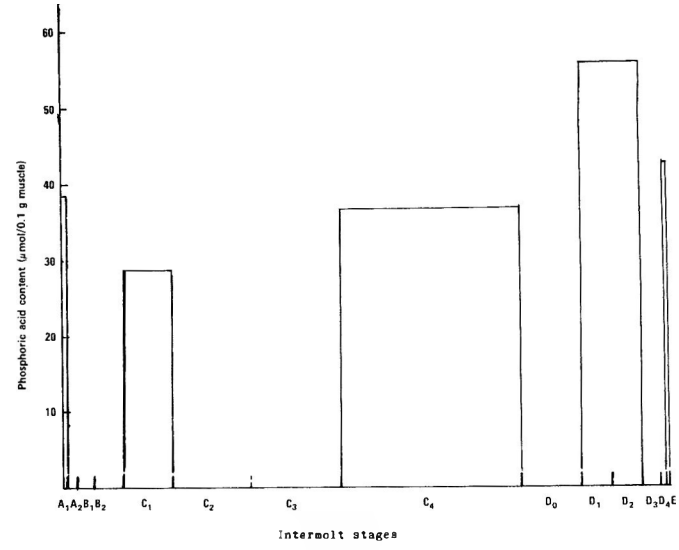

Fig. 3–Change in phosphoric acid content of horsehair crab throughout intermolt stages.

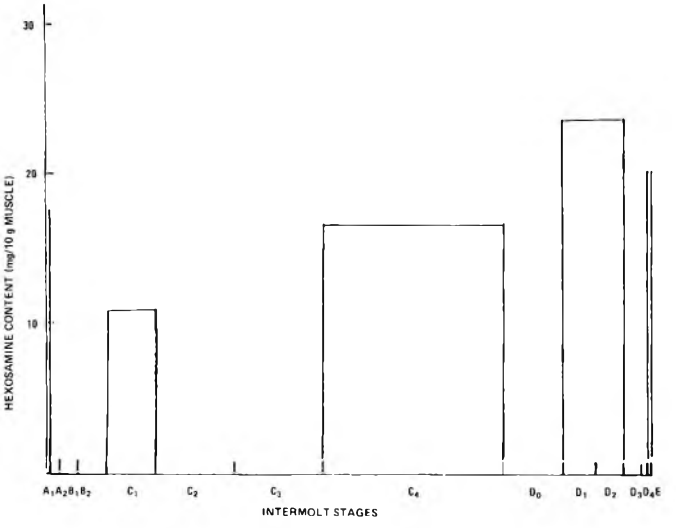

Fig. 4–Change in hexosamine content of horsehair crab muscle throughout intermolt stages.

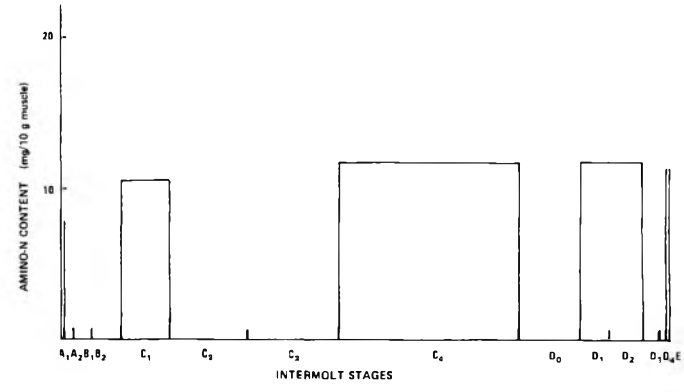

Fig. 5–Change in amino-N content of horsehair crab muscle throughout intermolt stages.

mating the solution at 660 mμ by means of Hitachi Perkin-Elmer 139 UV-VIS Spectrophotometer (AOAC, 1965).

Hexosamine content was determined by the method of Rondle and Morgan (1955). This method involves hydrolyzing the ground muscle with 4 N hydrochloric acid in a sealed glass ampule (φ 7 mm by 70 mm) for 15 hr at 105°C, filtering the hydrolyzate, neutralizing, mixing the solution with acetylacetone and sodium carbonate, heating for 20 min in a boiling bath, cooling under running water, shaking the solution gently with ethanol and Ehrlich's reagent, heating for 10 min at 70°C, cooling at room temperature and estimating the colored solution at 530 mμ by means of the spectrophotometer.

Buffering capacity was estimated by adding the ground muscle to deionized water, filtering and adjusting pH value of the filtrate to 3.20 with 0.033 N hydrochloric acid. The solution was mixed with an aliquot of 0.033 N barium hydroxide and pH value estimated. The amount of amino acid was determined by the standard Pope-Stevens' method.

Soaking crab muscle in water. The 1st leg muscle without shell was placed in a porcelain tray and washed with running deionized water at the rate of 10 ml/sec for 10 min. The procedure was repeated 2 or 3 times under similar conditions, to prepare the repeat soaking sample.

RESULTS

THE CALCIUM, phosphoric acid, hexosamine and amino acid contents in crab leg muscle during the intermolt stages are shown in Figures 2–5, respectively. In these figures, the intermolt cycle is marked on the X-axis with 5 stages from A to E with percent duration, as classified by Drach (1939). The calcium contents varied from 1.1 mg/g (stage C_1) to 2.2 mg/g (stage D_1-D_2) during the intermolt cycle. This indicates that calcium accumulation continues from stage C to stage D for subsequent shell calcification after molting, and suggests that the higher calcium level is still maintained at stage E.

Phosphoric acid and hexosamine levels varied similarly to the calcium content throughout the intermolt cycle. Thus, phosphoric acid and hexosamine may, like calcium, be involved in shell classification and chitin synthesis.

The amino acid content was, however, somewhat different from other compositions, and its level was almost constant throughout the intermolt cycle, varying slightly from 0.8 mg/g (stage A_1) to 1.1 mg/g (stage C_4). Presumably, the amino compounds in crab muscle are not affected by the physiological change, since many other organic and inorganic reserves relate to the molting.

Buffering capacity of horsehair crab muscle in different intermolt stages is shown in Figure 6. The buffering capacity of stage E crab is obviously larger than that of stage C_4 crab. Figure 7 shows that the buffering capacity of muscle of stage E was reduced by repeated washing so as to resemble that from crab at stage C_4.

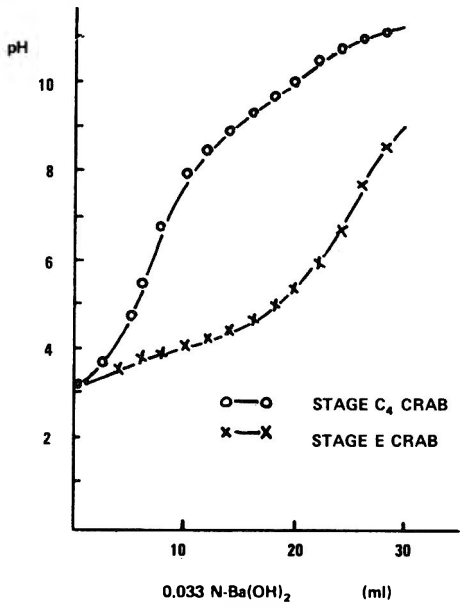

Fig. 6—Buffering capacity of horsehair crab muscle in different intermolt stages.

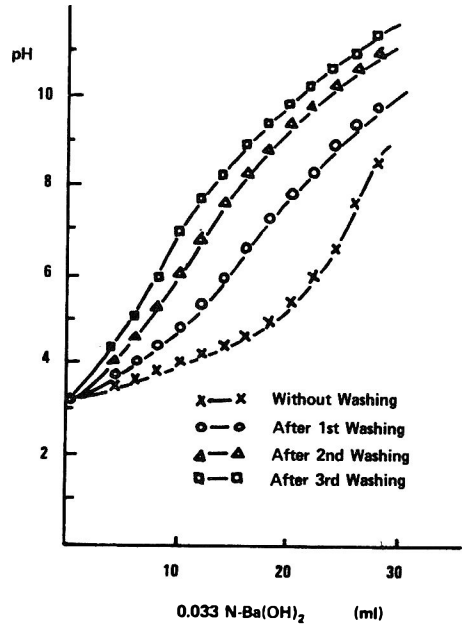

Fig. 7—Effect of washing on buffering capacity of soft-shell (stage E) horsehair crab muscle.

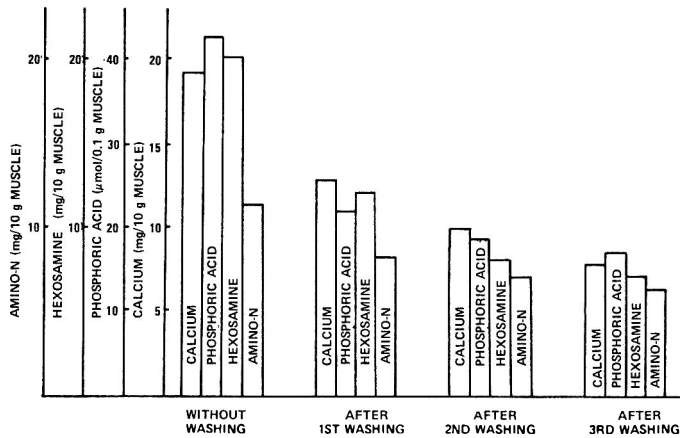

Fig. 8—Effect of washing on chemical compositions in horsehair crab muscle.

The amounts of calcium, phosphoric acid, hexosamine and amino acid which had accumulated were decreased by washing (Fig. 8). It is likely, therefore, that these constituents contribute to the buffering capacity of crab muscle.

DISCUSSION

THE RELATIONSHIP between chemical composition and the buffering capacity of horsehair crab at the intermolt stages has not previously been reported because of the difficulty concerned with artificial cultivation of this crab. Present results show that the buffering capacity does vary throughout the intermolt cycle according to certain changes in the amounts of calcium, phosphoric acid and hexosamine, and perhaps other compounds, but not the amino acids. This fact leaves scope for variation in the pH during the cycle.

Washing the muscle decreases the buffering capacity and results in a lowering of the pH value, as it removes substances from the muscle. From this it may be deduced that if papercrab (which has an excessively high pH) is used as the raw material for canning, there should be substantial periods of washing.

There is always danger that food given artificially is not what the crab would have selected in the open sea, so that the body constituents and physical activity of the cultivated crab might be different from those of crabs fed naturally. After considering this point, it was decided that food given in this experiment would be a mixture of flesh from several fish species, so that the effect of artificial feeding might be reduced.

It may be wondered whether paper-crab washed with water for weakening the buffering capacity is any less desirable as an article of canned food. Further work is planned to answer this question, and also to find out which constituent among the organic reserves will effect the strongest buffering capacity.

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M. YAMAGATA, K. HORIMOTO and C. NAGAOKA
Japan Frozen Foods Inspection Corporation, Tokyo 100, Japan

ACCURACY OF PREDICTING OCCURRENCE OF GREENING IN TUNA BASED ON CONTENT OF TRIMETHYLAMINE OXIDE

SUMMARY—To predict green tuna after cooking, a practical measurement to select green tuna using 100 pieces of iced and clipper-frozen yellowfin tuna was discussed. The most suitable sampling portions, the lowest level of trimethylamine oxide (TMAO) and the total trimethylamine content (TTMA) in tuna muscles were examined. It was recognized that the prediction of green tuna after cooking would be possible by determining TTMA content at the superficial layer of the central dorsal raw muscle (E portion). The lowest level of TMAO-N was 8–9 mg%, and that of TTMA-N was 11–12 mg% in an iced and 9 mg% in a clipper-frozen green tuna. The predictive measurement accuracy was 96% on the lowest level of TMAO-N or TTMA-N.

INTRODUCTION

GREENING of tuna has been a serious quality problem for tuna fishermen, freezers and canners. It is possible to predict green tuna before cooking by determining the trimethylamine oxide (TMAO) content in raw meat, since the presence of TMAO is an important factor of greening tuna meat (Nagaoka and Suzuki, 1964, and Koizumi and Hashimoto, 1965).

To accurately predict green tuna, 4 points must be noted. First, to predict green tuna by determining TMAO before cooking, the most suitable sampling portions must be considered using many samples. This is necessary, since greening action during cooking differs in various parts of fish bodies in relation to the TMAO content (Yamagata et al., 1969). Secondly, the lowest level of TMAO-N content in raw green meat must be found. Thirdly, it must be determined whether total trimethylamine (TTMA) determination without trimethylamine (TMA) determination in raw meat is equally effective in predicting green tuna. The fourth point is that the accuracy of the predictive measurement must be calculated.

In this paper, the most suitable sampling portions and the lowest level of TMAO and TTMA content in raw meat, for the purpose of selecting green tuna before cooking, were determined.

EXPERIMENTAL

Samples

100 samples (50 iced and 50 clipper-frozen fish) of yellowfin tuna (*Thunnus albacares*) were chosen for samples, frozen and stored at −25°C in a commercial cold-storage warehouse. These samples were divided into 3 groups:

Group 1. 50 pieces of iced fish, in which TTMA-N mg% had been determined in the superficial dorsal meat (E portion of Fig. 1), were chosen for samples. These consisted of 20 pieces with TTMA-N mg% below 3.0 mg%, 10 pieces between 3.1 and 5.0 mg%, 10 pieces between 5.1 and 9.0 mg% and 10 pieces with TTMA-N mg% above 9.1 mg%.

Group 2. This group consisted of 20 pieces of clipper-frozen fish, chosen by the above method, which had TTMA-N mg% above 10.0 mg%.

Group 3. 30 pieces of clipper-frozen fish were chosen as follows: 6 pieces with TTMA-N less than 3.0 mg%; 5 pieces with TTMA-N between 3.1 and 5.0 mg%; 6 pieces between 5.1 and 9.0 mg%; 7 pieces between 9.1 and 13.0 mg% and 6 pieces with TTMA-N greater than 13.1 mg%.

The reason for selecting the samples by the TTMA content is that the quantity of green tuna is rarely large in usual lots.

TTMA and TMAO determination

About 20 g of the muscle of each portion of the fish (Fig. 1, E, H and T) was removed in the frozen state, packed in polyethylene casing with dry ice and used for analysis. The TTMA and TMAO content was determined by the microdiffusion method (Yamagata et al., 1969).

TMA determination

The TMA content was also determined by the microdiffusion method of Beatty and Gibbons (1937).

Cooking procedure and sensory evaluation of cooked meat color

The head and tail of each fish were removed, the fish muscle from each portion cut horizontally along true red muscle into 2 halves then cooked for 220 min at a temperature of 104°C in a retort. The average temperature of the muscle after cooking was 79.2°C at the center and 85.7°C at the superficial layer. Each half-muscle was then longitudinally divided in half, resulting in 4 quarters of each portion. After cleaning, the color of each cooked loin was evaluated on a 5-point scale: 1 = normal; 2 = slightly dark-brown; 3 = partially green; 4 = slightly green and 5 = moderately or extremely green. The cooked whole meat (4 loins) was then evaluated by summing the scores for each of the quarters, where A = a total score of 4–5 points, B = 6–8 points, B′ = 9–12 points, C = 13–16 points and D = 17–20 points.

RESULTS & DISCUSSION

Sampling portions for evaluation of green tuna

Because of the method of harvesting tuna, where gaff hooks may injure meat from the fish body, it was thought that tail-end muscles would be suitable portions for obtaining muscle samples. But Yamagata et al. (1969) recommended that the interior portion of dorsal muscle be taken for assessing green tuna before cooking. To confirm this recommendation, research on the relationships between TMAO content of each portion of raw meat was conducted, using groups 1 and 2 (70 pieces of fish). The most suitable sampling portion for predicting green meat color was determined using

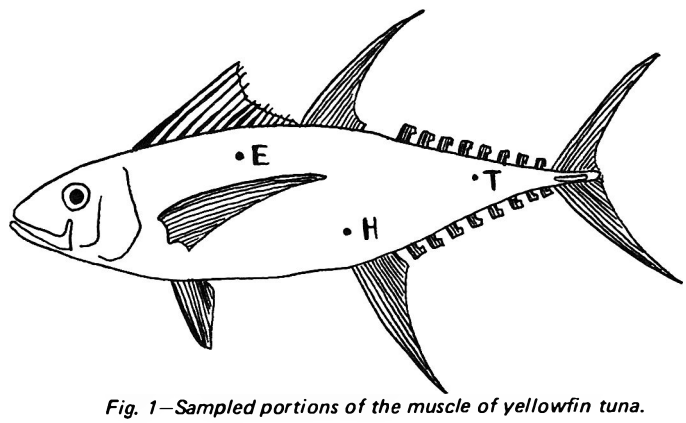

Fig. 1—Sampled portions of the muscle of yellowfin tuna.

Sampled portions: *E: 2–3 cm deep from the superficial dorsal meat, at the section of seventh spine of the first dorsal fin.*

H: 2–3 cm deep from the superficial ventral meat, near the anus.

T: 2–3 cm deep from the superficial tail meat, at the section of fourth dorsal finlet.

Table 1–Correlations between TMAO content[a] and color grades of cooked iced and clipper-frozen meat.

TMAO-N mg %	Color grades	A	B	B′	C	D	Total
	13.0 and Above				5	14	19
	12.0–12.9				2	2	4
	11.0–11.9					2	2
	10.0–10.9			1	2	2	5
	9.0–9.9			2	6		8
	8.0–8.9			1	1		2
	7.0–7.9			5			5
	6.0–6.9		2	3			5
	5.0–5.9		2	3			5
	4.0–4.9	1	2	2			5
	3.0–3.9	3	5	1			9
	2.0–2.9	2	4	1			7
	1.0–1.9	9	5				14
	Below 0.9	7	3				10
	Total	22	23	19	16	20	100 Pieces
							r = 0.936

[a]TMAO contents were determined at E portion of the iced and clipper-frozen fish.

Table 2–Correlations between TTMA Content[a] and color grades of cooked iced meat.

TTMA-N mg %	Color grades	A	B	B′	C	D	Total
	13.0 and Above				2	1	3
	12.0–12.9						0
	11.0–11.9			1	1		2
	10.0–10.9			1			1
	9.0–9.9			3	1		4
	8.0–8.9			3			3
	7.0–7.9		2	1			3
	6.0–6.9		1	1			2
	5.0–5.9		2				2
	4.0–4.9	2	4				6
	3.0–3.9	2	3	1			6
	2.0–2.9	7	5				12
	1.0–1.9	4	2				6
	Below 0.9						0
	Total	15	19	11	4	1	50 Pieces
							r = 0.885

[a]TTMA contents were determined at E portion of the fish.

epaxial (E), hypoxial (H) and tail-end (T) portions, as indicated in Figure 1.

The correlation coefficient (r) between TMAO content and cooked meat color of the fish for each E, H and T portion is 0.960, 0.864 and 0.837, respectively, as shown in Figure 2. It was thus concluded that the E portion is the most suitable sampling portion for predicting green tuna, as previously reported by Yamagata et al. (1969). Removing the sample from this portion of the fish body did no damage, since only 10–20 g of muscle was removed. In this examination it has not been discussed, because the TMAO content of the muscles adjacent to fin, head and belly was deeper than of other ordinary muscles (Yamagata et al., 1969).

Lowest level of TMAO-N for selection of green tuna

For selection of green tuna, the lowest level of TMAO-N content resulting in green tuna must be determined. Correlations between TMAO content in raw meat (E portion) and cooked meat color score are shown in Table 1, with a correlation coefficient (r) of 0.960. When color scores of C and D are considered to represent fish with undesirable color, the predictive measurement accuracy is 96% on a TMAO-N level of 8 or 9 mg%. It was determined that no difference is found, for the purpose of selecting a fish lot, between lots with TMAO-N content of 8 and those with 9 mg%.

Lowest level of TTMA-N for selection of green tuna

The lowest level of TTMA content in raw meat for the purpose of selecting the green tuna before cooking was determined using iced and clipper-frozen yellowfin tuna. Correlations between TTMA content in raw meat (E portion) and cooked meat color score of the iced fish are shown in Table 2, with a correlation coefficient (r) of 0.885. The predictive measurement accuracy is 96% with a TTMA-N content of 11 or 12 mg%. The lowest level of TTMA becomes higher than that of TMAO, for an iced fish is not fresh when compared to a clipper-frozen fish. The maximum TMA-N content in the E portion of the raw iced fish muscle was 4.0 mg%, with an average of about 2.0 mg%. It was found that the lowest levels of TTMA-N in iced fish are approaching the sum of the least amount of TMAO-N and TMA-N in raw meat.

Correlations between TTMA content in clipper-frozen raw meat (E portion) are given in Table 3, with a correlation coefficient (r) of 0.930. The predictive measurement accuracy was found to be

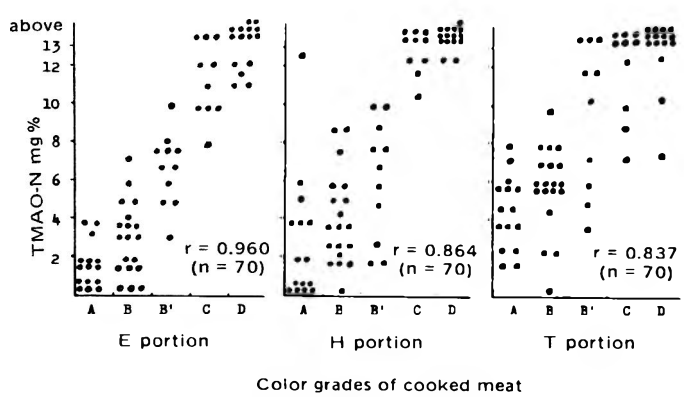

Fig. 2–Relations between TMAO content of each sampled portion in raw meat and color grades of cooked meat.

Table 3–Correlations between TTMA content[a] and color grades of cooked clipper-frozen meat.

TTMA-N mg %	Color grades	A	B	B′	C	D	Total
	13.0 and Above				3	14	17
	12.0–12.9				2	1	3
	11.0–11.9				2	3	5
	10.0–10.9			1	2	1	4
	9.0–9.9			1	3		4
	8.0–8.9			1			1
	7.0–7.9			1			1
	6.0–6.9		1	1			2
	5.0–5.9			2			2
	4.0–4.9	1	1				2
	3.0–3.9	1	1	1			3
	2.0–2.9	2	1				3
	1.0–1.9	2					2
	Below 0.9	1					1
	Total	7	4	8	12	19	50 Pieces
							r = 0.930

[a]TTMA contents were determined at E portion of the fish.

96% at a TTMA content of 9 mg%. In clipper-frozen fish, the lowest level of TTMA content in raw meat for selecting the green tuna before cooking was no different than TMAO selection, since the TMA-N content in the E portion of the raw meat is a maximum of 0.9 mg% and averages about 0.25 mg%. Failures of prediction in each table may be related to myoglobin content of the fish (Yamagata et al., 1970).

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W. J. ZIMMERMANN
Veterinary Medical Research Institute, College of Veterinary Medicine
Iowa State University of Science and Technology, Ames, Iowa

SALT CURE AND DRYING-TIME AND TEMPERATURE EFFECTS ON VIABILITY OF *Trichinella spiralis* IN DRY-CURED HAMS

SUMMARY—An official USDA dry-cure ham processing procedure was evaluated for efficacy in devitalizing trichinae. The procedure consisted basically of a 40-day salt cure followed by 10 days of drying at 95°F or above. Within the cure period, marked loss of viability was noted only for trichinae in muscle with brine concentrations approaching 8%. The drying temperature was the critical factor in devitalizing trichinae. 28 days' cure followed by 6 days of drying at 98°F destroyed viability of all parasites regardless of brine concentration. Other effective drying time-temperature combinations included: 125°F—4 days; 70°F—35 days and 40°F—190 days.

INTRODUCTION

PROCEDURES used to destroy or devitalize *Trichinella spiralis* larvae in ready-to-eat pork products include heat, freezing or curing procedures. Although a large majority of hams are precooked to at least 148°F, a significant number are processed by slow-cure procedures and known variously as country-style, Italian-style, southern-style or Smithfield-type hams.

Extensive studies were carried out by Ransom et al. (1920) to determine the effect of curing procedures on the viability of trichinae in various pork products. Trichinae in hams were rendered innocuous by either of the 2 following basic procedures: (1) The hams were cured by means of dry salt, applied at the rate of 4 lb per cwt of product, for a 40-day period at a temperature no lower than 40°F, followed by smoking or pale-drying for 10 days at a temperature no lower than 95°F; or (2) the hams were cured on the basis of 3 days' cure per lb of individual hams, with salt applied at a rate of 4 lb per cwt, with pickle also injected. This was followed by 48 hr of smoking at a temperature not lower than 80°F and finally by 20 days' drying at a temperature not lower than 45°F. Ransom et al. (1920) concluded that salt and drying temperature were primary factors affecting the trichinae. The salt undermines the viability of the parasite by dehydration, possible toxicity and lowering resistance to temperatures which would not normally affect the parasite.

Gammon et al. (1968) investigated another dry-cure procedure for hams and shoulders. The procedure consisted basically of the pork being dry-cured at 2 days per lb, followed by 30 days' hanging for salt equalization, rinsing, drying, smoking for 24 hr at 90–100°F and aging at 75°F. 4 wk of aging proved effective in negating the infectivity of the parasites. Crouse and Kemp (1969) used somewhat similar procedures for heavy hams and shoulders and found 3 weeks' aging to be effective in eliminating viable trichinae.

Allen and Goldberg (1962) determined that salt concentration had a direct effect on trichina viability. Trichinae were infective in ground pork to which salt was added and then stored in refrigerator for the following time and salt concentration combinations: 3 1/3% salt for 31–60 days (average 38); 2 1/2% salt for 49–79 days (average 64) and 2% salt for 63–92 days (average 80). Trichinae persisted

Table 1—Effect of standard curing-drying procedure on Trichinella spiralis viability in hams.

		Days						
	Salt	28	35	40	40 +	40 +	40 +	40 +
	Drying (98°F)				3	6	8	10
Muscle	No. hams	2	3	3	3	3	3	4
Gracilis	Mean salt (%)	5.19	5.32	5.18	5.42	3.9	4.33	2.7
	Mean moisture (%)	57.2	55.7	57.5	57.7	62.9	59.8	57.8
	Mean brine (%)	8.35	8.71	8.25	8.57	5.85	6.86	4.4
	Transmission	+1,+1	+1,-,-	+1,+1,-	-,-,-	-,-,-	-,-,-	-,-,-,-
Adductor	Mean salt (%)	2.40	3.52	3.56	2.22	2.47	2.76	2.8
	Mean moisture (%)	69.9	66.2	69.9	70.3	71.2	65.6	71.5
	Mean brine (%)	3.32	5.05	5.10	3.06	3.36	4.04	3.8
	Transmission	+2,+1	+3,+3,+2	+2,+1,+1	+2,+1,+1	-,-,-	-,-,-	-,-,-,-
Semimembranosus	Mean salt (%)	2.84	2.84	3.42	1.68	2.06	2.78	2.4
	Mean moisture (%)	69.7	63.5	65.6	68.03	70.3	65.1	68.2
	Mean brine (%)	3.92	4.30	4.96	2.41	2.85	4.10	3.5
	Transmission	+2,+2	D[a],+1,+1	D,+2,+1	+2,+1,+1	-,-,-	-,-,-	-,-,-,-
Biceps femoris	Mean salt (%)	0.34	0.43	0.50	0.64	0.59	0.76	0.7
	Mean moisture (%)	71.3	67.8	72.2	65.2	64.5	64.5	65.5
	Mean brine (%)	0.48	0.63	0.69	0.97	0.91	1.16	1.0
	Transmission	+4,+2	+4,+3,+3	+4,+2,+2	+3,+3,+2	-,-,-	-,-,-	-,-,-,-
Vastus intermedius	Mean salt (%)	0.41	0.95	1.04	0.83	1.52	1.83	2.1
	Mean moisture (%)	75.8	71.03	73.3	72.0	74.6	66.3	69.2
	Mean brine (%)	0.53	1.32	1.40	1.14	2.00	2.68	3.0
	Transmission	+4,+4	D,D,+3	D,D,+4	D,D,+4	-,-,-	-,-,-	-,-,-,-

[a]D–died.

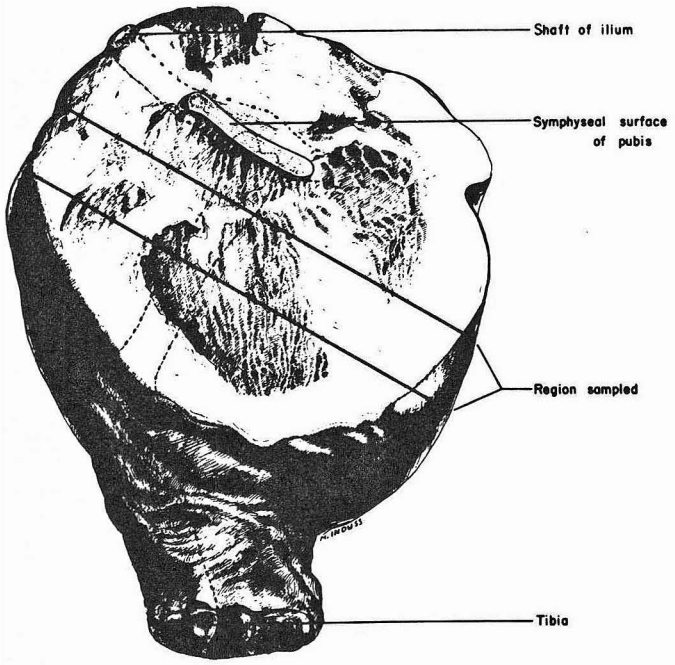

Fig. 1–Drawing of ham indicating region sampled.

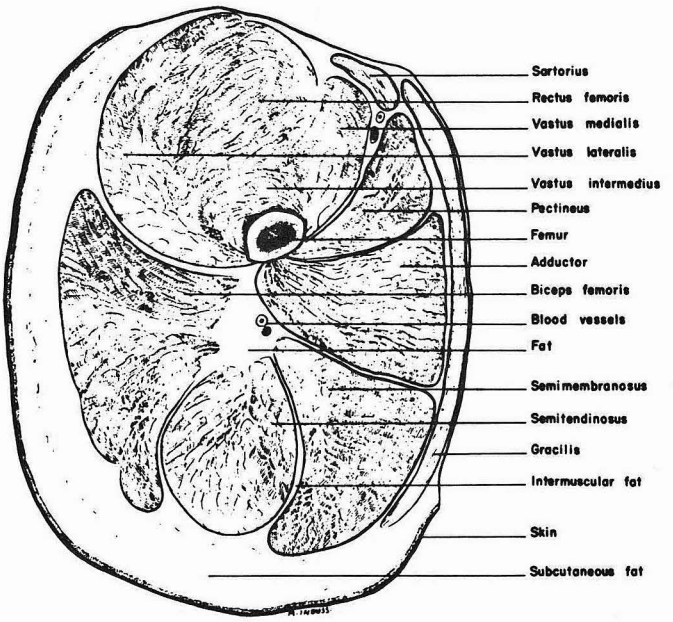

Fig. 2–Relative muscle location in ham slices examined in study.

longer in chunks of meat than in ground pork.

The basic procedures evaluated by Ransom et al. (1920) serve as a basis for the regulations of USDA's Consumer and Marketing Service (Consumer Protection Program) regarding ready-to-eat, dry-cured hams. The purpose of the following studies was to re-evaluate the first of these basic procedures and to determine the effect of salt, moisture and drying temperatures on the viability of *T. spiralis* larvae in hams.

MATERIALS & METHODS

THE SWINE from which the hams were obtained for these studies were from the disease-controlled herd maintained for experimental purposes at the Veterinary Medical Research Institute, Iowa State University. They were raised under conditions which minimized the possibility of prior exposure to infection with trichinae.

Infections were obtained by feeding the swine rat flesh containing encysted trichinae. The infective dosage used was approximately 500,000 *T. spiralis* per pig. The swine were infected 2–6 months prior to slaughter; at slaughter they generally weighed 300 lb or more. The diaphragms of all swine were examined at slaughter by the artificial digestion-Baermann technique. Trichinae per gram counts varied from 320–6,100 with a mean of 2,157. The trichinae-per-gram counts for muscle tissue in hams were approximately 20–25% of that for the diaphragm.

The basic curing procedure investigated in this study as written by the USDA (1965) is as follows:

"The hams shall be cured by a dry-salt curing process not less than 40 days at a temperature not lower than 36°F. The hams shall be did down in salt, not less than 4 pounds to each hundredweight of hams, the salt being applied in a thorough manner to the lean meat of each ham. When placed in cure the hams may be pumped with pickle if desired. At least once during the curing process the hams shall be overhauled and additional salt applied, if necessary, so that the lean meat of each ham is thoroughly covered. After removal from cure the hams may be soaked in water at a temperature not higher than 70°F for not more than 15 hours, during which time the water may be changed once; but they shall not be subjected to any other treatment designed to remove salt from the meat, except that superficial washing may be allowed. The hams shall finally be dried or smoked not less than 10 days at a temperature not lower than 95°F."

These studies were generally carried out using only the minimal conditions, or less, as prescribed above. In all but trial 7, only 4 lb of salt per cwt of ham were used in the initial application; 2 lb per cwt were added on overhaul 14 days after initial application. Exposure to salt was 40 days or less. Pickling solution was not pumped into the hams except for 2 groups in trial 2, when 4 oz of pickle at 100° strength (salometer) was injected into both the shank and along the flank side of the femur. The hams were not skinned, except for 2 groups in trial 2, to provide minimal area for salt penetration. The basic curing temperature was 36–38°F. At completion of the salt cure, the hams were soaked in water at 60–70°F for a period of 15 hr to allow maximum salt loss. The hams were not smoked but underwent pale-drying at various temperatures and time intervals. In trials 1 and 2, the drying temperature was 98°F, while in later trials the drying temperatures were varied to cover a range from 40–125°F. The hams were generally large, from 16.7–33.4 lb, with average size about 25.4 lb.

After salt cure and drying, the hams were sliced as shown in Figure 1. The initial cut was made parallel to, and 1 in. from, the symphyseal surface of the pubis. The second cut was made 3 in. ventrally from the initial cut. In trials 1 and 2, 11 muscles were examined for salt content, moisture content and trichina viability. These muscles are shown in Figure 2. In trials 3–7, the muscles examined were limited to 5, since results of the initial trials indicated that the muscles could be categorized into high-, medium- and low-salt muscles with related moisture and trichina viability.

Salt and moisture analyses of each muscle were made utilizing the official methods of the Association of Official Agricultural Chemists (1955). The salt analysis consisted basically of adding 0.5 N silver nitrate solution, boiling with nitric acid, followed by titration with 0.1 N ammonium thiocyanate solution. Moisture analyses were made by drying samples in vacuo at 95–100°C for 5 hr. Brine concentrations for the selected muscles were determined by dividing salt concentration by salt concentration plus moisture concentration and expressing as percent.

Trichina viability was determined by feeding 10 g of each muscle to albino rats kept in individual cages. Rats were sacrificed 30–35 days after feeding. The diaphragms were removed and a portion of the right ventral quadrant examined by the trichinoscopic method using a Leitz trichinoscope. The average number of trichinae in 5, 3-mm-diameter areas of the diaphragm samples was determined. The infections were graded as follows: up to 10 trichinae per area, +1; 11–30 trichinae, +2; 31–50 trichinae, +3 and over 50 trichinae, +4. Negatives were verified by digestion of the skinned and eviscerated carcasses using the artificial digestion-Baermann technique (Zimmermann et al., 1961). If the carcass was positive on digestion only, they were also classified as +1.

EXPERIMENTAL & RESULTS

Trial 1

The initial trial was an evaluation of the

Table 2—Effect of the method of salt administration on viability of Trichinella spiralis in hams.

	Salt	Days 21				21 + 6				28 + 6				40				40 + 6			
	Drying (98°F)																				
Muscle	Treatment	Salt %	H_2O %	Brine %	Trans.	Salt %	H_2O %	Brine %	Trans.	Salt %	H_2O %	Brine %	Trans.	Salt %	H_2O %	Brine %	Trans.	Salt %	H_2O %	Brine %	Trans.
Gracilis	NS,NI	5.15	67.9	7.05	+1	3.26	70.5	4.42	–	2.08	63.15	3.19	–	4.14	61.15	6.34	+1	5.03	73.3	6.42	–
	NS,I	5.62	66.4	7.80	+1	3.81	67.6	5.34	–	3.96	55.6	6.65	–	4.47	69.0	6.08	+1	2.98	63.35	4.49	–
	S,NI	5.41	57.5	8.60	+1	1.09	66.75	1.61	–	5.57	54.75	9.25	–	5.77	59.2	8.88	–	4.38	69.35	5.94	–
	S,I	5.7	54.65	9.45	–	3.82	70.6	5.14	–	5.71	58.9	8.83	–	6.22	49.0	11.26	–	4.17	56.95	6.82	–
Adductor	NS,NI	2.23	76.3	2.84	Died	1.44	75.8	1.87	–	1.67	70.0	2.33	–	1.90	75.6	2.45	+3	2.68	76.4	3.38	–
	NS,I	3.44	75.1	4.38	+2	3.59	71.75	4.77	–	3.93	68.4	5.44	–	2.96	70.2	4.04	+2	2.83	72.25	3.76	–
	S,NI	1.96	69.15	2.76	+2	1.03	84.1	1.22	–	2.40	62.55	3.69	–	4.0	68.75	5.49	+1	1.58	77.55	1.99	–
	S,I	2.95	69.7	4.06	+2	2.95	66.25	4.27	–	3.99	59.4	6.29	–	4.43	69.8	5.96	Died	3.26	71.6	4.35	–
Semimembranosus	NS,NI	2.69	77.8	3.34	+2	2.53	73.6	3.32	–	1.40	69.4	1.97	–	1.61	68.9	2.28	+3	2.45	72.4	3.27	–
	NS,I	1.04	72.4	1.42	+1	2.94	65.55	4.30	–	2.49	63.9	3.75	–	1.8	67.3	2.60	+4	3.36	67.05	4.77	–
	S,NI	2.25	68.85	3.16	+2	0.51	74.1	0.68	–	1.48	62.8	2.30	–	4.02	75.75	5.03	+1	0.81	73.7	1.08	–
	S,I	1.03	70.05	1.45	Died	2.58	65.35	3.80	–	3.33	66.4	4.77	–	2.8	61.8	4.33	+1	3.63	68.9	5.00	–
Biceps femoris	NS,NI	0.17	73.7	0.23	Died	0.41	71.9	0.57	+1	0.54	73.8	0.72	–	0.26	69.55	0.37	+3	0.76	76.15	0.98	–
	NS,I	0.76	73.75	1.02	+2	1.36	72.2	1.85	–	2.83	58.45	4.61	–	1.14	66.9	1.67	+2	1.77	69.65	2.47	–
	S,NI	1.37	68.05	1.98	+3	0.93	73.05	1.25	–	1.59	61.25	2.53	–	1.37	64.65	2.07	+2	1.68	78.85	2.08	–
	S,I	2.35	67.9	3.35	+1	1.77	64.45	2.67	–	3.39	61.95	5.18	–	2.04	68.35	2.89	+2	2.43	56.35	4.13	–
Vastus intermedius	NS,NI	0.57	80.8	0.70	Died	1.13	83.4	1.34	+1	0.79	68.4	1.14	–	0.95	79.5	1.18	Died	1.02	80.9	1.24	–
	NS,I	0.49	71.6	0.68	Died	2.28	72.9	3.03	–	2.66	66.9	3.82	–	1.27	69.1	1.80	Died	2.93	68.7	4.09	–
	S,NI	0.89	71.6	1.23	Died	0.98	76.7	1.26	–	2.16	64.6	3.23	–	1.02	73.8	1.36	Died	1.72	77.85	2.16	–
	S,I	1.27	75.7	1.65	Died	1.97	57.05	3.34	–	2.23	66.1	3.26	–	1.47	67.0	2.14	+2	2.72	72.1	3.63	–

Note: NS—Nonskinned; S—skinned; NI—noninjected; I—injected.

basic curing procedure under minimal conditions. Salt was applied at 4 lb per cwt initially, 2 lb per hundred on overhaul at 14 days. The curing temperature was 36°F, the drying temperature, 98°F. 21 hams were used in the study. Results are shown in Table 1. Although 11 muscles were analyzed from each ham for salt, moisture and trichinae viability, only 5 are listed in the Table, since the muscle could be grouped according to high, medium and low salt content. The moisture content of the meat and the infectivity of the parasites within each group varied only slightly. The high-salt muscles were the gracilis, sartorius and pectineus. Medium-salt muscles were the adductor, semimembranosus, vastus lateralis, rectus femoris and vastus medialis. The low-salt muscles were the biceps femoris, vastus intermedius and semitendinosus.

After 28 days of curing, trichinae were viable in each muscle. However, the viability as indicated by rat diaphragm evaluation did vary with the salt concentration of the muscle. Low viability was noted for the gracilis, where the mean brine concentration was 8.35%. The viability of the larvae increased with the relative decrease in brine concentration, with a high level of infectivity being evident for the biceps femoris and the vastus intermedius. The level of infectivity was relatively unchanged for the muscles, with the exception of the gracilis, through the entire 40 days of exposure to salt. Noninfectivity occurred for 2 of the 3 gracilis samples fed to rats on day 35 and in 1 of 3 gracilis samples fed on day 40, the termination of the curing process.

3 days of drying at 98°F had little effect on the viability of the larvae except in the gracilis muscle, where all samples were noninfective. 2 of 3 rats died when fed samples of the vastus intermedius. 6 days of drying rendered all trichinae nonviable regardless of brine concentration, which ranged from a mean of 5.85% in the gracilis down to only 0.91% in the biceps femoris. Samples fed after 8 and 10 days of drying were also noninfective.

Trial 2

Evaluation of the basic procedure was continued. Since skinning of the hams and injection of a brine solution were optional, these factors were included in trial 2. The effect of a shortened cure with a 6-day drying period was also included; 20 hams were utilized. Results are indicated in Table 2.

Results for 21 days of curing closely paralleled those obtained for 28 days of curing in trial 1, with the infectivity of the parasite generally being inversely related to the brine concentration. However, exposure of hams cured for 21 days to 6 days of drying at 98°F brought about an almost total loss of viability, with only light transmission occurring for the biceps femoris and the vastus intermedius in a nonskinned, noninjected ham. Exposure to 28 days of salt and 6 days of drying rendered all parasites nonviable even when skinning and injection were not used.

Results for 40 days of curing without drying indicated a high degree of transmission with again, the exception of the gracilis. Addition of a 6-day drying period at 98°F once again resulted in noninfectivity of the larvae.

Trial 3

28 days of curing plus 6 days of drying at 98°F had proved effective in eliminating the infectivity of the trichinae in trial 2. Therefore

Table 3–Effect of cure period, drying temperature and time on viability of Trichinella spiralis in hams.

		Days										
	Salt	21	21	21	28	28	28	35	35	35	35	35
	Drying	5	6	7	5	6	7	5	6	7	14	28
Muscles	Drying temp (°F)											
Adductor	70	+2	+3	Died	+2	+3	+2			+2	+2	+1
	98	+1	–	–	–	–	–	–	–	–		
Semimembranosus	70	+2	+3	Died	+2	+2	+2			+3	+2	+1
	98	+1	–	–	+1	–	–	–	–	–		
Semitendinosus	70	+3	+2	Died	+2	+3	+3			+2	+2	+2
	98	+2	–	–	+2	–	–	+1	–	–		
Biceps femoris	70	+3	+3	Died	+3	+3	+2			+2	+3	+2
	98	+2	–	–	+1	–	–	+1	–	–		
Vastus intermedius	70	+4	Died	Died	Died	Died	Died			Died	Died	+2
	98	+2	+3	+1	+2	–	–	+1	–	–		

trial 3 was designed to determine the drying periods necessary to devitalize trichinae in hams after curing periods of 21, 28 and 35 days. 2 drying temperatures were used: 70 and 98°F. The semitendinosus muscle was substituted for the gracilis muscle in this trial only. Results are shown in Table 3.

Results for drying at 98°F were similar to those obtained in the 2 previous trials. All muscle samples were infective following 21 days of curing and 5 days' drying. When the drying period was extended to 6 and 7 days, infectivity was lost for all muscles except the vastus intermedius. 6 days of drying was effective in destroying viability when preceded by 28 and 35 days of curing. A light to moderate degree of transmission was noted for both curing periods when the drying period was limited to only 5 days.

Drying at 70°F had a markedly lesser effect on viability. Even when curing at 35 days was followed by drying for 28 days, a low level of transmission still occurred.

Trial 4

Since the drying temperature seemed to be a primary factor in determining the relationship of drying time with the viability of the parasite, 4 drying temperatures were used: 98, 105, 115 and 125°F. 1 ham was sampled daily at each temperature level through 6 days of drying. All hams had prior salt exposure of 36 days. Results are indicated in Table 4.

Table 4–Effect of selected drying temperatures on viability of Trichinella spiralis in hams.

		Days					
	Salt	36	36	36	36	36	36
	Drying	1	2	3	4	5	6
Muscles	Drying temp (°F)						
Gracilis	98	+1	+1	–	–	–	–
	105	–	Died	–	+1	–	–
	115	+1	–	–	–	–	–
	125	Died	–	–	–	–	–
Adductor	98	+2	+2	+2	+2	–	–
	105	+1	Died	+1	–	–	–
	115	+2	–	–	–	–	–
	125	+2	–	–	–	–	–
Semimembranosus	98	+1	+2	+2	+1	–	–
	105	+1	+1	+1	–	–	–
	115	+2	–	–	–	–	–
	125	+2	–	–	–	–	–
Biceps femoris	98	+2	Died	+3	+3	+1	–
	105	+3	+2	+2	–	–	–
	115	+3	+1	+1	–	–	–
	125	+3	–	+1	–	–	–
Vastus intermedius	98	+2	+2	Died	–	–	–
	105	+2	Died	+1	–	–	–
	115	+1	–	–	–	–	–
	125	+2	–	–	–	–	–

A basic time-temperature relationship was indicated by the results of this trial. The drying time necessary to render the parasite noninfective for the various temperatures was: 98°F–6 days; 105°F–5 days; 115°F–4 days and 125°F–4 days. In regard to the 4-day period for 125°F, no transmission occurred for all samples fed on day 2, but the biceps femoris initiated a light infection when fed on day 3. In the 105°F series, only the gracilis muscle proved infective on day 4, a finding which was somewhat unexpected due to the surface exposure to salt.

Trial 5

Time-temperature relationships for temperature increments of 10°F ranging from 40–90°F were studied in trial 5 utilizing 16 hams. No minimal drying times were determined for 90 or 80°F, since infectivity was lost when the initial sample was obtained on day 14 for 90°F and on day 21 for 80°F. Loss of infectivity occurred between days 31 and 43 at 70°F. Samples for hams dried at 60°F were positive on day 51, negative on day 56 and lightly positive in both the biceps femoris and vastus intermedius on day 63. The 50°F sample was lightly infective in only the biceps femoris on day 98. Trichinae were infective at 182 days in hams kept at 40°F but were noninfective on days 198 and 204.

Trial 6

An attempt was made to determine more closely the time-temperature relationships for a drying temperature of 80°F. 6 hams were held in salt for 35 days, with the salt applied at a rate of 4 lb per cwt. The hams were not overhauled. A single ham was examined on days 14, 15, 17, 19, 21 and 22. Viable trichinae were obtained at feeding from only the biceps femoris fed on day 14.

Trial 7

3 pairs of hams were used, with salt being applied at the rate of 3 lb per cwt to 1 of each pair and at 5 lb per cwt to the other. The hams were in salt for 35 days and then dried at 80°F. After 14 days of drying, viable trichinae were present only in the vastus intermedius of the ham from the 3 lb per cwt group, whereas infective trichinae were present in all muscles but the gracilis of the 5 lb per cwt group. After 17 days of drying, infective larvae were present in the vastus intermedius and biceps femoris of both of the paired hams. No transmission occurred after 20 days' drying.

The difference in rate of salt application had little influence on the salt concentration of the selected muscles in the paired hams. The con-

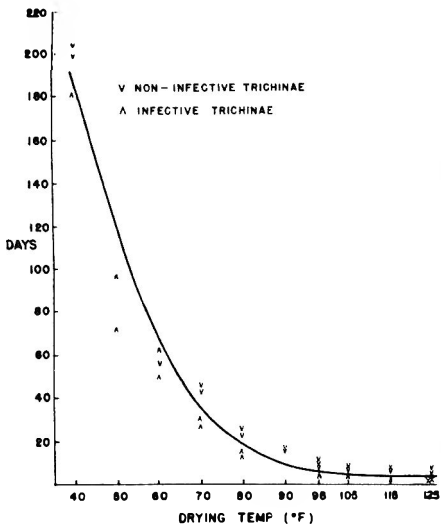

Fig. 3—Time-drying temperature relationship for loss of infectivity of Trichinella spiralis in hams.

centration of salt in the muscles of hams from the 3 lb per cwt grouping often exceeded that of the other group. The maximum percentage difference was 0.98%, with most differences being less than 0.2%.

Figure 3 represents the time-temperature relationships indicated by these studies for a temperature range of 40 through 125°F.

DISCUSSION

THIS STUDY has confirmed that the regulations imposed by Consumer Protection Programs, C&MS, USDA, on the processing of ready-to-eat pork products prepared under their supervision are safe and adequately protect the public from trichinosis. Indeed, the margin of safety for the procedure evaluated is such that the required curing-drying period of 50 days is about 50% more than the 28 days of curing and 6 days of drying deemed effective by results of this study. Use of minimal salt application and of nonskinned and oversize hams in this study further emphasizes the safety of this product. Although occasional human outbreaks originate from the ingestion of uncooked, dry-cured hams, these hams have been processed by plants and individuals not subject to federal regulations. The Wholesome Meat Act of 1967, markedly increasing the number of ham processors subject to these regulations, will sharply reduce the public health aspect of this problem.

The pork processing procedures studied by Ransom et al. (1920) were undoubtedly standard during the early part of this century. However, marked changes in technology have occurred since. Gammon et al. (1968) have stated that the present regulations are not conducive to the production of a uniformly high quality product. 1 of the major problems facing the USDA is that few ham processors exactly follow either of the prescribed methods. This becomes especially acute with the advent of the Wholesome Meat Act. Each processor has variations designed to improve quality and flavor as well as to reduce production costs. Many Southern processors allow hams to dry subject to ambient seasonal temperature variations. Occasional small, local Northern processors carry out a majority of their ham and sausage processing during winter in noninsulated smokehouses with resulting cold smoke and, therefore, little effect on the viability of trichinae which may be present in the product. Thus, how can these procedures be evaluated? Although this study must be regarded as preliminary, subject to more extensive and controlled evaluation, Figure 3 would indicate the probability of a time-temperature relationship which, when more fully detailed, could serve as a possible guide for helping to evaluate the efficacy of at least some of the procedures utilized. Thus, if hams are dried at ambient temperatures of 70–80°F, a drying period approximating 35 days would be necessary, whereas if the smokehouse temperature in winter was only 50°F, up to 110 days of drying may be required to ensure freedom from infectious trichinae.

It is somewhat difficult to assay the relationship of salt and moisture content to drying time and temperature in rendering the parasite innocuous. Although salt alone has been found effective for destroying trichinae in ground or chopped meat, the period of salt exposure would have to be extended over a prolonged, impractical period to be totally effective in hams. In these studies salt alone did not destroy trichinae in hams through a curing process of 40 days, except in the surface muscles when the brine content approximated 8%, and then the results were sporadic. Even increased exposure to salt, as induced by skinning and injection of pickle in trial 2, had little additive effect on the rate of devitalization of the parasite. Similarly, little contrast was noted in trial 7, when the effects of salt application rates of 3 and 5 lb per cwt of hams were compared. However, as proposed by Ransom et al., salt must be considered an important factor in the devitalization of the parasite through possible toxic action and dehydration of the parasite. The role of moisture content is also difficult to define. As the drying of the ham proceeds, the moisture tends to migrate from the deeper, low-salt muscles to the surface of the exposed muscles. Thus, the moisture content of the muscles near the surface actually increases and the relative salt content decreases when the drying process proceeds. Even with this equalization process, a range of 5% in salt content, 10% in moisture and 8.5% in brine concentration will be noted at the time all parasites become nonviable.

The drying temperature thus seems to be the primary factor in destruction of the parasite, with the rate of devitalization being directly related to temperature. Regardless of brine content ranges obtained in these studies, the parasite became noninfective when exposed to specific time-drying temperature relationships. This does not mean to imply that salt is not essential. 1 possibility would be that the metabolic activity of the parasite is related to temperature and that increased activity at higher temperatures allows the toxicity of the salt to act more readily.

Although the artificial digestion procedure is highly effective for detection of trichinae in fresh pork samples, it is somewhat less efficient when used to evaluate the viability of trichinae in partially cured pork products (Allen and Goldberg, 1962; Ransom et al., 1920). There is a period during the processing when the trichinae will exhibit movement, albeit sometimes feebly, but will not be infective. In this study, results of the digestion procedure and rat feeding trials closely paralleled during the curing period, but varied during the drying period at about the time the parasite became noninfective. Microscopic examination of the parasites would reveal living trichinae somewhat paler and more granular than normal in appearance. The feeding of a corresponding ham sample to rats would often give negative results. These findings emphasize the need for use of a xenodiagnostic test for correctly determining the effect of processing methods on *T. spiralis*.

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U. S. KUMTA and S. S. MAVINKURVE
Bhabha Atomic Research Centre, Biochemistry & Food Technology Division, Trombay, Bombay-85, India

RADIATION SENSITIVITY AND BIOCHEMICAL CHARACTERISTICS OF MICROFLORA OF BOMBAY DUCK (Harpodon nehereus)

*SUMMARY—The microbial flora of unirradiated and irradiated (0.5 Mrad) Bombay duck (*Harpodon nehereus*) was differentiated into organisms as spoilers and non-spoilers based on ability to liquify gelatin; ferment glucose; and produce indole, H_2S and urease. In spoiling unirradiated fish, there was a predominance of* Vibrio, Aeromonas *and* Proteus *spp while* Micrococci *and* Achromobacter *spp were the major surviving groups in irradiated Bombay duck stored for 15 days at 10°C. The predominant spoilers,* Proteus vulgaris *and* Aeromonas hydrophila, *produced large amounts of TMA and TVBN and were radiation sensitive as indicated by D_{10} values of 8.6 and 5.4 Krad respectively.* Micrococcus luteus *was relatively biochemically inert and radiation resistant, the D_{10} value being 88 Krad.*

INTRODUCTION

IT HAS BEEN consistently reported that total bacterial count, which serves as a parameter of spoilage of flesh foods, may not be indicative of incipient spoilage of radiation pasteurized fishery products (Spinelli et al., 1964; Licciardello et al., 1967). Our studies on Bombay duck indicated that unirradiated fish stored at 10–12°C developed putrid odors within 4 days, with a concomitant rise in total bacterial count. However, irradiated Bombay duck (0.4 Mrad) exhibited a high organoleptic score after 14 days storage at 10–12°C, although the total bacterial count had reached the same level as spoiled unirradiated samples (Sawant et al., 1967). These changes were also reflected in low levels of trimethylamine nitrogen (TMAN) and total volatile basic nitrogen (TVBN) indicative of freshness of irradiated samples.

Bacteriological studies (Mavinkurve et al., 1967) showed that the flora of unirradiated Bombay duck during spoilage comprised mainly *Proteus, Bacillus, Aeromonas, Micrococci*, and *Niesseria* while *Micrococci* and *Bacilli* were predominant in the irradiated samples stored at 10–12°C for 14 days. Such marked changes in terminal microflora have been reported in radiation pasteurized fishery products (Kazanas, 1966; Licciardello et al., 1967).

Several factors, including among others, initial flora, differences in substrates due to variations in fish species, radiation dose, and packaging and storage conditions influence the flora of irradiated fish. Hence it was essential to determine the microbial and associated biochemical changes occurring in microflora of Bombay duck immediately after irradiation and during storage with a view to differentiating such changes from those progressively leading to the spoilage of unirradiated Bombay duck.

Lerke et al. (1965) differentiated isolates from fish as spoilers and non-spoilers on the basis of their growth and related characteristics using fish press juice medium. In the present studies, Bombay duck drip was used as a test medium to investigate the spoilage potential of some of the isolates from Bombay duck.

This paper provides evidence on the differences in the types of organisms and their biochemical characteristics associated with unirradiated and irradiated Bombay duck fillets. Radiation sensitivity of, and the relationship between growth and formation of metabolites, TMA and TVBN, by cultures of *Proteus vulgaris, Acromonas hydrophila* and *Micrococcus luteus* were also investigated.

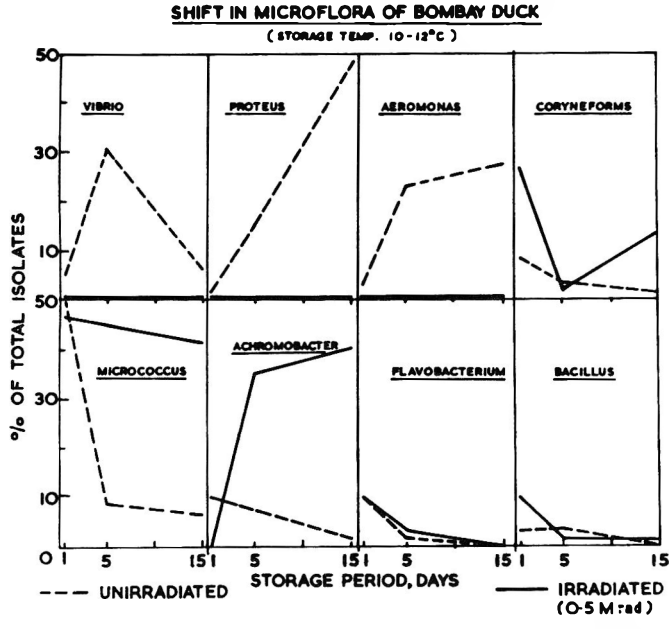

Fig. 1—Shift in microflora of Bombay duck. Organisms isolated from unirradiated and irradiated (0.5 Mrad) Bombay duck during storage at 10–12°C were characterized and identified according to the methods described previously (Mavinkurve et al., 1967). The percentage of microbial groups was calculated from the total number of isolates from respective sample.

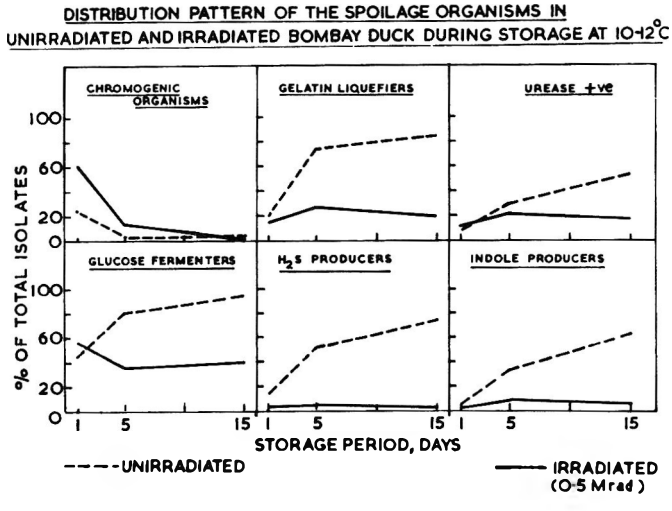

Fig. 2—Distribution pattern of the spoilage organisms in unirradiated and irradiated Bombay duck during storage at 10–12°C. The organisms showing positive tests have been expressed as percent of total isolates from unirradiated and irradiated (0.5 Mrad) Bombay duck fillets. Isolates were characterized as described previously (Mavinkurve et al., 1967).

Table 1—Morphological and biochemical characteristics of the predominant organisms isolated from unirradiated and irradiated Bombay duck stored at 10–12°C.

Culture No.[a]	Colony characteristics	Gram's staining and morphology	Motility	Oxidase	Mode of attack on Glucose[b] (Hugh and Leifson) Aerobic	Mode of attack on Glucose[b] (Hugh and Leifson) Anaerobic	Fermentation[b] of Glucose	Fermentation[b] of Lactose	Fermentation[b] of Maltose	Fermentation[b] of Mannitol	Nitrate reduction	Gelatin liquefaction	Formation of H_2S	Formation of Indole	Formation of Urease	Formation of Catalase	Methyl red	Voges-Proskauer	
333	Greyish white, translucent, Growth with swarming	Gram-negative coccobacillary to bacillary forms	actively motile	–	AG	AG	AG	–	AG	–	+	+	+	+	+	+	+	+	*P. vulgaris*
318	Off white, smooth, opaque, rich growth	Gram-negative long bacilli	motile	+	AG	AG	AG	–	AG	AG	+	+	+	+	–	+	+	–	*A. hydrophila*
235	Off white, smooth opaque rich growth	Gram positive cocci in clusters	non-motile	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	*M. luteus*

[a] *P. vulgaris* and *A. hydrophila* were isolated from unirradiated and *M. luteus* from irradiated Bombay duck. The biochemical characteristics of the organisms were studied according to the methods described in the text.

[b] AG dentoes the formation of acid and gas from carbohydrates.

MATERIALS & METHODS

EVISCERATED and beheaded Bombay duck fillets were sealed in polythene bags and irradiated at 0.5 Mrad in a Gamma cell 220 at a dose rate of 0.83 Mrad/hr. Unirradiated fillets served as control. Samples were stored at a temperature of 10–12°C and analyzed at intervals of 1, 5 and 14 days for total bacterial count (TBC) and for qualitative changes in the microflora.

Microorganisms were isolated and characterized according to the methods described previously (Mavinkurve et al., 1967) and were identified according to the schemes of Shewan et al. (1960), Edwards and Ewing (1962) and Masurovsky et al. (1963).

Biochemical characteristics of the isolates

All the isolates, 178 from unirradiated and 132 from irradiated (0.5 Mrad) Bombay duck during storage at 10–12°C, were subjected to biochemical tests which included hydrogen sulphide and indole production, gelatin liquefaction, urease activity and fermentation of carbohydrates.

Selection of the organisms

For a comparative study of the spoilage potential of the predominant organisms, strains of *P. vulgaris* and *A. hydrophila* were isolated from unirradiated Bombay duck fillets stored for 5 days at 10–12°C and a strain of *M. luteus* from irradiated (0.5 Mrad) fillets stored for 15 days at 10–12°C.

Growth patterns of the organisms in fish drip medium

Drip medium was prepared using fresh Bombay duck. Bombay duck were eviscerated, cleaned with tap water, sealed in polythene bags and frozen overnight at –30°C. The drip collected after thawing the fish was filtered through glass wool, diluted with 0.5% NaCl solution in the proportion of 1 to 3, and sterilized by Seitz filtration. The medium was stored at 0°C until required. Total nitrogen content of the drip was determined by nesslerization (Umbreit et al., 1957).

Growth studies

1 ml of an 18-hr old culture of *P. vulgaris* was inoculated in 100 ml of fish drip medium, mixed, and incubated at 30°C in a shaker water bath at 125 rpm. Samples were withdrawn at regular intervals and analyzed for turbidity as an index of bacterial growth and for TVBN, TMAN and 'tyrosine' value using the methods described previously (Sawant et al., 1967).

Similar studies were carried out with cultures of *A. hydrophila* and *M. luteus*.

Effect of irradiated medium on growth pattern of the spoilers

The growth patterns of *P. vulgaris*, *A. hydrophila* and *M. luteus* were studied in terms of turbidity and formation of TVBN in fish drip medium irradiated at 0.5 Mrad.

Radiation sensitivity of microorganisms

Bacterial cells grown for 18 hr in tryptone-glucose-yeast extract broth at 30°C in a shaker water bath at a speed of 125 rpm were suspended in 0.1M phosphate buffer, pH 7.0. The cell density was adjusted to 10^7–10^8/ml. The cell suspension in buffer was dispensed in sterile test tubes which were then exposed to different doses of gamma irradiation in a Co^{60} source (Gamma Cell 220, Atomic Energy of Canada Limited) with a dose rate of 0.17 Mrad/hr. The radiation dose ranged from 5–40 Krad for *Proteus* and *Aeromonas* and from 10–450 Krad for *Micrococcus*.

Viable cell counts in the unirradiated and irradiated cell suspensions were determined using TGE agar and the percentage survival was calculated for each dose.

RESULTS

Changes in the microbial flora due to irradiation and storage

Figure 1 depicts the qualitative changes in the generic distribution of microflora of irradiated and unirradiated Bombay duck stored at 10–12°C for 15 days.

In unirradiated samples, during 5 day storage period, there was a marked reduction in *Micrococci, Coryneforms* and *Flavobacterium* spp while *Vibrio, Proteus* and *Aeromonas* spp showed a rapid rise. On 15th day storage, *Proteus* spp and *Aeromonas* spp were most predominant organisms.

Chromogenic *Micrococci* were the major survivors in irradiated Bombay duck. However, as storage progressed, the increase in the *Micrococcus* spp in irradiated samples was contributed both by non-chromogenic as well as chromogenic *Micrococci*. Coryneforms decreased after irradiation but comprised 10% of the total isolates on storage for 15 days.

It was interesting to note that there was a rapid increase in *Achromobacter* spp in irradiated Bombay duck during storage, accounting for 35% and 41% of the total isolates on 5 and 15 days respectively.

Biochemical characteristics of the microorganisms

Figure 2 summarizes the distribution pattern of the organisms with respect to the biochemical characteristics concerned with the spoilage potential of unirradiated and irradiated Bombay duck during storage at 10–12°C. Prior to spoilage, the unirradiated fish included spoilers as well as non-spoilers with reference to indole, H_2S, NO_2 production, urease and proteolytic activity. During further storage, however, there was a selective growth of the spoilers and the flora consisted mostly of the proteolytic and biochemically active organisms.

In contrast, the flora of samples immediately after irradiation consisted

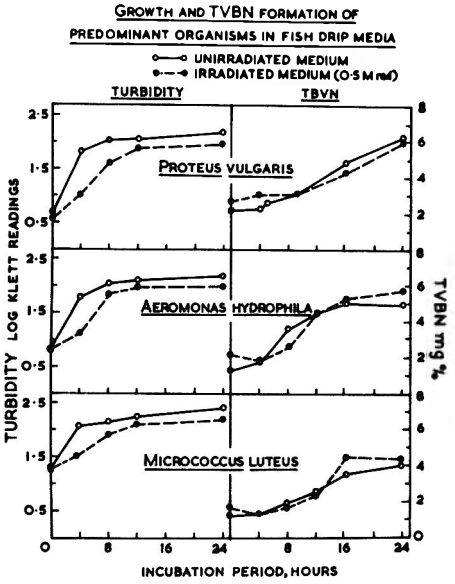

Fig. 3–Growth and TVBN formation of predominant organisms was followed in unirradiated and irradiated (0.5 Mrad) Bombay duck drip media incubated at 30°C with continuous shaking. Despite the lag and suppression in the growth of the cells, TVBN formation is not affected in irradiated medium. M. luteus exhibits low TVBN formation compared with A. hydrophila and P. vulgaris.

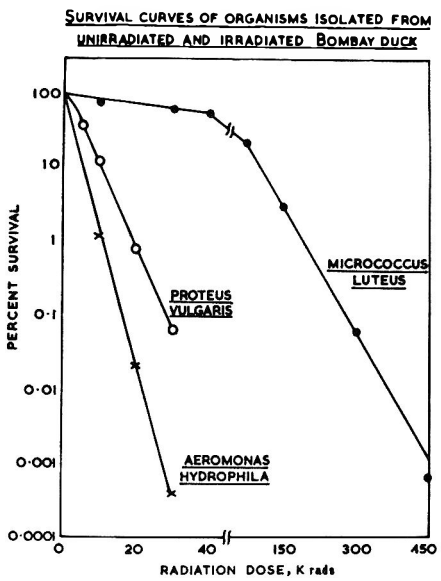

Fig. 4–Survival curves of organisms isolated from unirradiated and irradiated Bombay duck. Organisms were irradiated in phosphate buffer, pH 7.0, at varying doses and percent survival determined. P. vulgaris and A. hydrophila are highly radiation sensitive, D_{10} values being 8.6 and 5.4 respectively; the D_{10} value of M. luteus is 88 Krad.

mostly of chromogenic organisms and was devoid of rapid spoilers. During storage there was also no significant increase in spoilage organisms.

Characteristics of selected organisms

Table 1 shows the biochemical and morphological characteristics of the three selected microorganisms. Strains of *P. vulgaris* and *A. hydrophila* isolated from spoiled fish can be considered as biochemically active because of their ability to liquefy gelatin, produce indole and hydrogen sulphide, and their ability to ferment carbohydrates. In contrast, *M. luteus* did not show gelatin liquefaction, carbohydrate fermentation, indole and hydrogen sulphide production and thus could be denoted as biochemically inert in terms of spoilage activity.

Figure 3 shows the growth pattern and TVBN formation by *P. vulgaris, A. hydrophila* and *M. luteus* in unirradiated and irradiated (0.5 Mrad) fish drip medium.

The growth of *Aeromonas* spp, *Proteus* spp and *Micrococcus* spp was retarded initially in the irradiated medium. These organisms exhibited a lag for about 4 hr in irradiated medium as against immediate rapid growth in the control medium. Though an increase in turbidity was observed thereafter, growth was less than in the unirradiated medium throughout the incubation period of 24 hr. Despite less turbidity, TVBN formation was not significantly affected in the irradiated medium.

Aeromonas spp and *Proteus* spp showed a rapid rise in TVBN content concurrent with an increase in turbidity. A lag of 12 hr in the growth of *M. luteus* was followed by a slight increase in TVBN to 4.3 mg% after 24 hr, as against 7.5 mg% TVBN observed in cultures of *P. vulgaris*.

The influence of aeration on the formation of TMA by the organisms in fish drip medium was investigated. Cultures of *A. hydrophila* and *M. luteus* did not exhibit significant changes in TMA content throughout the incubation period of 24 hr with or without aeration. Thus the initial value of 0.06 mg% increased only up to 0.09 mg%. In contrast, unaerated cultures of *P. vulgaris* showed a rapid rise in the production of TMA; the initial value of 0.07 mg% increased to 0.54 mg% after 24 hr incubation. *P. vulgaris* cells incubated with continuous shaking, however, did not show appreciable rise in TMA content.

Radiation sensitivity of the microorganisms

Figure 4 shows the differences in the radiation sensitivities of *P. vulgaris, A. hydrophila* and *M. luteus*. D_{10} values were respectively, 8.6, 5.4, and 88.0 Krad.

DISCUSSION

THE SEQUENCE OF events which lead to deteriorative processes in unirradiated fishery products is largely due to microbial factors. Prior to spoilage, the microbial flora is heterogenous in nature but due to varying growth rates and differences in adaptability to environmental conditions, certain microorganisms increase in their numbers (Mavinkurve et al., 1967; Licciardello et al., 1967). Further, since such organisms are biochemically active, a series of reactions leads to putrefaction of the tissue. Thus, the predominance of *Proteus, Vibrio* and *Aeromonas* spp. (Fig. 1) and their associated activities of proteolysis, formation of hydrogen sulphide and indole production bring about rapid deterioration of unirradiated Bombay duck. The ability to form hydrogen sulphide and to hydrolyze gelatin has been used as criteria for enumeration of spoilage bacteria from fish (Chai et al., 1968).

Correlation between degree of spoilage and products of metabolism of rapid spoilers has been studied extensively (Lerke et al.; 1965, Lobben and Lee, 1968; Shaw and Shewan, 1968). The studies of Lobben and Lee (1968) indicated that species of *Pseudomonas, Flavobacterium*, and *Achromobacter* varied with respect to their growth and TVA and TVB formation in Rockfish and that a bacterial load of 10^8/g or more is needed to produce detectable amounts of TVA and TVB. *P. vulgaris* and *A. hydrophila* isolated from spoiled Bombay duck produced relatively large amounts of TVBN but showed marked differences in the formation of TMAN.

The radiation pasteurization process does not reduce all the microbial groups uniformly in a fixed proportion for a particular radiation dose due to the differences in radiation sensitivities, repair mechanisms and dose modifying factors (Schmidt-Lorenz and Farkas, 1961; Thornley, 1963; Quinn et al., 1967; Serianni and Bruce, 1968). The D_{10} value for *Micrococcus luteus* is 88 Krad, yet it was found to survive in Bombay duck exposed to 0.5 Mrad. Organisms of the *Achromobacter* spp accounted for 35 to 41% of the total isolates from irradiated Bombay duck stored for 5 and 15 days at 10–12°C. *Achromobacter* spp is considered to be one of the radiation sensitive organisms. Schmidt-Lorenz and Farkas (1961) reported D_{10} values ranging from 8–18 Krad. Thornley (1966) has shown that in spoiled irradiated chicken (0.5 Mrad), *Achromobacter* spp account for 20% of the isolates. In the previous studies on the composition of microbial flora of irradiated Bombay duck (0.4 Mrad) stored at 10–12°C, *Achromobacter* spp was not detected. This may be due to the lesser number of isolates examined.

Higher number of organisms of the order of 10^8 is used in experiments designed to derive D_{10} values. However,

in fresh Bombay duck the total bacterial load is of the order of 10^4/g and on irradiation with 0.5 Mrad therefore, the number of organisms surviving is much less of which *Micrococci* and *Achromobacter* spp account for the most. The rapidity with which these organisms are able to grow in fish is of considerable practical interest.

The survival of microbial groups relatively inactive in spoilage reactions forms the basis of the radiation pasteurization process for flesh foods. Thus, the radiation survivors grow rapidly bringing the total bacterial load of irradiated fish to a spoilage level of 10^8/g, the same as in unirradiated samples; however, fish samples are organoleptically acceptable (Spinelli et al., 1964; Sawant et al., 1967). Radiation dose forms an important processing criterion. Although a wide range of doses can eliminate active microbial groups and lead to extension in shelf life (Sawant et al., 1967), it is essential to select the dose which does not reduce the organoleptic attributes by degrading the radiation sensitive food components.

Due to the biochemically inactive nature of *M. luteus* and other *Micrococcus* species it is difficult to evaluate their precise role in determining the storage properties of irradiated Bombay duck. *M. luteus*, one of the major survivors in irradiated Bombay duck did not contribute to the formation of TMA. Castell and Anderson (1948) have also reported the inability of species of *Micrococcus, M. aureus* and *M. citreus* to reduce TMAO.

The terminal spoilage of irradiated sea foods is characterized by sweetish, stale odors, which are markedly different from those commonly noticed in spoiling unirradiated fillets. Studies on the nature of metabolites produced by radiation survivors and their relationship, if any, with organoleptic attributes would be useful in monitoring the shelf-life of radiation pasteurized fishery products.

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M. G. E. HAMED and A. N. ELIAS
Food Technology Division, National Research Centre, Cairo, Egypt

EFFECT OF OXYTETRACYCLINE AND CHLORTETRACYCLINE ON SURVIVAL OF THE TREMATODE Heterophyes sp. IN THE FLESH OF MULLET CAUGHT IN BRACKISH EGYPTIAN WATERS

SUMMARY—Mullet, Mugil cephalus, *caught from brackish Egyptian waters, serve as an intermediate host for* Heterophyes *parasite which renders such fish hazardous to health. The objective of this work was to show the effectiveness of antibiotics, used to prolong the storage life of fresh fish, as parasiticides. To achieve this, both whole mullet and small portions of infected mullet tissue were dipped in oxytetracycline (OTC) and chlortetracycline (CTC) solutions of different concentrations ranging from 30–4000 ppm. The rate of parasite destruction, very slow at low concentrations of either antibiotic, increased at much higher concentrations of antibiotic. However, a concentration of antibiotic sufficiently high to destroy the parasite completely cannot be used for the preservation of fresh fish because of the high concentration of antibiotic in the fish tissue after treatment. Although these findings are negative, they have a place in the literature.*

INTRODUCTION

CONSIDERABLE amounts of mullet, *Mugil cephalus,* are caught from brackish Egyptian waters. Mullet serve as the most common intermediate host for the worms of *Heterophyes* (Nasr, 1941). This parasite gains entrance to man's body or to fish-eating animals through consumption of fish containing the live encysted metacercariae and this causes abdominal pains in its hosts.

Recently, experiments were carried out to show the effect of food-processing methods upon the survival of the encysted parasite found in the fish muscles (Elias et al., 1968; Hamed and Elias, 1969). It was found that insufficient grilling of fish does not completely destroy the cysts of *Heterophyes;* therefore, these fish are hazardous to consumers. Moreover, this parasite survived for 33 hr when infected mullet were stored at −10 or −20°C. Viability was greater at refrigerator temperatures, i.e., 9 days, but the fish developed a strong putrid odor (Hamed and Elias, 1969). The parasite has also been found to stand relatively high salt concentrations (Elias et al., 1968).

Many antibiotics have been used recently to prolong the storage life of fresh fish (Tarr et al., 1950; 1952). Antibiotics of the "broad-spectrum" type, i.e., chlortetracycline (aureomycin) and oxytetracycline (terramycin), have been used either as a dip or in ice. Previous studies showed that dipping of mullet containing the encysted metacercariae of *Heterophyes* in chlortetracycline solution (30 ppm) for 2 hr did not affect the viability of the parasite. Moreover, ice treated with oxytetracycline (5 ppm), when used for the preservation of mullet, had no harmful effect on the survival of *Heterophyes* (Hamed and Elias, 1969).

This paper presents data relative to attempts to destroy a parasite of mullet through treatment with oxytetracycline and chlortetracycline. Further, it seems to present some challenges which have not been adequately investigated; for example, controlling of parasites in seafoods. Perhaps it will stimulate further effort in solving this most perplexing problem.

MATERIALS & METHODS

Fish

Almost all mullet caught from Lake Manzala in Egypt are infected with *Heterophyes.* Mullet taken from this lake were used in this investigation. Experiments were carried out with whole fish, from 20–25 cm in length, and with small parts of fish tissue (1 by 1 by 0.5 cm) to ensure complete penetration of antibiotics.

Antibiotic solutions

Oxytetracycline (OTC) (micronized) and chlortetracycline (CTC)-HCl were used. Both antibiotic preparations were dissolved in distilled water to give final concentrations of 30, 60, 90, 150, 300, 500, 2000 and 4000 ppm.

Treatment of fish

Whole mullets were arranged in suitable glass vessels and enough antibiotic solution added to cover the upper surface of the fish. Vessels containing the fish and antibiotic solution were then stored at 6 ± 2°C for varying periods according to the concentrations of the antibiotic. In the experiments with small portions of fish muscles, the area near the tail of infected mullet was cut into small pieces using forceps and knives. This area of the fish body usually contains large numbers of the encysted metacercariae of *Heterophyes.* The small portions of fish tissue were placed in glass beakers followed by the antibiotic solutions, then held at 6 ± 2°C. Periodically, samples from both whole mullet and small parts of fish dipped in the different antibiotic solutions were withdrawn for examination.

Microscopic detection of *Heterophyes* cysts in fish tissue

Approximately 0.25–0.50 g of fish muscle was pressed between 2 glass microscope slides. The movement of the worms inside the cysts indicated that the parasite was still alive. For the examination of whole mullet, the samples were taken from the muscle tissue near the tail. In each determination 10 cysts were selected at random and examined to follow the rate of killing of the parasite as affected by the type and concentration of antibiotics as well as the duration of dipping.

Assay of the antibiotic concentration in fish

In each examination 2 fish were used. 5.0 g of skinned muscle of each fish were taken aseptically with knives and forceps. Samples from the 2 fish, i.e., 10.0 g, were put in a sterile blender and mixed with 90 ml of 0.1 M citrate buffer (pH 5.2) for 5 min. The resulting mixture represented the first obtainable dilution, i.e., $\frac{1}{10}$th dilution. Further dilutions were prepared using the same buffer solution. Suitable dilutions were taken for determination of the antibiotic concentration by Tomiyama's pad method using *Bacillus cereus* var. *mycoides* (Tomiyama et al., 1957).

RESULTS & DISCUSSION

A—Experiments with whole mullet

Both the skin and flesh of fish serve as effective barriers to the penetration of antibiotics to the depth necessary to effect the desired results. The present study represents an attempt to destroy a parasite of mullet through treatment with oxytetracycline (OTC) and chlortetracycline (CTC). The daily examination of mullet immersed in OTC and CTC solutions having concentrations of 30, 60, 90 and 150 ppm revealed that such concentrations of antibiotics did not completely destroy the parasite. The failure of such low antibiotic concentrations to destroy all parasites rendered the fish hazardous to health.

In 30 ppm of OTC almost all cysts examined contained live metacercariae during the first 3 days; whereas, the concentration of this antibiotic in fish tissue amounted to 13 ppm after 24 hr of dipping. The antibiotic concentration in mullet stored in 150 ppm CTC solution for 1 day reached as much as 63 ppm; meanwhile, 8 of 10 cysts examined were found still alive.

From the aforementioned results, OTC and CTC under the conditions reported do not act as effective parasiticides. Hence, it was further suggested to study the effectiveness of these antibiotics upon the survival of *Heterophyes* when higher concentrations are used for dipping of

Table 1—Viability of Heterophyes found in flesh of whole mullet dipped in relatively higher concentrations of OTC and CTC solutions and the antibiotic concentration in fish tissue.

Dipping time in hours	Concentrations of antibiotic solutions used for dipping of whole mullet											
	300 ppm			500 ppm			2000 ppm			4000 ppm		
	A		B	A		B	A		B	A		B
	+	−	ppm	+	−	ppm	+	−	ppm	+	−	ppm
	Oxytetracycline (OTC)											
3	10	0	10	10	0	15	10	0	210	8	2	300
6	10	0	90	10	0	120						
8							8	2	300	7	3	600
9	8	2	100	9	1	150						
12	9	1	105	10	0							
21							7	3	850	4	6	1500
24	8	2	125	8	2	220	4	6	1000	2	8	1600
29	7	3	130	8	2	225						
36	8	2	135	7	3	230						
48	7	3	160	7	3	260						
	Chlortetracycline (CTC)											
3	10	0	8	10	0	12	8	2	205	7	3	350
6	10	0	100	9	1	130						
8							7	3	310	5	5	600
9	9	1	105	8	2	165						
12	8	2	110	8	2	180						
21							6	4	1000	2	8	1600
24	8	2	120	7	3	230	3	7	1100	1	9	1700
29	8	2	125	7	3	233						
36	9	1	140	6	4	240						
48	8	2	150	6	4	255						

A = Results of the microscopic examination of 10 cysts.
B = Antibiotic concentration in fish tissue.
+ = Live worm; − = killed worms.

whole mullet. To achieve this, 2 separate experiments were conducted using 300, 500, 2000 and 4000 ppm from the 2 types of antibiotics, and samples from treated fish were taken periodically for microscopic examination and for the assay of antibiotic concentration in fish tissue. Results of these experiments are summarized in Table 1. They clearly indicate that the encysted metacercariae of *Heterophyes* were highly resistant to both OTC and CTC antibiotics. Concentrations of 300 and 500 ppm had no marked effect on the viability of the parasite, whereas concentrations of 2000 and 4000 ppm had a moderate effect on it. Only about 1/3 of the cysts were killed when 300 and 500 ppm of both antibiotics were applied for 48 hr. In higher concentrations, i.e., 2000 and 4000 ppm, the rate of destruction distinctively increased after 24 hr. For example, 2 of the 10 cysts examined remained alive when whole mullet were dipped for 24 hr in 4000 ppm OTC solution. Similar results were obtained with CTC. The concentration of antibiotics in fish tissue resulted in a marked increase during the first 24 hr but this increase was less marked during the second 24 hr. For instance, the antibiotic concentration in the tissue was 125 and 220 ppm after dipping for 24 hr in 300 and 500 ppm OTC solutions, respectively, and the corresponding values after 48 hr were 160 and 260 ppm. However, these concentrations did not affect the viability of *Heterophyes*, as previously discussed. When higher concentrations (i.e., 2000 and 4000 ppm) were used, the rate of killing markedly increased, with an increase in antibiotic concentration. The concentration reached 1600 ppm in the tissue immersed in 4000 ppm OTC solution for 24 hr. Similar results were obtained for CTC (Table 1).

These findings indicated that the antibiotics OTC and CTC did not act as effective parasiticides under the conditions reported in these studies. From a practical food quality standpoint, such high antibiotic concentrations cannot be considered for dipping of fish, due to food regulatory limits of 5 ppm of antibiotics in fish tissue.

B—Experiments with small portions of mullet

To obtain more direct contact of the antibiotics with *Heterophyes*, small pieces of infected fish tissue were soaked in antibiotic solutions of varying concentrations. The data in Table 2 indicate the numbers of live and killed cysts from 10 cysts examined in each subsample. As in the previous experiment with whole mullet, antibiotics of low concentrations, i.e., 30, 60, 90 and 150 ppm, had no marked effect on the survival of *Heterophyes*. At these concentrations (6–8) cysts were found after 8 days. However, when higher concentrations of OTC and CTC were used, the rate of killing was increased and the parasite was completely destroyed after 6 and 8 days, respectively. OTC and CTC showed similar trends. The duration after which the cysts were completely killed was reduced to 1 and 5 days when

Table 2—Viability of Heterophyes found in small portions of fish soaked in OTC and CTC solutions (results from 10 cysts).

Dipping time in days	Concentrations of antibiotic solutions used for dipping (ppm)															
	30		60		90		150		300		500		1000		2000	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
	Oxytetracycline (OTC)															
0.5															3	7
1	10	0	9	1	9	1	8	2	8	2	9	1	8	2	0	10
2	9	1	10	0	9	1	8	2	8	2	7	3	7	3	0	10
3	10	0	9	1	7	3	8	2	7	3	8	2	4	6		
4	9	1	8	2	8	2	8	2	7	3	4	6	3	7		
5	9	1	9	1	8	2	7	3	6	4	1	9	0	10		
6	8	2	9	1	7	3	7	3	6	4	0	10	0	10		
7	9	1	8	2	6	4	7	3	2	8	0	10				
8	8	2	7	3	6	4	6	4	0	10						
9									0	10						
	Chlortetracycline (CTC)															
0.5															3	7
1	10	0	9	1	8	2	7	3	10	0	8	2	9	1	2	8
2	9	1	9	1	7	3	7	3	9	1	8	2	6	4	0	10
3	8	2	8	2	7	3	8	2	8	2	8	2	5	5	0	10
4	8	2	8	2	7	3	7	3	8	2	6	4	5	5		
5	9	1	7	3	8	2	8	2	7	3	4	6	0	10		
6	9	1	7	3	7	3	7	3	5	5	0	10	0	10		
7	8	2	8	2	7	3	6	4	2	8	0	10				
8	7	3	7	3	6	4	7	3	0	10						
9									0	10						

+ = Cysts containing live worms.
− = Cysts containing killed worms.

solutions of 2000 and 1000 ppm of OTC were used, respectively. The corresponding values for CTC were 2 and 5 days (Table 2).

Results of the microscopic examination of infected mullet portions dipped in antibiotic solutions confirm the findings obtained previously with the treatment of whole mullet. It was obvious that the rate of killing was slightly higher when small portions of fish tissue were immersed in the antibiotic solutions as compared to the treatments. This may have been due to the easier penetration of the antibiotics into the smaller portion tissue.

Although the findings in this study were negative, they suggest the urgent necessity for further efforts in solving this most perplexing problem in seafoods.

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LINDA S. MILLER and J. F PRICE
Dept. of Food Science, Michigan State University, E. Lansing, Mich.

EXTRACT RELEASE VOLUME (ERV) RESPONSES WITH ASEPTIC AND INOCULATED PORK

*SUMMARY The extract-release volume (ERV) phenomenon, previously reported as a reliable rapid indicator of bacterial spoilage in meat, was observed for pork muscle obtained aseptically and for that inoculated with pure and mixed cultures of bacteria over a 20-day storage period at 2° and 10°C. ERV values obtained using pork longissimus dorsi muscle were found to be inversely related to bacterial numbers regardless of population type (homogeneous or heterogeneous) of the microorganisms. The correlations between ERV and bacterial numbers did not denote reliable prediction of the bacteriological quality of pork by ERV. Under conditions of mixed culture sample contamination the correlation between ERV and bacterial numbers was much higher than for pure culture sample contamination with psychrophilic, anaerobic, or acid-producing bacteria. Minor initial differences in ERV between control (aseptic) and inoculated samples indicated that ERV responded to growth of the bacteria rather than to their presence per se. Differences in ERV that were attributable to sample source (animal differences) by analysis of variance test indicated there was a wide range of hydration capacities in pork longissimus dorsi muscle samples. This disparity minimized the significance of the influence of bacterial growth on the ERV response for practical applications. Contrary to the usual ERV response observed with meat spoiled under refrigerator conditions, ERV was found to increase with storage time and bacterial growth when pork was contaminated with a putrefactive anaerobe (*Clostridium perfringens*) and stored at 35°C. Though pH appeared to influence the magnitude of the changes observed in ERV, decreases in ERV with bacterial growth or storage time could not be explained solely as a pH effect.*

INTRODUCTION

THE EXTRACT RELEASE volume (ERV) phenomenon has been studied as a means of evaluating changes in meat that result from microbial growth and spoilage. One suggestion was that ERV could serve as a rapid test to indicate the microbial quality of ground beef. Jay and Kontou (1964) showed that ERV decreased as spoilage proceeded and bacterial population increased. This relationship of ERV to bacteria numbers was relatively consistent with ground beef spoiling by its natural microflora, and organoleptic condition paralleled bacterial population (Jay and Kontou, 1964). These workers suggested that an ERV of 25 ml (corresponded to approx. 16 million organisms per g) should be a probable limit to differentiate between organoleptically acceptable and unacceptable beef.

Investigative work done as early as 1964 (Price, J. F., Wisidagama, C. and Bratzler, L. J.—unpublished data) in our laboratories indicated that a wide range of ERV (0–40 ml) could be observed with different batches of ground pork exhibiting similar organoleptic spoilage and total counts. Further, the fat content of ground beef in the range of 4–40% appeared to have influenced the magnitude of ERV depression during refrigerated storage. Differing ERV patterns with different storage temperature conditions (4 and 16°C) (Price, Wisidgama and Bratzler, 1965—unpublished data) and differing culture maintenance practices (Borton, 1966) was thought to be due to differences in microflora.

This study was undertaken to evaluate the ERV phenomenon as an index of the bacteriological condition of pork, and to evaluate the effects of variations in the microflora on ERV using applied procedures for obtaining aseptic muscle (Borton, 1966; Davis, 1965).

EXPERIMENTAL

Sampling procedure

Pairs of 68–127 kg hogs obtained from the Michigan State University farms were slaughtered after being held overnight without feed. Each animal was electrically stunned and hoisted by one rear leg while the forelegs, sticking area, and ventral fore quarters were scrubbed with a warm bactericidal soap (Phisohex, Winthrop Labs, N.Y.) solution. Liquid soap was applied to the area full strength and followed by a 30 sec scrub with brush, a water rinse, and a 30 sec scrub with 1:100 soap dilution which was scraped away (no rinse). The hog was stuck with a sterile knife and conventionally scalded and dehaired. Prior to evisceration, the carcass was washed with bactericidal soap and rinsed with 100% ethanol. After evisceration, the body cavity was rinsed with 100% ethanol and flamed. The entire unsplit carcass was again rinsed with ethanol and stored in a 2–4°C cooler.

Sample excision

Each carcass was rinsed with ethanol after 24- or 48-hr storage and the shoulder portion removed at the third rib. The remainder of the carcass was placed belly-down on a paper-covered table in a 3°C cooler having minimal air currents. All surgical equipment and sample containers were sterile and the operators wore sterile surgical gloves.

One incision was made down the midline of the loin backfat cover. After peeling away the backfat cover from the surface of the longissimus dorsi with one sterile knife, another sterile knife was used to excise slices of loin muscle which were aseptically transferred to sterile containers for grinding. Control samples were taken from one side and pork to be inoculated was taken from the opposite longissimus dorsi muscle.

Sample inoculation

Control muscle slices were ground aseptically in a sterile grinder. 10 ml of sterile water (buffered dilution blanks, American Public Health Assn. 1958) were added as the 1200–1400g of meat was extruded. Regrinding facilitated mixing the sample. The ground tissue was transferred aseptically to 14 sterile screw-topped containers. Half the containers were stored at 2°C; the rest at 10°C. Muscle for inoculation was also ground in a sterile grinder but had 10.0 ml of a specific bacterial suspension added after which the sample was reground. These tissues were stored in the same manner as the control samples.

Inocula preparation

All inocula were prepared from water washings of APT (All Purpose plus Tween) agar slants of the bacteria used with the exception of *C. perfringens* which was grown in fluid thioglycollate medium The inocula consisted of either 0.1 ml or 1.0 ml of a 1:100 dilution of turbid washings in 9.9 ml sterile phosphate-buffered water.

Sample treatment

Muscles excised from at least two different pigs were used in each phase of the study. Primary interest was in the effects of (1) a pure culture psychrophile; (2) pure culture lactic acid formers; (3) mixed culture psychrophiles; and (4) a pure culture putrefactive anaerobe. The treatment design is presented in Table 1.

Bacterial numbers

Standard procedures described by the American Public Health Association (1958) were used. The effects of actively growing *C. perfringens* on the original test parameters of ERV, pH, and bacterial numbers were studied because the original experimental conditions were recognized as being nonconducive to the growth and enumeration of this organism. Therefore, after inoculation with *C. perfringens*, samples were stored at 35°C in a nitrogen-flushed desiccator along with control samples and were analyzed after 0, 2, 4, 6, and 8 days of storage. Plating of the samples for quantitation of bacterial numbers was done in both SPS (sulfite-polymyxin-sulfadiazine) and APT (all purpose plus Tween) agars under anaerobic and aerobic conditions. In this manner unintentional contaminants, aerobic or anaerobic, could be detected in addition to the organisms added. Inoculated samples were expected to show the

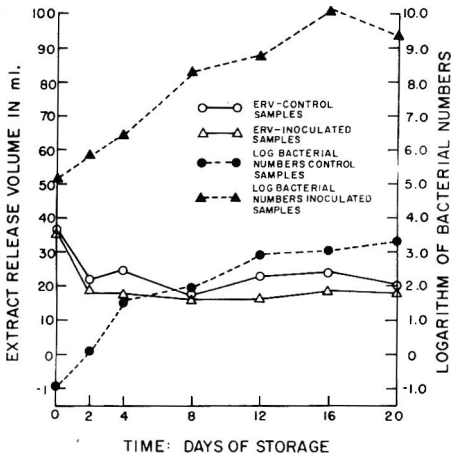

Fig. 1–Extract-release volume (ERV) and logarithm of bacterial numbers for control and P. fluorescens inoculated pork–phase 1, animal 1.

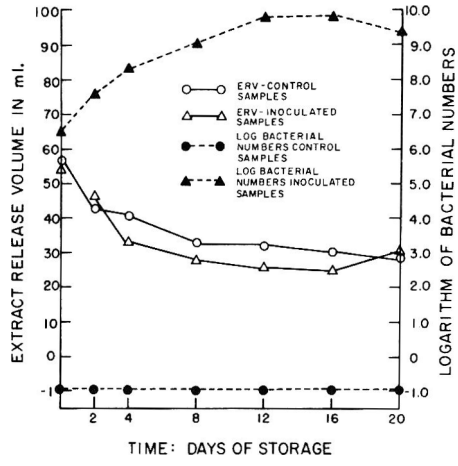

Fig. 2–Extract-release volume (ERV) and logarithm of bacterial numbers for control and P. fluorescens inoculated pork–phase 1, animal 2.

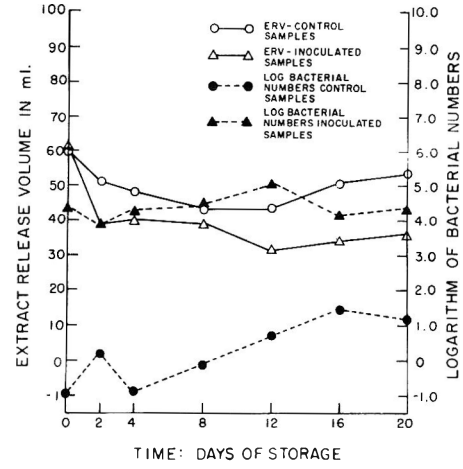

Fig. 3–Extract-release volume (ERV) and logarithm of bacterial numbers for control and S. faecalis inoculated pork–phase 2, animal 3.

effects of both anaerobic bacterial growth and high temperature storage on the test parameters while the control samples, if obtained bacteria-free, would show only temperature effects.

Extract-release volume

A slight modification of the procedure of Jay and Kontou (1964) was used. Duplicate 25g samples of each of the four sample treatments (control, 2°C or 10°C storage; inoculated, 2°C or 10°C storage) were tempered for 1 hr at 5°C and were then blended for 2 min with 100 ml of 0.1M phosphate buffer. The slurry was filtered through Whatman No. 1 filter paper folded to yield eight sides. The amount of filtrate collected after 15 min was reported as the extract-release volume (ERV) and was recorded as the average of the duplicate samples. Extract pH was measured with a Beckman Zeromatic pH meter. All extractions were performed in a 5°C cooler.

Statistical analysis

Data obtained were subjected to basic statistical analysis which yielded simple correlation coefficients over all phases and within each phase. Analysis of variance within phases was accomplished to ascertain significant sources of variation.

RESULTS & DISCUSSION

THE CONTROL SAMPLES, which were intended to be aseptic, exhibited either nondetectable or extremely low levels of initial contamination (Fig. 1–7). Outgrowth was observed in only 3 of the 13 control sample sets represented in these figures. The special procedures for procuring aseptic raw meat samples were therefore considered adequate for the purposes of this study. Because a few of the control samples exhibited outgrowth the data for each sample source (animal) were charted separately. However, data for the two storage temperatures (2° and 10°C) for each sample source in a given phase of this study were combined for illustrative purposes in Figures 1–6. Bacterial growth patterns were influenced by storage temperature to the extent that bacterial populations in the inoculated samples reached higher maximum levels 4 to 8 days sooner at 10°C than at 2°C. Since the growth curves observed were essentially parallel the combination of the data for the two storage temperatures did not alter interpretation of the results in phases 1–3 of this experiment.

In those instances where no viable organisms were recovered from sets of low-dilution plates containing a total of 1.0g of sample, it was assumed that the bacterial populations were not 0 but were less than 0.1 cell per gram of sample, and the log of the bacterial numbers was assigned the value –1.0.

The experimental conditions of low temperature storage did not allow for adequate and consistent recovery of viable cells from the meat samples when *C. perfringens* was the contaminating microorganism (phase 4, animals 7 and 8). However, similar techniques were adequate for obtaining reasonable recovery of this anaerobe from pork when the samples were incubated at 35°C (animal 9, Fig. 7).

Table 1–Treatment design.

Inoculation phase	Source animal No.	Approx. inoculation level	Organism used	Storage temperature (°C)	Sampling times (days)
1–pure culture psychrophile	1	10^5/g	*Pseudomonas fluorescens*	2 & 10	0, 2, 4, 8, 12, 16 and 20
	2	10^6-10^7/g			
2–pure culture lactic acid formers	3	10^4/g	*Streptococcus faecalis*[1]	2 & 10	0, 2, 4, 8, 12, 16 and 20
	4	10^4-10^5/g	*Pediococcus cerevisiae*		
3–mixed "natural" flora	5	10^3-10^4/g	Mixed[2]	2 & 10	0, 2, 4, 8, 12, 16 and 20
	6	10^3-10^4/g	Mixed		
4–pure culture putrefactive anaerobe	7	10^2-10^3/g	*Clostridium perfringens*	2 & 10	0, 2, 4, 8, 12, 16 and 20
	8	10^3-10^4/g	*Clostridium perfringens*		
	9	10^4-10^5/g	*Clostridium perfringens*	35	0, 2, 4, 6 and 8

[1] *S. faecalis* was included as a homofermentative lactic acid producer after trials with muscle from two pigs using *P. cerevisiae* resulted in failure to recover organisms from contaminated samples.

[2] The mixed culture was obtained from commercially prepared ground beef and used after only one transfer to artificial media. It was composed mainly of Gram-negative rods.

Phase 1

The changes in ERV and bacterial numbers in pork samples inoculated with *P. fluorescens* at two different levels are illustrated in Figures 1 and 2. ERV decreased over the storage period with the majority of the change occurring during the first 4 days of storage. Although the ERV of the inoculated samples reached lower levels than that of the control samples, the ERV for the aseptic control samples (Fig. 2) also decreased similarly with storage. Differences observed in ERV between control and inoculated samples at any level of contamination were not significant. Upon comparison of the initial ERV of samples from animal 1 (Fig. 1) with that of

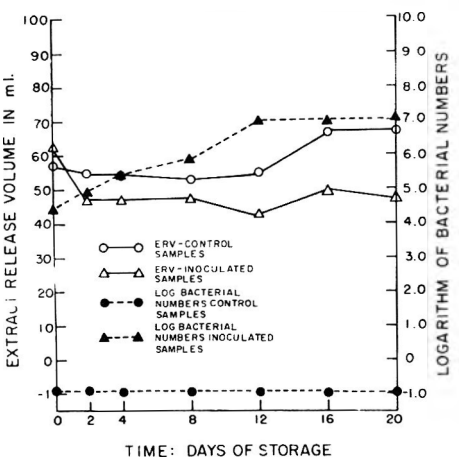

Fig. 4–Extract-release volume (ERV) and logarithm of bacterial numbers for control and P. cerevisiae inoculated pork–phase 2, animal 4.

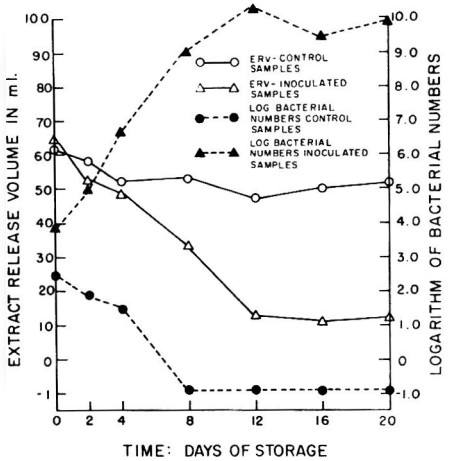

Fig. 5–Extract-release volume (ERV) and logarithm of bacterial numbers for control and Mixed Culture inoculated pork–phase 3, animal 5.

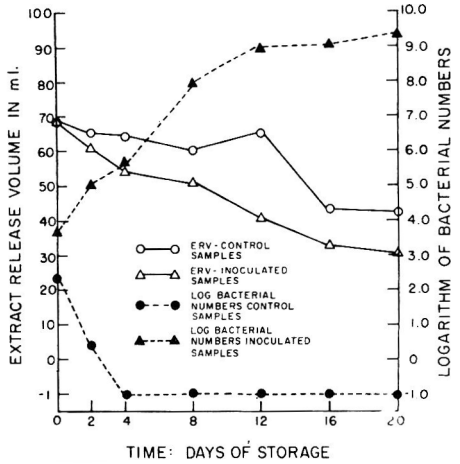

Fig. 6–Extract-release volume (ERV) and logarithm of bacterial numbers for control and Mixed Culture inoculated pork–phase 3, animal 6.

animal 2 (Fig. 2) the striking differences attributable to sample source were readily apparent. These differences undoubtedly limited the value of the ERV phenomenon for estimation of the microbial quality of pork in phase 1. Sample source differences overshadowed the effects of inoculation level on the test parameters. Still, ERVs observed at these similar bacterial populations were quite different from one another. Apparently, factors other than bacterial growth were mainly responsible for ERV variation. The ERV of pork muscle contaminated with *P. fluorescens* was significantly correlated with time ($r = -.47$, $P \leqslant 0.01$) and with the log of bacterial numbers ($r = -.34$, $P \leqslant 0.01$). A positive correlation existed between ERV and pH in phase 1 ($r = 0.56$, $P \leqslant 0.01$).

Application of a pure culture of *S. faecalis* to fresh ground pork resulted in little or no increase in viable cell numbers over a 20 day storage period at 2° or 10°C. Although the bacterial populations of the samples remained fairly constant, decreases in meat slurry pH were observed, indicating that fermentation was probably occurring. ERV for inoculated samples decreased rapidly during the first 2 days of storage and changed little thereafter (Fig. 3), while slightly contaminated control samples showed slower decreases in ERV.

When *P. cerevisiae* was added to pork the maximum viable populations recoverable were between 10^6/g at 2°C and 10^8/g at 10°C storage. In this portion of the study both control sample sets were aseptic (Fig. 4). However, in contrast with observations of other control ERV trends (Fig. 2, 4, and 6) the ERV for these control samples increased at the 16 and 20 day sampling periods. This difference could not be explained satisfactorily. Little change was observed in the ERV of *P. cerevisiae*, inoculated samples after the initial rapid decline (Fig. 4). The pH of the meat slurries of the inoculated samples decreased by 0.4 pH unit after 8–16 days indicating considerable fermentation activity.

With the use of lactic acid fermenting bacteria, the interrelationships between ERV and time ($r = -.16$, $P = 0.23$) and ERV and log bacterial numbers ($r = -.45$, $P \leqslant 0.01$) were low, accounting for little of the variation observed. ERV and pH were positively interrelated ($r = 0.35$, $P \leqslant 0.01$).

The ERV for control samples in phase 3 (Figures 5 and 6) did not deviate grossly from those patterns observed for the aseptic control samples in other phases of this study. However, the ERV of pork inoculated with a mixed culture of psychrophilic microorganisms decreased nearly linearly with time (Fig. 5 and 6). These findings were consistent with the observations of Jay and Kontou (1964) for ground beef spoiled by normal psychrophilic microflora. Lower ERV values corresponded to the higher bacterial populations recovered.

The pH reading of the meat slurries in the control sample sets decreased only slightly during storage (0.1 – 0.2/pH unit). The pH of inoculated pork increased during storage, with the greatest differences between inoculated and control sample pH (0.3–0.8 pH unit) occurring at 10°C storage from the 12th to the 20th day. Alkaline reactions indicated proteolytic changes in the samples. This was in agreement with typical patterns of low-temperature spoilage by psychrophiles. Declines in ERV were parallel to these pH increases ($r = -.71$, $P \leqslant 0.0005$) and lowest ERV values were observed when pH was at the maximum. Though

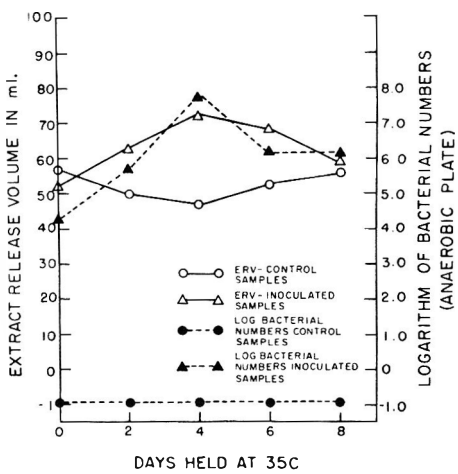

Fig. 7–Extract-release volume (ERV) and logarithm of bacterial numbers for control and C. perfringens inoculated pork–phase 4, animal 9.

the degree to which ERV was influenced by pH per se was not readily discernible, the growth of typical psychrophilic populations of microorganisms in pork was associated with pH increase and ERV decrease.

In this phase of the study low ERV values were associated with active bacterial growth. That the mere presence of bacterial cells did not elicit ERV response was evident because the differences in control and inoculated sample ERV at days 0 and 2 of storage were slight. Therefore, the decline in ERV (increase in apparent hydration capacity of the meat proteins) was probably a result of active growth of the organisms and not just due to their presence in the tissue. With mixed culture inoculation, ERV was significantly correlated with time ($r = -.65$) and with the log of bacterial

numbers ($r = -.64$, $P \leqslant 0.01$). These correlations more closely approached that of Jay and Kontou (1964) ($r = -.808$) than did any observed in this study. The direction of the relationships is noteworthy. Approximately 40% of the variation in bacterial numbers could be accounted for by the ERV response. The lack of a higher relationship was explained in part by analysis of variance. Variation in bacterial count could be attributed largely to inoculation treatment differences. However, sample source, temperature, and time of storage also exhibited important influences on ERV. The negative correlation of ERV with pH ($r = -.71$, $P \leqslant 0.0005$) indicated agreement with the known effects of pH on hydration capacity of muscle proteins.

In pork samples from animals 7 and 8 which were inoculated with *C. perfringens*, the presence of aerobic bacteria in both control and inoculated samples was rarely observed. The experimental storage conditions (2° or 10°C) were obviously not conducive to survival of the organism and the erratic recovery of viable cells precluded meaningful interpretation of the data. In general, there were no differences between the ERVs of the control and inoculated samples. In fact, the patterns of ERV for these samples were very similar to the trends observed for all aseptic samples.

The ERV patterns of control and *C. perfringens* inoculated pork held at 35°C are illustrated in Figure 7. Control ERV values decreased slightly during the first days of storage and then stabilized or increased in the absence of microbially induced degradation. High temperature storage of the aseptic meat appeared to cause protein precipitation in the exudate and a change in the pigment proteins. Proteolytic degradation was apparent in the *C. perfringens* inoculated samples held at 35°C. Liquefaction of the tissues was extensive and the foul odors produced were typical of putrefactive anaerobic spoilage. The response of ERV to high temperature putrefactive spoilage in pork was directly in opposition to that observed for pork inoculated with mixed psychrophiles at refrigerator temperatures. Higher bacterial populations and increasing putrefaction were accompanied by significant increases in ERV.

The meat-buffer slurries of the control samples did not appear physically different from the control slurries observed in phases 1–3, but the slurries of the inoculated samples were markedly different. In phases 1–3 the larger populations of bacteria in the meat were associated with slurries of increasingly thick and viscous character which released progressively decreasing amounts of extract. This effect could have been the result of bacterially caused alterations in the hydration capacity of the muscle proteins or the consequence of water-binding metabolic by-products of the organisms added. However, slurries of pork containing large populations of *C. perfringens* were thin and serous and released larger amounts of extract as the bacterial populations of the meat increased. The typical psychrophilic spoilage of meat is also proteolytic; however, it is possible that the extent of proteolysis occurring dictates whether ERV decreases or increases. More extensive proteolysis observed with meat spoiled with the putrefactive anaerobe *C. perfringens* could indicate that the meat proteins were sufficiently digested to destroy most of their water-binding capacity.

The extract-release volume phenomenon could be an index of the hydration capacity or water-holding ability of pork muscle tissue. If this is correct, then the effects of bacterial growth upon the moisture imbibing characteristics of pork are quite variable depending upon the type of contaminating microorganisms and the conditions for their growth.

As an index of bacterial numbers or extent of microbial spoilage in pork the ERV phenomenon was of no practical value. While the ERV test may be applied as a crude index of spoilage conditions of refrigerated "normal" pork, other rapid tests such as resazurin reduction time (Saffle et al., 1961) are more reliable as predictors of the microbiological status of the meat. ERV of pork longissimus dorsi muscle was subject to extensive variation due to the wide range of water-holding capacity inherent in the meat. Furthermore, different species of bacteria did not elicit the same ERV response with growth.

Low temperature growth of *P. fluorescens*, *S faecalis*, and *P. cerevisiae* appeared to result in decreased ERV even in the absence of pH increases. A dramatic decrease in ERV with concomitant increase in pH was evident upon low temperature mixed microbial spoilage of the pork. However, the putrefactive degradation of pork by *C. perfringens* at high temperatures resulted in increased ERV with pH increases as well.

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JAMES E. THOMSON
USDA, Market Quality Research Div., ARS, Beltsville, Maryland 20705
and LAWRENCE A. RISSE
USDA, Transportation and Facilities Research Div., ARS, Orlando, Florida 32807

DRY ICE IN VARIOUS SHIPPING BOXES FOR CHILLED POULTRY: EFFECT ON MICROBIOLOGICAL AND ORGANOLEPTIC QUALITY

SUMMARY—Comparisons were made of dry ice and water ice in shipping boxes for chilled chickens. Three types of boxes were tested: wax-resin-coated corrugated fiberboard, expanded polystyrene foam, and wirebound wood-veneer. Microbial counts, CO_2 concentration, and off-odor development were determined. Microbial counts on poultry stored at 0.5°C for up to 9 days were not significantly different as a function of box type or coolant. Counts on poultry stored at 4.4°C were significantly greater at 9 days on poultry stored in fiberboard boxes with dry ice than on poultry with water ice in fiberboard boxes or polystyrene boxes with dry ice; at 3 and 6 days there were no significant differences. At 5.2°C, counts were significantly smaller in polystyrene boxes with dry ice than in either wirebound boxes with water ice or fiberboard boxes with dry ice. An off-odor not characteristic of spoilage odor could develop in CO_2 atmosphere storage earlier than spoilage odor in an air atmosphere if storage temperature was low (0.5°C). At higher temperature (5.2°C), spoilage odor in air occurred earlier than CO_2-related off-odor in CO_2 atmosphere.

INTRODUCTION

CARBON DIOXIDE as a preservative for poultry meat has been used with varying results. Smith (1934) and Lea (1934) found that holding uneviscerated chickens at about minus 0.5°C in 96% CO_2 was unsatisfactory; swelling of tissues, discoloration, and autolysis of tissues by enzymes occurred. Ogilvy and Ayres (1951) found improved storage life of chicken in up to 25% CO_2. Wabeck et al. (1967) found that no benefit occurred when 0.5 kg of dry ice was added to 6.8 kg of water ice in wirebound containers; temperature rise was as rapid whether or not dry ice was present. Adding dry ice to water ice in corrugated boxes, however, extended temperature rise time by 6 hr as compared to these boxes with water ice alone. Wabeck et al. (1968) found that growth of psychrophiles on chicken was inhibited by 10 or 20% CO_2, but coliforms remained relatively stable. Off-odor, of a type different from usual spoilage off-odor was noted on chicken in CO_2, and usually occurred earlier in storage than the spoilage off-odor of chicken in an air atmosphere.

Lemmons (1966) described a commercial system of utilizing CO_2 in which about ½ lb of dry ice "snow" was placed in corrugated boxes, an absorbent pad was placed on the "snow" and the poultry was packed in the box. Good product appearance and characteristics were found in commercial shipments. Burkhart (1966) described a commercial system in which prepackaged chicken was shipped in corrugated boxes utilizing dry ice "snow" as coolant.

Baker (1957) compared chickens dry packed (without either water or dry ice) to chickens standard ice-packed, both stored at 2°C, and found no important difference in appearance or microbial count. Dry-packed chicken was preferred by a taste panel. May et al. (1966) found slower microbial growth on ice-packed than dry-packed at 3.3° to 5.6°C storage. Shantz et al. (1967) found that at 4.4°C storage, microbial counts were about the same in dry and ice packs until 4 days of storage, but by 7 days of storage psychrophile counts were higher in dry than in ice packs.

The purpose of this study was to evaluate the use of dry ice under actual or potential commercial conditions. Relations of type of shipping box and coolant to shelf life as determined by microbiological condition and off-odor development were studied.

EXPERIMENTAL

Experiment 1

In a commercial poultry processing plant, full boxes of packed chicken fryers with giblets weighing 1.0 to 1.1 kg per bird, were removed from the line at the point between the filled-box weighing scale and the box-icing operation. The birds were removed from the boxes and repacked rapidly into test boxes. When repacked, temperature in a deep portion of the breast meat of these birds averaged 5°C. The test boxes consisted of four combinations of box material and coolant as follows:

Box material	Coolant
Wax-resin-coated corrugated fiberboard	9.1 kg water ice
Wax-resin-coated corrugated fiberboard	1 kg dry ice "snow"
Expanded polystyrene foam	0.5 kg block dry ice
Expanded polystyrene foam	1 kg dry ice "snow"

An absorbent pad measuring 39.5 × 54.0 cm was placed in the bottom of each test box in which dry ice was used as the coolant. This pad consisted of a top layer of high wet-strength, low absorbency kraft paper, and 10 layers of high wet-strength, high absorbency cellulose wadding.

To determine the general level of microbial counts on the chicken carcasses in the processing plant during the period of repacking, 14 birds were sampled. The breast-swab method was used, wherein a 12.3 sq cm circular area, delineated by a sterile circular fiber template held on the breast skin, was swabbed with a moistened sterile cotton swab for 30 sec, then the swab tip was placed aseptically into 99 ml sterile 0.1% peptone.

Twelve of each of the four box type-coolant combinations were packed.

To pack the boxes in which water ice was used as coolant, about 23 chickens (about 25 kg) were placed breast up, legs toward center in two layers in each box. The appropriate amount of water ice was placed on top of the poultry, and the box was closed.

To pack the boxes in which dry ice "snow" was used as coolant, the 1 kg of "snow" was first dispensed into the bottom of each box from a hooded nozzle leading from a specially modified CO_2 "snow" forming machine. The absorbent pad was then placed over the "snow," and about 25 kg of poultry was placed in the box, and the box was closed.

To pack the boxes in which a block of dry ice was used as coolant, the 0.5 kg block was first placed in a separate small expanded polystyrene "ice bucket" with loosely fitting top, the absorbent pad was then placed in the bottom of the box to be packed, the "ice bucket" containing the dry ice block was placed in a corner of the box, then about 25 kg of poultry was placed in the box, and the box was closed.

All boxes were loaded in a mechanically refrigerated truck and transported approximately 150 mi from the plant to the cold-storage facilities at Beltsville, Maryland. Temperature in the truck was 0.5° ± 3.3°C during the trip.

Upon arrival at Beltsville, 6 boxes of each box type-coolant combination were distributed into a controlled-temperature storage room at 0.5° ± 0.5°C, and 6 into a room at 4.4° ± 0.5°C. Three stacks each 2 boxes high of each box type-coolant combination were placed on wooden racks in the storage rooms. Stacks of each type were distributed throughout the room to minimize the effect of possible air temperature variations.

Percent gaseous CO_2 concentration was measured every day of storage in all boxes in which dry ice was used as coolant. CO_2 determination was by the Ranarex portable CO_2 indicating instrument manufactured by the Permutit Company. Atmospheres inside the boxes were sampled by inserting the plastic sampling tube of the instrument through a hole punched in the top of each box. This hole was kept sealed between samplings with a rubber stopper and adhesive tape.

Temperatures were obtained in the storage room air in both rooms, in the air inside the

Table 1—Microbial counts on breast skin of chicken carcasses in shipping boxes (Experiment 1).[a]

Storage temp (°C ± 0.5°C)	Box material	Coolant	Days stored 3	6	9	Avg of all
0.5	Fiberboard	9.1 kg water ice	3.9 abcd	3.9 abcd	4.3 cde	4.0 ab
	Fiberboard	1 kg dry ice "snow"	3.6 ab	3.6 ab	4.3 cde	3.8 a
	Polystyrene	0.5 kg dry ice block	3.9 abcd	3.6 ab	4.2 bcde	3.9 a
	Polystyrene	1 kg dry ice "snow"	3.9 abcd	3.4 a	4.0 abcd	3.8 a
4.4	Fiberboard	9.1 kg water ice	3.8 abc	3.9 abcd	5.7 g	4.5 d
	Fiberboard	1 kg dry ice "snow"	3.9 abcd	4.5 def	6.9 h	5.1 e
	Polystyrene	0.5 kg dry ice block	3.9 abcd	4.0 abcd	5.1 fg	4.3 cd
	Polystyrene	1 kg dry ice "snow"	3.9 abcd	4.0 abcd	4.7 efg	4.2 bc
Avg of both	Fiberboard	9.1 kg water ice	3.8 ab	3.9 ab	5.0 e	4.3 b
	Fiberboard	1 kg dry ice "snow"	3.8 ab	4.1 bc	5.6 f	4.5 c
	Polystyrene	0.5 kg dry ice block	3.9 ab	3.8 ab	4.6 d	4.1 a
	Polystyrene	1 kg dry ice "snow"	3.9 ab	3.7 a	4.3 cd	4.0 a
0.5	Avg of all	Average of all	3.8 ab	3.6 a	4.2 d	3.9 a
4.4	Avg of all	Average of all	3.9 bc	4.1 cd	5.6 e	4.5 b
Avg of both	Avg of all	Average of all	3.9 a	3.9 a	4.9 b	

[a]Mean log of total organisms by swab method per sq cm. Among means enclosed by lines, those followed by different letters are significantly different at the 5% level.

Table 2—Microbial counts on breast skin of chicken carcasses in shipping boxes (storage at 5.2°C ± 2.0°C) (Experiment 2).[a]

Box material	Coolant	Box stack position	Days stored 3	6	9	Avg of all	Avg each box material-coolant combination
Fiberboard	1 kg dry ice "snow"	top	3.9	6.2	7.5	5.9 b	6.0 bc
		bottom	4.1	6.5	8.0	6.2 bc	
Polystyrene	1 kg dry ice "snow"	top	4.0	5.2	7.8	5.7 b	5.2 a
		bottom	3.9	4.4	6.0	4.7 a	
Polystyrene	none	top	4.2	7.2	8.4	6.6 c	6.4 c
		bottom	4.1	6.2	8.2	6.2 bc	
Wirebound	9.1 kg water ice	top	4.1	6.0	7.7	5.9 b	5.9 b
		bottom	3.8	5.9	7.6	5.8 b	
Avg of all	Avg of all	top	4.3	6.5	8.1	6.3 b	
		bottom	4.1	5.9	7.5	5.8 a	
		Average	4.0 a	6.0 b	7.7 c		

[a]Mean log of total organisms by swab method per sq cm. Among means enclosed by lines, those followed by different letters are significantly different at the 5% level.

boxes, and in deep portions of the carcasses in top fiberboard and polystyrene boxes in the 0.5°C storage room by thermocouples of a 20-point potentiometer recorder.

After 3, 6 and 9 days of storage, 1 stack of 2 boxes of each box type-coolant combination was removed from each storage room, and the breast skin surface of four birds in each box was swabbed for microbiological condition. Serial dilutions were made from original samples, plated in duplicate on tryptone glucose yeast extract agar, and incubated at 20°C, for 72 hr.

The entire experiment was replicated twice. Analysis of variance and Multiple Range Test (Duncan, 1955) were applied to the data.

Experiment 2

24 full boxes of ice-packed chicken fryers, weighing 1.0 to 1.1 kg per bird, were obtained at the same processing plant as in experiment 1. The boxes were then transported to Beltsville by truck. Average temperature in a deep portion of the breast meat upon arrival at Beltsville was 2°C. The birds were then rapidly repacked into test boxes.

Four combinations of box material and coolant were evaluated:

Box material	Coolant
Wax-resin-coated corrugated fiberboard	1 kg dry ice "snow"
Expanded polystyrene foam	1 kg dry ice "snow"
Expanded polystyrene foam	None
Wirebound wood-veneer	9.1 kg water ice

The dry ice "snow" was formed by crushing chips from a block of dry ice to very fine powder.

An absorbent pad of the same type as used in experiment 1 was placed in the bottom of all boxes except the wirebound with water ice.

To determine the general level of microbial counts on the carcasses at the start of storage, 12 birds were sampled by the breast-swab method during repacking into test boxes.

The birds were repacked by the same procedure as in experiment 1.

The test boxes were then stacked 2 high of the same type for a total of 12 stacks on wire racks in a storage room maintained at 5.2° ± 2.0°C. Stacks of each type of box were distributed throughout the room to minimize the effect of possible variations in air temperature in the room.

Percent gaseous CO_2 was measured as in experiment 1. CO_2 concentration in polystyrene box with no coolant was also measured.

Temperatures in the storage room air and in the deep portion of carcasses in each box type were obtained by thermocouples of a 20-point potentiometer recorder.

After 3, 6 and 9 days of storage, 1 stack of 2 boxes of each type was removed from the room. Microbiological condition was determined as in experiment 1.

The presence, intensity and character of off-odor were determined by three experienced investigators as each box was opened after storage.

The entire experiment was replicated twice, and statistical analyses as in experiment 1 were applied.

Experiment 3

Four fryer chickens were stored at 0.5°C, and four at 5.2°C. Two at each temperature were in a CO_2-containing atmosphere in glass jars, and two were in air. The CO_2 atmosphere was measured and adjusted daily to conform approximately to the rate of change in CO_2 concentration which had occurred in polystyrene boxes with dry ice in experiments 1 and 2. Degree and character of off-odors developing each day for 16 days of storage were determined by an experienced investigator.

Experiment 4

128 chicken fryers without giblets, frozen and held at −29°C about 3 months before the start of this experiment, were thawed in running tap water at 7°C in a 200-gal vat until temperature in a deep portion of the meat was 3°–4°C. The vat was emptied of water, the birds drained for 5 min, and 24 birds were then placed in each of 4 boxes as follows:

Box material	Coolant
Wirebound wood-veneer	9.1 kg water ice
Wax-resin-coated corrugated fiberboard	0.5 kg dry ice "snow"
Wax-resin-coated corrugated fiberboard	2.3 kg dry ice "snow"
Expanded polystyrene foam	0.5 kg dry ice "snow"

All boxes except the wirebound were tightly strapped with filament tape. The boxes were stored at 3°C, and opened briefly at 7, 10 and 13 days for microbiological and organoleptic examination; presence or absence and character of off-odors on the birds and in the atmosphere in the box were also determined. Microbiological examination, CO_2 concentration measurements and statistical analyses were as in experiment 1.

RESULTS & DISCUSSION

Microbiological changes

All microbial counts herein reported

are expressed as the mean logarithms of the actual counts.

The microbial counts on the breast skin of the chickens sampled at the processing plant in experiment 1 for replicate one were 3.8 ± 0.1 (logarithmic mean ± standard error) per square centimeter, and for replicate two were 4.0 ± 0.1. Counts during the period of test box packing in experiment 2 for replicate one were 3.9 ± 0.1, and for replicate two were 3.3 ± 0.1.

In experiment 1 (Table 1), chickens stored at 4.4°C showed greater counts (P = 0.01) than those stored at 0.5°C. Chickens stored in fiberboard boxes with dry ice "snow" as coolant showed greater counts (P = 0.05) than those in fiberboard with water ice, which in turn showed greater counts (P = 0.05) than those in polystyrene boxes with either "snow" or block dry ice. There was no significant difference in counts between polystyrene with dry ice in the form of 1 kg of "snow" and polystyrene with dry ice in the form of a 0.5 kg block. The greater count in fiberboard boxes with dry ice was evident only at 4.4°C storage temperature; at 0.5°C, the fiberboard with dry ice was not significantly different from the other box types. Also, until after 6 days of storage at either temperature, the difference in counts between fiberboard with dry ice and the other box types was inconsequential. At 9 days of storage at 4.4°C the count was significantly greatest in the fiberboard with dry ice (P = 0.05), smallest in the polystyrene with dry ice (P = 0.05), and intermediate in fiberboard with water ice (P = 0.05). Because the CO_2 dissipated so rapidly from the fiberboard boxes, dry ice in boxes of this type was not as effective as water ice in retarding microbial growth at 4.4°C storage. At 9 days of storage at 0.5°C counts were not significantly different from those at 6 days at 4.4°C (P = 0.05).

In experiment 2 (Table 2), the overall microbial count was greater than in experiment 1 because of a higher and more variable storage temperature. The storage rooms used in experiment 1 were in a well insulated, centrally conditioned facility in which temperature control was highly accurate and precise. These rooms were not available for experiment 2, so a less well insulated and controllable room was used. The room temperature variability in experiment 2 was not random, however; the compressor turned on and off regularly every 30 min so that the room temperature cycled in a consistent manner above and below the mean temperature throughout the experiment.

In experiment 2 (Table 2) the count was significantly greater after 6 days than 3 days (P = 0.01) and greater after 9 days than 6 days (P = 0.01).

Polystyrene boxes with dry ice showed significantly smaller counts (P = 0.05) than either wirebound with water ice or fiberboard with dry ice (Table 2). Polystyrene with no coolant showed significantly greater counts (P = 0.05) than polystyrene with dry ice or wirebound with water ice.

Significantly greater overall counts (P = 0.05) were found in the top boxes of the stacks than the bottom in experiment 2 (Table 2). This was due primarily to the consistent difference in count in the top from that in the bottom polystyrene boxes with dry ice. This difference increased in magnitude over the storage period. A tendency toward this pattern, with polystyrene boxes had been noted in experiment 1, but was not statistically significant.

In the bottom polystyrene boxes with dry ice, counts were smaller because the box lids were firmly held in place by the boxes higher in the stack, so that gaseous CO_2 leakage around the lids and out of the box was minimized. Greater CO_2 retention in bottom boxes was confirmed by CO_2 measurement, as will be noted later. Counts should be smaller in the top boxes of this type if they were fabricated with very tightly fitting lids, were strapped, or if the lids were weighted or pressed down in some manner.

In experiment 4 (Table 3) overall counts were significantly greater after 10 than after 7 days (P = 0.01) and greater after 13 than after 10 days (P = 0.01). Polystyrene boxes with dry ice showed significantly smaller counts than the other box types (P = 0.01). The use of 2.3 kg of dry ice in the fiberboard box did not result in smaller counts than 0.5 kg of dry ice in fiberboard or 9.1 kg water ice in the wirebound box; these three box types were not significantly different.

Table 3–Microbial counts on breast skin of chicken carcasses in shipping boxes (storage at 3°C) (Experiment 4).[a]

Box material	Coolant	Days stored 7	10	13	Avg of all
Wirebound	9.1 kg water ice	4.9	5.7	7.3	6.0 a
Fiberboard	0.5 kg dry ice "snow"	4.9	7.0	8.1	6.7 a
Fiberboard	2.3 kg dry ice "snow"	4.6	6.3	7.8	6.2 a
Polystyrene	0.5 kg dry ice "snow"	3.7	4.7	4.8	4.4 b
	Average	4.5 a	5.9 b	7.0 c	

[a]Mean log of count per sq cm. Among means enclosed by lines, those followed by different letters are significantly different at the 1% level.

CO_2 concentration changes

Fiberboard boxes in which dry ice was

Table 4–Percent CO_2 content of atmosphere in polystyrene shipping boxes after specified storage period (Experiment 1).[a]

Storage room temp	Form of dry ice	Box stack position	Days stored 1	2	3	4	5	6	7	8	9
0.5° ± 0.5°C	0.5 kg Block	Top	41	17	6	2	2	1	1	1	0
		Bottom	60	57	34	22	17	15	14	12	9
0.5° ± 0.5°C	1.0 kg "snow"	Top	17	10	6	5	3	2	1	1	0
		Bottom	40	30	21	20	16	14	11	9	8
4.4° ± 0.5°C	0.5 kg Block	Top	33	9	6	3	2	1	0	0	0
		Bottom	69	48	27	21	15	12	10	8	6
4.4° ± 0.5°C	1.0 kg "snow"	Top	12	11	4	2	2	1	1	0	0
		Bottom	46	34	23	22	19	14	11	10	7
		Mn	40 a	27 b	16 c	12 cd	9 de	7 de	6 de	5 de	4 e

[a]Means followed by different letters are significantly different at the 5% level.

used showed less than 1% CO_2 content in atmosphere after 1 day of storage, and continued to show this low content throughout storage.

Percentages of gaseous CO_2 in polystyrene boxes with dry ice as coolant did not differ significantly as a function of storage room temperature.

Content of CO_2 in polystyrene boxes was significantly greater ($P = 0.05$) when a block of dry ice, rather than "snow" was used. This was most evident the first few days of storage (Table 4). Probably, because of the slower sublimation rate of block dry ice, the evolution of CO_2 was extended over a longer period.

The bottom polystyrene boxes in the stacks showed significantly greater CO_2 content than the top boxes ($P = 0.01$). Box lids on the bottom boxes were held down tightly, while leakage of CO_2 gas could progress quite rapidly around the loosely fitting lids of top boxes.

CO_2 content of polystyrene boxes declined significantly during the 9-day storage period ($P = 0.01$). CO_2 content in the polystyrene boxes using dry ice "snow" as coolant in experiments 2 and 4 changed in approximately the same way as in experiment 1.

In polystyrene boxes in which no coolant was used, bottom boxes accumulated up to 4% CO_2 after 6 days of storage, and between 6 and 8% after 9 days. This CO_2 probably evolved from microbial growth and need be considered only in the latest stages of storage, however. Under the conditions of this experiment, CO_2 from microbial growth was measurable only when the carcasses had reached a state of advanced spoilage.

Off-odor development

In experiment 2, carcasses in polystyrene boxes with dry ice "snow" as coolant showed only very slight spoilage off-odor after 9 days of storage; those in polystyrene boxes with no coolant showed slight spoilage off-odor after 6 days, and very strong after 9 days of storage; those in fiberboard boxes with dry ice "snow" showed slight spoilage off-odor after 6 days, and strong (but less than polystyrene with no coolant) after 9 days of storage; those in wirebound boxes with water ice as coolant showed no spoilage off-odor in 6 days, but very strong off-odor in 9 days. No off-odors except those which could be characterized as typical spoilage odors were noted in experiment 2.

At 1°C in 10 and 20% CO_2 atmospheres, Wabeck et al. (1968) found "sweet" or "fruity" off-odors, unlike spoilage odors at about 10 or 12 days of storage. This usually occurred earlier than the characteristic off-odor of spoiled poultry stored in air. The off-odor of carcasses in CO_2 atmosphere, which was less objectionable than off-odor of carcasses in air atmosphere, was attributed to growth of an organism that is normally suppressed by spoilage organisms. Experiments 3 and 4 further evaluate this phenomenon and relate it to the conditions of storage in the present study (data not shown).

In experiment 3 at 0.5°C in CO_2 atmosphere, the sweetish, not characteristically spoilage, odor was noted on carcasses after 8 days. This odor, which remained and intensified over the entire 16-day storage period, was not replaced or supplemented by typical spoilage odor. At this temperature in air, very slight typical spoilage odor was noted after 8 days. The odor remained slight until about 13 days of storage, when it became strong.

At 5.2°C in CO_2 the sweetish odor was first evident after 7 days of storage and remained strong until after 14 days of storage, when spoilage odor could also be detected. Typical spoilage odor was strong after 15 days. At this temperature in air, slight typical spoilage odor was first evident after 5 days, and was strong after 7 days of storage.

In experiment 4, at 3°C no off-odors of any type were noted at 7 days. At 10 days, a slight spoilage off-odor was noted in wirebound boxes with 9.1 kg water ice and in fiberboard boxes with 2.3 kg of dry ice, and a strong spoilage off-odor was noted in fiberboard boxes with 0.5 kg of dry ice. The sweetish, CO_2-related off-odor was apparent in the polystyrene box by 10 days of storage. At 13 days of storage, spoilage off-odor was strong in all boxes except polystyrene with 0.5 kg of dry ice; in the last type, CO_2-related off-odor was very strong.

The off-odor which may appear under CO_2 storage, although less repulsive than typical spoilage odor, can be a problem under certain conditions. At low (near freezing) storage temperatures in CO_2 atmosphere, this off-odor may appear much earlier than spoilage odor in air. At higher storage temperatures, spoilage odor in air may occur earlier than off-odor in CO_2, but only by a day or so.

The CO_2-related off-odor could be a problem only in storage facilities or tightly closed boxes in which relatively high concentrations of CO_2 are maintained over several days.

The basis of CO_2 atmosphere-related off-odor is yet to be completely elucidated. In addition to selective inhibition of spoilage organisms, unusual metabolism of certain spoilage types, or action of tissue enzymes might be involved.

No color change or effect not within the normal range for commercially produced fryer chickens, was found in any carcasses in these experiments.

CONCLUSIONS

DRY ICE in boxes which could not be sealed to minimize CO_2 escape (fiberboard boxes), could be less effective, and was never more effective, than water ice in fiberboard or wirebound wooden boxes in retarding microbial growth on fryer chickens. Dry ice, in boxes sealed tightly enough to allow CO_2 buildup (polystyrene boxes), was more effective than water ice in fiberboard or wirebound wooden boxes in retarding microbial growth; however, an off-odor on the carcasses associated with high CO_2 content in the atmosphere in the polystyrene boxes might occur.

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C. R. SCOTT and H. O. SMITH
Packaging Research Laboratory, The Dow Chemical Company, Midland, Michigan 48640

COTTAGE CHEESE SHELF LIFE AND SPECIAL GAS ATMOSPHERES

SUMMARY—Cottage cheese samples were stored at 3–4°C for 10–12 days in special all-glass containers with purified carbon dioxide, nitrogen and air atmospheres. Shelf-life quality of the cheese was measured by taste panel scoring and by bacterial counts of the top centimeter of product. Carbon dioxide slightly decreased the bacterial counts but it produced an acid or tart cheese. Nitrogen did not significantly decrease the bacterial counts nor affect the taste of the cheese.

INTRODUCTION

THE LITERATURE on cottage cheese shelf life contains varying opinions regarding the efficacy of replacing headspace air with various gas atmospheres or vacuum, or both. Zimmerman and Kester (1960) found hermetically sealed containers, vacuum packaging and packaging in inert gases successful means to retard surface spoilage. Tsantilis and Kosikowski (1960) evaluated the effect of vacuum and atmospheres of nitrogen or carbon dioxide on the shelf life of cottage cheese. The cottage cheese with carbon dioxide had a greatly reduced yeast and mold count and retained a fresh flavor even after an extended storage period. Taylor et al. (1965) found that nitrogen flushing did not provide consistent improvement in shelf life of cottage cheese which had been packaged in a dairy plant. A long-term storage experiment at room temperature by Krcal (1968) showed real reduction in microbial deterioration of cottage cheese by carbon dioxide and nitrogen whereas the carbon dioxide treatment was "preferable."

This report describes a further evaluation of the effect of carbon dioxide and nitrogen atmospheres on the shelf life of cottage cheese under controlled conditions. Polystyrene plastic containers for years have been accepted as suitably inert and excellent for packaging of cottage cheese. For this study, however, special glass containers were designed to eliminate other possible factors that may have affected the flavor of the cheese in the earlier studies.

The effects of vacuum on the storage life of cottage cheese were not included in this study because present commercial containers are not suited to vacuum packaging. In addition, it is commercially easier to create an oxygen-poor atmosphere by a gas-flush procedure than by vacuum.

EXPERIMENTAL

General

Commercial tubs of cottage cheese with lids removed were placed in specially constructed glass containers, a selected gas flushed through to displace all headspace air and the containers sealed for refrigerated storage. At the end of the storage period the containers were sampled for analysis of the headspace gas. The cheese was sampled for bacteria counts and finally was evaluated by a taste panel.

Source and type of cottage cheese

Creamed, small-curd cheese was used. Experiments were carried out separately on cottage cheese from 2 different firms. The cheese, commercially packaged in 16-oz polystyrene tubs with polystyrene lids, was transported on ice to the laboratory. The testing began 4–12 hr after the commercial packaging.

Glass containers

The special glass containers, as shown in Figure 1, were prepared from 2-liter Pyrex resin reaction kettles (Corning 6947). The bottom portion which held the tub with cheese measured 13 cm inside diameter and 6 cm deep. The cheese cartons with lids removed measured 11.8 cm in diameter and when placed into the unit were nearly flush with the flange. The kettle lid contains 4 female taper joints, 1 in the center and the other 3 arranged symmetrically around the periphery. The center joint and 1 on the periphery were plugged with glass stoppers and were not used. 1 of the remaining joints was fitted with a 2-way glass stopcock, mounted on the corresponding male joint, and the other with a 3-way glass stopcock, prepared in similar fashion. The plugs of both stopcocks were of Teflon. A gas-flow deflector on the latter joint distributed the gases more uniformly throughout the closed container and prevented the gas flow from striking 1 part of the cheese surface more than another. To clean and deodorize, the units were heated to 565°C in a glass annealing oven, rinsed with hot tap water and dried at 150°C in a nonodorous oven.

A high-purity, low-odor glycerine was used as the sealing medium for all the ground-glass surfaces. The empty containers were chilled while sealed, but were equipped with glass breathing tubes containing calcium chloride and activated charcoal to minimize contamination with moisture and odorants. The outside surfaces of tubs of cottage cheese were checked for cleanliness and lack of odors before the tubs were placed in the glass containers.

Gases

Prepurified nitrogen, carbon dioxide and air for the special atmospheres were obtained from typical cylinder gases, but were deodorized by passing each gas through 22 cm of activated charcoal, Barnaby-Cheney, type M-1, 10–20 mesh. Each gas was introduced into the appropriate glass container with cottage cheese by flushing 2 min at 1,500 cc/min. At the end of the gas-flushing step, headspace samples were obtained in evacuated 20-cc glass ampules by proper manipulation of the 3-way stopcocks. Slight additional gas flushing then restored the atmospheric pressure of the headspace. Headspace samples were also collected at the end of the storage period before the containers were opened to the ambient atmosphere.

Gas analysis

Gases in the ampules were analyzed for their content of carbon dioxide, nitrogen and oxygen by means of a Consolidated CEC 21-104 mass spectrometer.

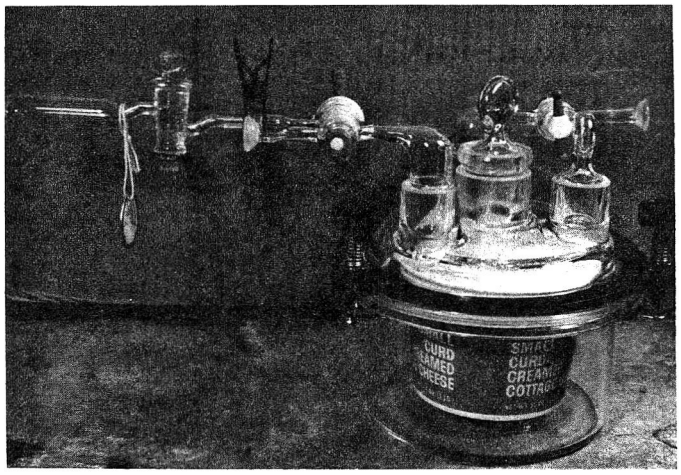

Fig. 1—Special glass container for studying the effect of selected gas atmospheres on the quality of stored cottage cheese. An ampule for gas sampling is shown attached at the left to 1 arm of the 3-way stopcock.

Storage conditions

The special glass containers with the open tubs of cottage cheese and with the special gas atmospheres were stored 10, 11 or 12 days at 3–4°C. The storage area was either dark or under greatly reduced light.

Bacteria counts

10 g of cottage cheese from the top centimeter of each sample were placed in a sterile pint Mason jar containing 90 ml of sterile buffered dilution water and fitted with a blender assembly head for a Hamilton Beach bar mixer. The cottage cheese sample in the buffer was macerated by operating the blender 60 sec at low speed. Aliquots of this mixture were appropriately diluted with sterile buffered dilution water to give the required agar plate counts utilizing Plate Count Agar (Difco Laboratories). The plates were incubated at 30°C for the mesophilic or total plate count and 7°C for the psychrotrophic counts.

Taste panel evaluation

At the end of the storage period, and after sampling for the headspace analysis and the bacteria count, the top portion (approximately 1 cm) of the cheese was transferred to deodorized jars and carefully mixed. Judges ranked and scored the coded samples using the flavor portion of the American Dairy Science Association (ADSA) cottage cheese scorecard. The best score on this card for flavor is 40. Ranking was emphasized over scoring and was accomplished by requesting the judges to give a different score to each of the 3 samples.

RESULTS & DISCUSSION

Special headspace atmospheres

Table 1 presents the results of analyses of the headspace atmospheres. When air was added initially, oxygen decreased slightly while carbon dioxide increased slightly, as would be expected where microbial growth occurs. When nitrogen was introduced into the headspace, oxygen increased (significantly in 2 of the 4 experiments) while the nitrogen content decreased. Introduction of carbon dioxide into the headspace resulted in a decrease of that gas in 2 of the 3 experiments. This decrease was attributed to an uptake of carbon dioxide by the cottage cheese, as will be noted in the discussion on flavor changes.

The configuration of the glass container lid caused a greatly increased headspace compared with that of a commercial cottage cheese package. The resulting large volume of selected gas, however, should have accentuated any specific effect on bacterial growth or flavor.

Table 1—Compositions of headspace gases in special glass containers with cottage cheese.

Cottage cheese source	Gas added initially	Number of samples	Mean mole percent composition of headspace gases[a]					
			N_2	O_2	CO_2	N_2	O_2	CO_2
			After 3 days' storage			After 10 days' storage		
Firm A (June)	Air	4	78.3	20.7	0.1	78.6	20.1	0.4
	N_2	2	93.6	3.5	2.7	93.2	4.3	2.3
	CO_2	2	12.1	3.7	83.9	15.7	4.6	79.4
			After 3 days' storage			After 11 days' storage		
Firm A (October)	Air	4	77.5	21.6	0.08	76.8	21.4	0.86
	N_2	2	98.4	1.6	0.05	92.0	7.0	0.69
	CO_2	2	37.9	9.9	51.7	33.5	8.7	57.3
			Initial (0 days)			After 11 days' storage		
Firm B (October)	Air	3	77.4	21.6	0.05	77.5	19.8	1.9
	N_2	3	97.2	2.7	0.01	91.3	6.8	1.6
	CO_2	2	16.5	3.3	80.0	33.0	8.6	58.1
			Initial (0 days)			After 12 days' storage		
Firm B (November)	Air	3	76.6	22.2	0.30	77.3	21.1	0.67
	N_2	3	94.3	5.3	0.18	86.1	6.4	0.64

[a]Difference from 100 mole percent is due mainly to argon.

Table 2—Effect of special gas atmospheres on numbers of bacteria recovered from cottage cheese stored 11–12 days at 3°C.

Cottage cheese source	Gas added initially	Number of samples	Mean number of bacteria per gram of cheese determined from agar plates incubated at		
			30°C[a]		7°C[b]
			Initial	After storage	
Firm A (October)	Air	4	5×10^2	4×10^7	4×10^7
	N_2	2		4×10^6	4×10^6
	CO_2	2		2×10^4	4×10^3
Firm B (October)	Air	3	5×10^4	2×10^8	3×10^8
	N_2	3		7×10^7	8×10^7
	CO_2	2		6×10^7	5×10^7
Firm B (November)	Air	3	1×10^3	2×10^5	5×10^4
	N_2	3		1×10^5	4×10^4

[a]30°C for mesophilic bacteria.
[b]7°C for psychrotrophic bacteria.

Numbers of bacteria

Table 2 presents results of counts of viable bacteria at the end of the storage period under each selected gas. Where carbon dioxide was the headspace gas, a trend toward lowered bacteria count was apparent. The inhibitive effect of the carbon dioxide was greater for the cottage cheese with the lower initial count. These results are consistent with observations of Elliott and Michener (1965), who point out that psychrotrophic microorganisms causing spoilage of fleshy foods at low temperatures are particularly sensitive to carbon dioxide. In addition, they state that inhibition by the gas is most noticeable when the growth is slow, as at low-storage temperatures or with low levels of contamination.

The bacterial inhibition by carbon dioxide noted in these experiments was as pronounced for mesophilic as for psychrotrophic organisms. A lowered pH cannot be wholly credited for the inhibitory effect, since only a slight, if actual, lowering of pH occurred in the top centimeter of the cottage cheese over the 11-day storage period. On the other hand, a definite change occurred which was discernible by taste, as described below.

Nitrogen as the headspace gas also caused a trend toward decreased bacteria counts. In both carbon dioxide and nitrogen, the effect of decreased oxygen tension may lead to decreased aerobic bacterial growth.

The 3–4°C cottage cheese storage temperature was chosen to approximate good commercial marketing conditions, while the 10–12-day time permitted microbial outgrowths short of offensive surface appearance or gross odor and flavor changes. Storage in dark or dim light minimized flavor changes by light-induced autoxidation (Dunkley, 1968).

Flavor

Table 3 presents results of the taste panel evaluation of the experimental cottage cheese samples. The ADSA scores are included to show the cheese samples were all very acceptable but had relative levels of acceptance.

Storage of cottage cheese with carbon

dioxide as the headspace gas led to inferior flavor quality, in the opinion of the panel. Comments on these samples included "tart," "sour," "has a bite." This lower rank and low ADSA score of cottage cheese under carbon dioxide, however, may have been a response due to a regional idiosyncrasy. In fact, Reddy and Lindsay (1969) have recently described the addition to sour cream of compounds that can lead to formation of carbon dioxide with reported enhancement of the cultured flavor.

Cottage cheese from Firm A with nitrogen in the headspace ranked higher than that with air. In the cheese from Firm B, however, no significant difference resulted when nitrogen versus air was compared in the headspace. Thus, the advantage or disadvantage of utilizing nitrogen as the headspace gas may be greatly dependent upon the specific cheese product.

Results of these studies, carried out at 3–4°C for 10–12 days, indicate that packaging cottage cheese with either carbon dioxide or nitrogen will not greatly increase the refrigerated shelf life as measured by flavor scores or bacteria counts. However, if carbon dioxide were used, a changed flavor would be encountered with advantage or disadvantage according to regional preference.

Table 3—Flavor scores of cottage cheese stored 11–12 days at 3°C and under special gas atmospheres.

Cottage cheese source	Number of judges	Total number of judgments	Rank scores[a] of cottage cheese stored under			Mean ADSA[b] flavor scores of cottage cheese stored under		
			Air	N_2	CO_2	Air	N_2	CO_2
Firm A (June)	3	9	19*	26*	9*	37.4	38	35
Firm A (October)	5	6	12*	16*	8*	37.3	38.2	36
Firm B (October	9	9	22	22	10*	37	37.4	34.4
Firm B (November)	8	8	13	11	—	37.6	37.25	—

[a]Highest rank score is best in the opinion of the taste panel.

[b]American Dairy Science Association Scorecard for cottage cheese.

*Significantly different at the P = 0.05 level (Kramer and Twigg, 1966).

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CATHERINE G. CAVALETTO and HARRY Y. YAMAMOTO
Department of Food Science and Technology, University of Hawaii, Honolulu, Hawaii 96822

FACTORS AFFECTING MACADAMIA NUT STABILITY.
3. Effects of Roasting Oil Quality and Antioxidants

SUMMARY—Chemical and physical changes in roasting oil from a commercial processing plant and the relationship of these changes to quality and shelf-life of roasted macadamia kernels were investigated. Oil was sampled initially and after 2, 4 and 13 weeks of roasting. Generally, free fatty acid, color (absorbance at 550 nm), refractive index and viscosity of the roasting oil increased with use. Changes in iodine number and fatty acid composition indicated there was considerable oil exchange between roasting oil and macadamia kernels. Antioxidant loss in the roasting oil was rapid. No flavor differences were observed in kernels immediately after roasting in the various oil samples, and shelf-life was not appreciably affected by continuous use of the oil for as long as 13 weeks. A second study investigated the effects of vacuum-packing (0, 15 and 24 in.) and direct antioxidant application (approximately 76 ppm butylated hydroxyanisole and butylated hydroxytoluene) on the stability of dry-roasted macadamia kernels. Stability of antioxidant-treated kernels was greater than that of untreated kernels, regardless of vacuum level. Vacuum packing had no effect on antioxidant-treated kernels, but showed some benefit for untreated kernels.

INTRODUCTION

PRODUCTION of macadamia nuts in Hawaii has increased from 2.6 million pounds in 1960 to 10.5 million pounds in 1969. Production is expected to increase again sharply by 1972. The roasted kernels, considered by many to be among the finest of confectionery nuts, were at first mainly a gourmet item with limited distribution but now can be found in supermarkets throughout the United States. This wider distribution and the anticipated industry growth have increased the need for studies on factors affecting macadamia nut stability, so that staleness, rancidity and other off-flavors which sometimes occur can be prevented.

Effects of moisture, heat and light on the storage stability of raw and roasted macadamia kernels, *Macadamia integrifolia*, were reported previously (Cavaletto et al., 1966; Dela Cruz et al., 1966). It was shown that moisture content was the most important factor of those investigated and that kernels dried to about 1% moisture had the greatest stability.

Macadamia kernels are generally roasted in coconut oil with antioxidants added to extend the usable life of the oil and, hopefully, for some rancidity protection for the kernels. The degree of kernel protection offered by antioxidants and the effectiveness of various application methods have not previously been examined. Likewise, little has previously been known of the chemistry of the roasting oil and what its usable life might be.

We report herein 2 studies concerning macadamia nut stability. The first investigated the chemical and physical changes in roasting oil from a commercial plant and the relationship of these changes to quality and shelf-life of roasted kernels. The subsequent work determined the effect of vacuum packing and direct application of antioxidants to dry-roasted macadamia kernels.

MATERIALS & METHODS

Roasting oil study

Samples of fresh and used (2, 4 and 13 weeks) roasting oil were obtained from a commercial macadamia nut processor. This processor used a batch roaster charged with fresh coconut oil (76°F mp) containing 200 ppm each of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Fresh coconut oil with BHA and BHT was added as replacement oil periodically during the 13 weeks of continuous use.

Peroxide value was determined by the AOCS Official Method Cd 8-53 (1960) upon receipt of each oil sample. The remaining analyses were done after the last sample was collected. The initial, 2- and 4-week samples were held at −18°C until that time. Iodine number was determined by the Hoffman and Green (1939) rapid Wijs method. Free fatty acid was determined by titrating 10 g oil dissolved in 50 ml hot 95% neutral ethanol containing 0.1% phenolphthalein with 0.1 N NaOH. Refractive index at 40°C was determined with a Bausch and Lomb Abbé 3L refractometer. Fatty acid composition was determined by gas-liquid chromatography as described previously (Cavaletto et al., 1966) using a 5-ft by ¼-in. column with 15% DEGS on 60/80 acid-washed DCMS Chromosorb W. Operating conditions were as follows: column temperature, 200°C, detector and injector temperatures, 250°C and helium flow rate, 60 cc/min. BHA and BHT were determined by a modification of the Schwecke and Nelson method (1964). An 0.5-g oil sample was mixed with 5 ml ethyl-ether solution containing a known amount of DiBHA (100–250 μg), the ether completely evaporated over N_2 and approximately 5 μl of the residual oil chromatographed on an Aerograph 200 hydrogen-flame instrument. Absorbance at 550 nm was determined in a 1-cm cuvette on oil samples after centrifuging at 10,000 × g for 5 min.

Upon receipt of each oil sample, macadamia kernels were roasted in the oil at 260°F for 15 min and then centrifuged to remove excess roasting oil. Coconut oil adhesive (110°F mp) was added (1.1%), the kernels salted (1.5%) and vacuum sealed (approximately 24 in.) in No. 211 cylinder cans. Following roasting, the cans were held at 0°F. After the last sampling period, all of the roasted samples were thawed and one-half of the kernels used immediately

Table 1—Chemical and physical analyses on roasting oils from a commercial plant.

Analysis	Oil sample (weeks used) 0	2	4	13
Free fatty acid (meq NaOH/100 g)	0.30	1.83	4.15	2.92
Absorbance at 550 nm	0.03	0.46	1.01	0.75
Peroxide value	3.2	1.5	1.0	1.1
Refractive Index	1.4496	1.4524	1.4562	1.4580
Viscosity (cps)	31	34	40	41
Iodine No. (gI_2/100 g)	9.74	23.5	44.9	57.4

Table 2—Fatty acid composition of fresh and used roasting oil from a commercial plant and macadamia oil.

	Oil sample (weeks used) 0	2	4	13	Macadamia oil
Fatty acids (%)					
Lauric	57.0	47.3	28.6	17.2	Trace
Myristic	20.0	15.6	9.6	6.5	0.7
Palmitic	9.8	10.3	9.8	10.0	7.4
Palmitoleic	0.3	4.7	11.4	15.4	18.5
Stearic	2.8	2.7	2.8	2.5	2.8
Oleic	8.2	17.7	32.7	43.0	65.0
Linoleic	1.9	1.7	2.3	2.0	1.5
Arachidic	–	Trace	1.3	1.3	1.8
Eicosenic	–	Trace	1.3	2.1	2.3

Table 3–Estimates of the percentage macadamia oil present in used roasting oil, based on the lauric or palmitoleic acid content.

Oil sample (weeks used)	Percent macadamia oil		
	Lauric	Palmitoleic	Average
2	17	25	21
4	50	62	56
13	70	84	77

Table 4–Antioxidants in fresh and used roasting oil from a commercial plant. The control was a freshly prepared laboratory sample containing 200 ppm each of BHA and BHT.

	Oil sample (weeks used)				
	0	2	4	13	Control
Antioxidant (ppm)					
BHA	179	25	Trace	17	200
BHT	15	–	–	20	200

for flavor evaluation. The remaining nuts were stored at 100°F and evaluated after 4 and 8 months.

A 9-member panel scored flavor quality according to a 10-point scale described previously (Cavaletto et al., 1968) where 10 = highest quality. Analysis of variance was applied to the scores to determine significant differences.

Antioxidant-vacuum study

Macadamia kernels were roasted in a laboratory dry roaster at 325°F for 15 min. The dry roaster is comprised of a 12-in.-diameter, perforated stainless steel drum rotating (4 rpm) in a forced-draft oven.

Roasted kernels were divided into 2 lots, 1 coated with 1.6% coconut oil adhesive (110°F mp), the other with 1.6% coconut oil adhesive containing 0.9% Tenox 4 (20% BHA and 20% BHT) (Eastman Chemical Products, Inc., Kingsport, Tenn.). The latter resulted in approximately 76 ppm total antioxidants based on an average oil content of 76% in kernels. Both lots of kernels were then salted and sealed in No. 211 cylinder cans under one of the following conditions: 0, 15 or 24 in. vacuum. The samples were then stored at approximately 75°F until evaluated.

Immediately following roasting, the 2 treatments (with and without antioxidant) were evaluated by a panel of 8 experienced judges to determine initial flavor quality. After 1, 3, 6, 9 and 12 months all treatments were evaluated by the same panel. The judges evaluated the 6 treatments on each of 5 days at each storage interval for 30–40 evaluations per treatment at each interval. The flavor scoring scale just referred to was used and significant differences determined by analysis of variance.

RESULTS & DISCUSSION

Roasting oil study

Macadamia nuts are roasted in coconut oil at about 275°F to a light golden-brown color. Since only a small amount of roasting oil is consumed or lost in the process, it is replenished only periodically with small quantities of fresh oil and may be used for an entire season (6 to 7 months). Usually, antioxidants are added to the oil for protection of the oil itself and for carry-over to the kernels. Other than these generalized procedures, neither the roasting process nor the equipment used by the industry is standard at this time. The roasting oil used in this study was obtained from one of the larger macadamia nut processors which uses a batch roaster wherein the oil is heated by recirculation through a heat exchanger. Thus, the results reported in this paper apply only to this specific case, although they probably indicate the over-all trend.

Results of chemical and physical analyses of roasting oils after various periods of use are shown in Table 1. Generally, free fatty acids, color (absorbance at 550 nm), refractive index and viscosity of the roasting oil increased with use. This general pattern was reversed or at least decreased in the 13-week oil, reflecting addition of fresh replacement oil some time between the 4th and 13th week. These slow changes are probably due to the low moisture content of the kernels, relatively low roasting temperature and the high stability of coconut oil.

The iodine number showed a large change with use from a value of 9.74, typical for coconut oil (Thieme, 1968), to a high value of 57.4, approaching that of macadamia nuts (Cavaletto et al., 1966). Apparently, considerable exchange of fats between the macadamia kernels and cooking oil occurs during roasting. Further evidence for this exchange is seen in the fatty acid composition of the roasting oil, which changed from a pattern typical for coconut oil to one more like macadamia oil (Table 2). The percentage of macadamia oil in the roasting oil can be estimated by the laurate or palmitoleate content, since these fatty acids are characteristic of coconut and macadamia oil, respectively. Results of such estimates shown in Table 3 indicate that the amount of oil exchanged was very high. In 2 weeks the oil changed from 100% coconut oil to 21% macadamia oil and reached 77% macadamia oil after 13 weeks. This high rate of exchange, which has not been reported for other deep-fat cooking processes, is probably due to the high oil content of macadamia nuts (Cavaletto et al., 1966) and could be another factor in the relatively slow deterioration of the roasting oil.

Results of the antioxidant analyses are shown in Table 4. The fresh oil used in this study had approximately 200 ppm each of BHA and BHT (Tenox 4) added and no additional antioxidant was added except for the antioxidant in the replacement oil. Results show that, even in the fresh oil sample, considerable antioxidant loss or consumption had occurred, indicated by the very low BHT and slightly lower BHA value. It appears likely that peroxides present in the fresh oil consumed the BHT preferentially. Antioxidant loss in the roasting oil was very rapid. After only 2 weeks' use, the total antioxidant concentration was 25 ppm and after 4 weeks, only trace quantities were present. The 13-week sample had a total of 37 ppm, reflecting the fresh antioxidant added in the replacement oil. These low residual antioxidant levels in the oil are probably inadequate for protection of the roasting oil itself and for carry-over to the roasted nut. Thus, improvement in antioxidant quality control for the roasting oil is indicated.

Table 5–Average flavor scores for macadamia kernels roasted in oil from a commercial plant and after storage for 4 and 8 months.

Storage time (months)	Oil sample (weeks used)				
	0	2	4	13	
0	7.9	8.0	8.3	8.4	N.S.
4	7.0	7.9	6.8	7.2	**
8	6.5	7.1	6.6	6.6	N.S.

N.S.–Not significantly different.

**Significantly different at .01 probability level.

Results of flavor evaluations following roasting and after 4 and 8 months storage at 100°F are shown in Table 5. Kernels roasted in the various oil samples and evaluated after roasting showed no significant flavor differences. After 4 months of storage at 100°F, kernels roasted in 2 week oil scored significantly higher than all other samples, but no significant differences were found among the samples roasted in fresh, 4- and 13-week oil. After 8 months at 100°F, no significant flavor differences were observed. None of the observed flavor differences was very great, and all samples were scored in an acceptable range. Thus, it appears that continuous use of coconut oil for as long as 13 weeks in macadamia roasting is feasible, although some oil deterioration does occur.

Antioxidant study

Since antioxidant loss in cooking oil is rapid, an effective quality control procedure must be established to assure some carry-over to the roasted kernels. Another and perhaps more reliable method on antioxidant application would be the incorporation of antioxidants in the adhesive oil which is applied for improved sal

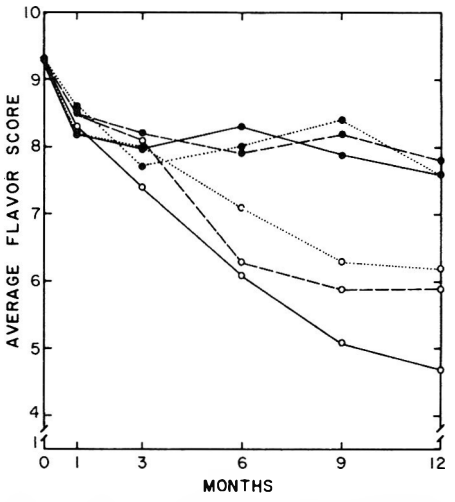

Fig. 1—Effect of antioxidants and vacuum levels on the flavor stability of roasted macadamia kernels. ○ = no antioxidants, ● = BHA + BHT, —— = air, - - - - = 15 in. vacuum, ····· = 24 in. vacuum.

adhesion after roasting or by the use of antioxidant-salt. Thus, the effectiveness of antioxidants applied in the adhesive oil for protecting macadamia kernels packaged in air and under 15 and 24 in. vacuum was tested. To avoid possible effects of roasting oil quality, dry-roasted macadamia kernels were used.

Results of the effects of antioxidant and vacuum levels on flavor stability are shown in Figure 1. The effect of added antioxidants on flavor quality is clearly seen after 6 months of storage, when all of the antioxidant-treated samples scored significantly higher than those without added antioxidants. After a slight decrease in flavor quality during the first month of storage, the antioxidant-treated samples changed very little during the remaining 11 months, regardless of vacuum level. Those samples without added antioxidant decreased in flavor quality continuously during the 12 months' storage period.

Vacuum level had no effect on flavor quality when antioxidants were added to the kernels. However, in treatments without antioxidants, it did have an effect, as flavor quality was directly related to vacuum level. Therefore, it is apparent that BHA and BHT are very effective in extending the shelf-life of roasted macadamia nuts when applied to sound kernels at the levels utilized in this study, and that these antioxidants provide better protection for macadamia kernels than vacuum packing.

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P. J. BECHTEL, M. P. PALNITKAR, D. R. HELDMAN and A. M. PEARSON
Department of Food Science, Michigan State University, East Lansing, Michigan 48823

BOUND WATER DETERMINATION USING VACUUM DIFFERENTIAL SCANNING CALORIMETRY

SUMMARY—Using a vacuum differential scanning calorimeter, it was possible to measure the relative bound water content of several food stuffs. Freeze-dried beef powder, freeze-dried whole egg powder, modified corn starch powder and non-fat dry milk were used. Results indicate that the relative bound water of food stuffs can be determined, but determination of total water from water binding energy will require further experimentation.

INTRODUCTION

MANY METHODS are available for moisture determinations. The most common techniques include thermogravimetric measurements, which have been adapted to all types of foods. Other methods for water determination include gas chromatography (Penther and Notter, 1964), nuclear magnetic resonance (Miller and Kaslow, 1963), conductivity (Christensen and Linko, 1963), capacitance (Zelery, 1960), and other chemical methods (Seaman et al., 1949). The above methods measure a physical parameter and relate it to moisture changes.

A newer method of moisture determination involves the use of a differential scanning calorimeter (DSC), or for some applications, a differential thermal analyzer (DTA). DSC can measure energy changes involved in a chemical transition, which in this case is water leaving the product. Once the energy is determined, it is a simple calculation to find the related water, provided all evaporating water has the same energy requirement. This type of differential temperature analysis has been widely used in the fields of cement chemistry, soil chemistry and the chemistry of other porous materials (Whitehead and Breger, 1950).

DSC can also be used with vacuum, where the driving force for water removal is a constant temperature and a reduction in pressure to a value lower than the moisture vapor pressure (Smyth and Adams, 1923; Stone, 1960). This technique has recently been used by Wadsö (1966) and Morawetz (1968) in the determination of heat of vaporization of selected organic compounds and water. These workers reported good results using small samples (0.3g or less) in a rapid determination (10–15 min), if the data were adjusted using an empirical constant. They reported greater errors with increasing vacuum and found the heat of vaporization within 0.1 Kcal per mole for the materials tested at moderate vacuum (10^{-1} mm of Hg). Under standard conditions both workers utilized isothermal programming and reduced vapor pressure to obtain the heat of vaporization.

The present study was undertaken to investigate the usefulness of DSC for determination of relative bound water states and associated energies in foods. This was accomplished by calculating the energy requirements necessary to remove various percentages of water from food samples.

MATERIALS & METHODS

Sample preparation

Four food samples were utilized: freeze-dried beef, freeze-dried whole egg, modified corn starch powder (Amaizo 721-A modified corn starch, American Maize), and non-fat dry milk (Land O'Lakes, low heat, spray non-fat dry milk, extra grade). Both the modified corn starch and the non-fat dry milk were used without further preparation. Powdered whole eggs were prepared from fresh whole eggs, which were freeze dried, powdered and held in a nitrogen atmosphere until used.

Frozen beef longissimus muscle (commercial grade) was thawed and trimmed of all excess fat and collagenous material. The muscle was cubed, refrozen in dry ice and freeze dried in a Stokes, model MT-110 freeze drier for 48 hr. The freeze-dried material was ground in a blender for 30 sec and stored under vacuum until utilized.

Moisture equilibration

Freeze-dried beef, freeze-dried whole egg, modified corn starch powder and non-fat dry milk were conditioned to five different moisture contents. Each moisture content represented an equilibrium condition at a given temperature. An Aminco air conditioning apparatus was used to condition the samples to 24, 41, 63 and 82% relative humidity at 27 ± 1°C. Thin layers of sample were placed on plates in the conditioning chamber for approximately 8 hr, and relative humidity conditions were monitored with an electric hygrometer during equilibration.

Samples equilibrated at >99% relative humidity were placed in a vacuum desiccator above a layer of water. A vacuum was then produced in the desiccator, and the products allowed to equilibrate for 48 hr at 22°C. All samples were used immediately or packed in vials and used within 4 hr.

Moisture determination

All samples were initially weighed in aluminum weighing dishes at room temperature and then placed in a drying oven at 100 ± 2°C for 48 hr. The dishes were then reweighed and the percent moisture was calculated. The weight of samples used for moisture determination ranged from 0.2–0.8g.

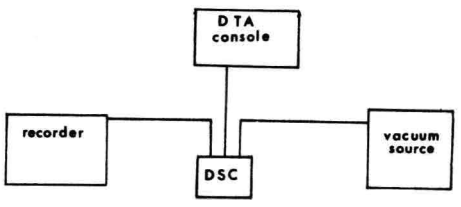

Fig. 1—Equipment assembly for vacuum DSC operations.

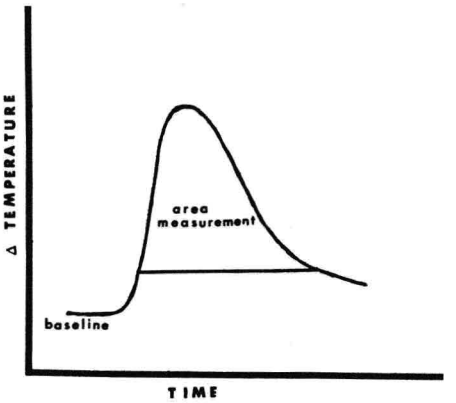

Fig. 2—Diagram showing the portion of vacuum DSC curve used for area calculations.

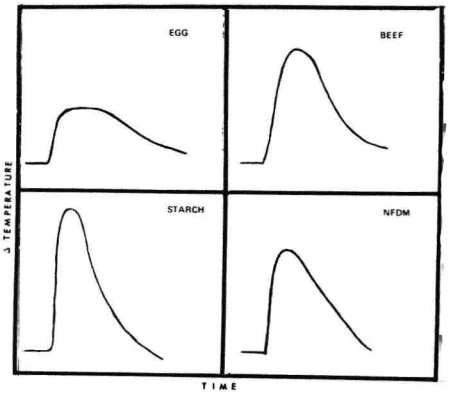

Fig. 3—Typical shape of vacuum DSC curve for each product at 82% R.H.

Isothermal differential scanning calorimetry

The apparatus for removal of water vapor and detection of the resultant heat loss is shown in Figure 1. It consists of a DTA console (DuPont – 900 DTA), a calorimeter cell (DuPont – No. 900350), a vacuum pump (Cenco Hyvac 7 – Central Scientific) and a recorder (Sargent – Model SR).

Operation of the DTA console was duplicated for each run. The program mode was isothermal, maintaining 32°C with an ice water reference. Deviations from the isothermal condition were recorded on the DTA console recorder with a sensitivity of 10°C per min. (x-axis). The calorimeter was cooled between experiments to maintain uniform isothermal conditions.

The external time base recorder indicated the difference in temperature between the reference cup and sample cup temperature. The chart speed was 1 in. per min, and the attenuation was varied with the recorder range selector.

The vacuum pump was set to pull a vacuum of 29 in. of Hg on the calorimeter cell within 15 sec. The calorimeter cell was equipped with a system allowing vacuum maintenance over long periods of time. To minimize vacuum errors, a valve was placed between the vacuum source and the cell. This allowed the pump to be turned on prior to creating vacuum in the cell, and also provided a means of breaking the vacuum.

The sample was introduced into the calorimeter using small aluminum cups (DuPont). Samples of 3–20 mg were packed into the cups as uniformly as possible. The large variation in sample weight was due to the differences in product density and conformation at various moisture levels. The emissivity difference due to weight variation were felt to be negligible due to the design of the sample cup.

Analysis

Figure 2 illustrates the typical area calculation. The calculation uses a constant percentage of the distance from the baseline to the curve peak for creation of a new baseline. Utilizing the (Fig. 2) new baseline, area is calculated on a time independent basis.

The equation for determining ΔH values is given in the DuPont 900 Differential Analyzer Instruction Manual:

$$\Delta H = (Z)\frac{(A\,\Delta\,T_S)}{(MC)}$$

- ΔH = enthalapy value (mcal per mg)
- Z = calibration coefficient (mcal per °C-min)
- A = peak area (sq in.)
- ΔT_S = Y-axis sensitivity (°C per in.)
- M = sample mass (mg)
- C = Chart speed (in. per min)

Using the above equation, the ΔH values for water removal can be determined if M is known. The heat of vaporization value of pure water is 578.6 g-cal per g, utilizing the above formula 19.8 sq in. of area per g of pure water was obtained. This value was used as a constant in calculating the amount of water removed from the product.

Table 1—Values for initial moisture content, percentage of moisture removed and percentage bound moisture determined by DSC using various products.

Initial moisture[a] %	Moisture removed[b] %	Bound Moisture[c] %	Relative humidity of sample %	Temp of equilibrium °C
	Nonfat Dry Milk (NFDM)			
24.6	11.5	13.1	99+	22
16.8	4.2	12.6	82	26
9.4	1.2	8.2	63	27
7.7	0.7	7.0	41	26
	Beef Powder			
36.8	26.9	9.9	99+	22
18.3	7.5	10.8	82	26
11.4	3.5	7.9	63	27
7.5	2.0	5.5	41	26
5.6	1.4	4.2	24	27
	Egg Powder			
33.7	15.2	18.5	99+	22
18.2	2.0	16.2	82	26
9.9	1.8	8.1	63	27
5.7	1.3	4.4	41	26
	Corn Starch			
25.2	4.7	20.5	99+	22
15.9	4.6	11.3	82	26
11.2	4.1	7.1	63	27
6.7	3.4	3.3	41	26

[a]Determined by dry oven.
[b]Moisture removed by DSC.
[c]Initial moisture – moisture removed by DSC = bound moisture.

RESULTS & DISCUSSION

THE BASIC SHAPE of the curves for each product is outlined in Figure 3. As shown, each product has an uniquely shaped curve. The general trend was for less curve symmetry at lower moisture values, and more symmetry at higher moisture contents. The degree of curve symmetry may be related to the time required to remove moisture. A more symmetrical shape indicates less product resistance to water removal. Corn starch powder had the most symmetrical curves at all moisture levels, while freeze-dried egg powder had the least symmetrical curves. A possible interaction not measured in this study was the effect of particle size on curve shape and bound water values.

Product transformation took place during moisture conditioning of the samples. The two products remaining relatively unchanged at all moisture levels were the freeze-dried beef and non-fat dry milk. These products generally remained as individual particles with minor clumping at higher moisture levels. Freeze-dried egg remained a powder at the two lower moisture levels (Table 1), but at 18.2% moisture clumping occurred and at saturation levels a moist globular structure was present. Globular structures should bind more water. This was verified by the large bound water value associated with the globular structure. Starch powder had the largest physical transformation of any tested product. At 11.2% moisture, clumping had occurred, and at 25.2% a tough gelatinous film was formed. These films reduced moisture loss, resulting in more bound water.

From the areas under the curves and the moisture data (Table 1) the following calculations were made and graphed (Fig. 4): % bound water in sample = % water in sample not accounted for by curve.

The term bound water as used herein does not have reference to monolayer films or other arbitrary definitions, but is defined as the water retained by the product upon using the vacuum DSC method.

Figure 4 shows that percent bound water decreases directly with the moisture level of the conditioned sample at a constant temperature. Of all products tested, only starch exhibited a constant slope. The unusual aspect of this graph is the lack of a constant bound water percentage which may indicate failure to account for the time relationship. Bound water, as defined in this paper, has been shown to systematically decrease with sample moisture content. This indicates that bound water determination in this study is relative rather than a constant value.

Difficulties in maintaining isothermal conditions and in maintaining reproducible vacuum were experienced. The energy values associated with water removal were calculated, but are not included due to the unsolved particle, vacuum and time relationships. Although the exact energetics were not discernible, the general trends are indicated by the percent bound

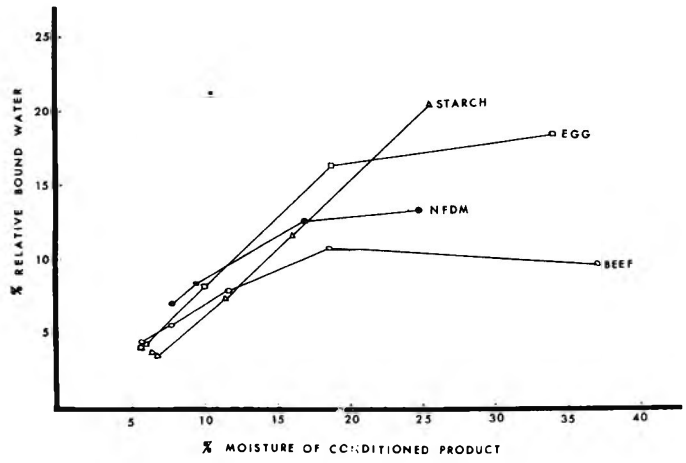

Fig. 4—Percentage moisture of conditioned products correlated with the percent relative bound water values determined by vacuum DSC. Percent relative bound water = percent of water in sample not accounted for by energy calculation from the curve.

useful in determining gross energy trends in water removal from food stuffs. Further work is in progress in an attempt to reduce experimental errors and calculations of water binding energies. If experimental errors can be reduced, the method would be applicable to calculations of bound water and the ensuing energetics.

water (Fig. 4). Products binding water best were egg and starch powders. Products rehydrating best were egg and beef powders.

Results from this work have yielded a methodology for determination of energy requirements for water removal from dry food products. The data at present are

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MARY E. ZABIK[1] and LE ROY DUGAN JR.
Department of Food Science, Michigan State University, East Lansing, Michigan

POTENTIAL OF FREEZE DRYING FOR REMOVAL OF CHLORINATED HYDROCARBON INSECTICIDES FROM EGGS

SUMMARY—The potential of freeze drying for residue removal from whole eggs and egg yolks was evaluated. Significant pesticide reductions of 79% for lindane, 37% for dieldrin, 57% for p,p'-DDT and 31% for o,p'-DDT-DDD occurred in freeze-dried whole eggs. Half the lindane was removed by freeze drying egg yolk, but only minimal removal occurred for the other pesticides. Approximately a 20% increase in p,p'-DDE occurred in both the freeze-dried whole eggs and yolks. The success of freeze drying for pesticide removal from eggs appeared related to both the vapor pressure of the pesticide and the amount of contamination in the whole egg.

INTRODUCTION

NO ONE can dispute the great contribution pesticides have made to the control of insect-borne diseases, to increased agricultural production and to the control of household pests, thus improving the quality of living the world over. Nevertheless, the almost universal occurrence of pesticide residues in soil, water, air and living organisms has led to the view that pesticides are environmental contaminants or, more spectacularly in some of the popular press, insidious, uncontrollable poisons (Mitchell, 1966). The chlorinated hydrocarbon insecticides have attracted particular attention because of their persistence and adverse effects on some birds and aquatic ecosystems.

Even though the mere presence of pesticide residues in living organisms does not necessarily jeopardize public health, prudent practice would be to minimize the pesticide residues in our food supply until more definitive studies elucidate the long-range effects of accumulating pesticide residues in human and animal tissues.

Mitchell (1966) cited the reduction of several chlorinated hydrocarbon pesticides from water by codistillation. Over 90% of aldrin and heptachlor codistilled, whereas only 30% of the lindane and p,p'-DDT was lost by this method. Dieldrin loss was 55% by codistillation. Gooding (1966) reported the removal of numerous organochlorine pesticides from crude oil during commercial processing. Smith et al. (1968) studied this removal further and found that the deodorization step was primarily responsible for the reduction. Kroger (1968) demonstrated that steam deodorization of butter oil at 180–195°C and 0.01–0.05 mm Hg for 5 hr completely removed naturally occurring contamination of heptachlor epoxide and dieldrin.

Bills and Sloan (1967) reported 95–99% of added lindane, heptachlor, heptachlor epoxide, aldrin, DDT, DDE and TDE was successfully removed from milk fat by a laboratory scale molecular distillation apparatus. Langlois et al. (1965) reported 50% losses of heptachlor epoxide and dieldrin by condensation, but Li and co-workers (1970) reported condensation and spray drying resulted in much smaller losses of organochlorine pesticides in milk. Using market milk, Ruzicka and co-workers (1967) stated that roller drying caused a 15% loss of dieldrin and p,p'-DDE, a 20% loss of β-BHC, p,p'-TDE and p,p'-DDT and a 25% loss in α-BHC, γ-BHC and heptachlor epoxide from whole milk. Spray drying was found to reduce added DDT and heptachlor by 62 and 95%, respectively, and fed lindane, heptachlor epoxide and dieldrin by 82, 70 and 56%, respectively (Langlois et al., 1964; 1965). Drum drying resulted in similar removal of all pesticides except DDT. Thus, the drying of eggs could also be useful in reducing the residue level. Whole egg and egg yolks from hens fed rations contaminated with lindane, dieldrin and p,p'-DDT were freeze dried. Residue values for the freeze-dried eggs were compared to those in the liquid eggs to assess the degree of removal.

EXPERIMENTAL

60 EGGS were collected over a 10-day period from 10, 10-month-old White Leghorn hens fed approximately 1 month on a standard laying ration contaminated with 25.0 ± 0.1 ppm each of lindane (99%, Applied Science Labs, Inc., State College, Pa.) [gamma isomer of 1,2,3,4,5,6-hexachlorobenzene dieldrin (Recrystallized 99+%, Shell Chemical Company, New York) (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonapthalene-HEOD) and p,p'-DDT (ESA pesticide reference standard, 99+%, City Chemical Corp., New York) (1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane]. These hens were maintained in individual cages (10 by 18 by 18 in.) in a room held at 25 ± 2°C.

After collecting, eggs were dated and held at 4–5°C until broken; any egg with a cracked shell was discarded. The first 2/3 of the eggs were broken, the albumen separated and the yolk collected after straining through cheesecloth to remove vitelline membrane and any contaminating albumen. After shelling, the last eggs collected were homogenized by blending for 3, 15-sec periods with a Waring Blendor operating at low speed.

3, 50-g samples each of whole egg and yolk were prepared for freeze drying by placing them in 400-ml beakers to a depth of approximately 3/4-in., covering securely with aluminum foil and freezing at −23°C overnight. The frozen samples were transferred to a Stokes Freeze-Dryer, Model 2003F-2, and freeze dried for 24 hr with a system pressure of 100 μ. During this period the platen temperature was ambient or below. The freeze-dried samples were removed from the beakers, ground with a mortar and pestle and the powder stored in tightly sealed glass vials at 4°C for solids and pesticide residue analyses. Portions of whole egg and yolk were reserved for analyses on the liquid material.

Percentage moisture was determined by drying duplicate samples to a constant weight

Table 1—Summary of the effect of freeze drying on pesticide residue contents of whole egg and egg yolk (ppm based on solids).

Egg	Pesticide	Liquid sample[a]	Freeze-dried sample[a]	z Statistic
Whole	Lindane	30.7 ± 0.4	6.4 ± 1.6	34.191***
	Dieldrin	104.3 ± 4.1	65.2 ± 8.8	8.405***
	p,p'-DDT	64.2 ± 1.3	27.3 ± 3.0	24.437***
	p,p'-DDE	12.5 ± 1.0	15.6 ± 1.6	3.274**
	o,p'-DDT-DDD	2.9 ± 0.0	2.0 ± 0.4	5.784***
	Total DDT	79.7 ± 2.2	44.9 ± 4.5	14.425***
Yolk	Lindane	27.3 ± 0.4	14.1 ± 3.8	8.380***
	Dieldrin	90.2 ± 2.8	89.5 ± 8.3	0.184
	p,p'-DDT	54.9 ± 3.6	49.7 ± 7.4	1.332
	p,p'-DDE	10.5 ± 0.1	12.7 ± 2.0	2.732*
	o,p'-DDT-DDD	2.5 ± 0.4	2.1 ± 1.1	0.703
	Total DDT	68.0 ± 3.8	64.5 ± 8.8	0.727

*Significant at the 5% level of probability.
**Significant at the 1% level of probability.
***Significant at the 0.1% level of probability.
[a]Mean and standard deviation.

[1]Present address: Department of Human Nutrition and Foods, Michigan State University, East Lansing.

at 60°C under vacuum (Steer et al., 1968). Sample sizes for duplicate pesticide residue analyses were 30 g for liquid whole egg, 25 g for whole yolk and 5 g for freeze-dried samples. Hexane, nanograde from Mallinckrodt Chemical Works, was used for all pesticide extractions and standard preparations. Methanol and acetone were glass distilled and gave no peaks which coincided with chlorinated hydrocarbon pesticides used in this study. Standard solutions of lindane, dieldrin and p,p'-DDT were prepared using the compounds as listed for feed contamination as well as standard solutions of o,p'-DDT (98+%, Analytical Standards, Pesticide Repository, Pesticide Research Laboratory, Perrine, Florida) and p,p'-DDE [1,1-dichloro-2,2-bis (p-chlorophenyl) ethene]. All glassware used for pesticide extraction was acetone-rinsed after thorough washing.

The liquid or freeze-dried samples were extracted with 200 ml of 1:1 hexane-methanol for 10 min in a Waring Blendor. The mixture was filtered through a Büchner funnel fitted with Whatman No. 1 filter paper and 15.0 ± 0.1 g acid-washed Hyflo Super Cel (Johns-Manville Products) to aid filtration. The Super Cel and precipitated protein were washed with 100 ml of 1:1 hexane-methanol followed by 4, 50-ml hexane washings to ensure complete removal of pesticides. Soxhlet extraction of the Super Cel and filter paper with hexane for 48 hr (20-min cycle) yielded no additional pesticides, verifying the adequacy of the washing procedure.

The hexane-methanol solutions were transferred to 500-ml separatory funnels and the methanol washed out with 4, 100-ml portions of 10% sodium chloride solution, after which the hexane was dried over anhydrous sodium sulfate for 30 min. No further cleanup or concentration was required for quantitative analyses. Preliminary studies with spiked egg samples established that this simplified method gave recoveries of 96+% for pesticides fed.

Gas-chromatographic analyses were carried out using a Beckman GC-4 chromatograph equipped with a discharge electron capture detector. It was fitted with a 6-ft (1.83 m) by 1/8-in. (3.5 mm) i.d. Pyrex column packed with 11% QF-1 plus 3% DC-200 on 60/80-mesh Gas Chrom Q and was operated at column, inlet and detector temperatures of 210, 260 and 280°C, respectively. Helium flow rates used were 40 ml/min for the column and 120 ml/min for the discharge side of the detector. Standards were injected at the beginning of each run, after every 10–15 samples and at the end of the run. Quantitations were based on the peak height of the standards and concentrations were expressed on a dry weight basis.

RESULTS & DISCUSSION

AVERAGE moisture contents of the freeze-dried whole eggs and egg yolks were 3.2 and 1.5%, respectively. To eliminate variation due to fluctuations in moisture, all pesticide data are reported as parts per million on a solids basis.

Pesticide residue analyses indicated that a portion of the p,p'-DDT fed the hens was dechlorinated to form DDE. The conversion of DDT to DDE by fowl has been reported in several other investigations (Cummings et al., 1966; 1967; Ritchey et al., 1967; 1969). A third peak was observed in the chromatogram of the DDT series, considered to be due to o,p'-DDT, since GLC analyses showed that the DDT reference standard used to contaminate the feed contained a minimal contamination of o,p'-DDT (approximately 0.03 ppm o,p'-DDT in a 1.0-ppm solution of p,p'-DDT). The proportion of o,p'-DDT to p,p'-DDT was slightly higher than this low contamination in the standard, so this peak may also represent minimal quantities of the DDT degradation product DDD [1,1-dichloro-2,2-bis (p-chlorophenyl) ethane], previously reported in chicken (Wesley et al., 1969; Ritchey et al., 1969). The column used to obtain the GLC analyses did not separate these 2 compounds. However, the very low values for this peak did not warrant further investigation.

The distribution of lindane, dieldrin and DDT compounds in the whole egg and egg yolk before and after freeze-drying is illustrated in Table 1. Reduction in pesticide contamination occurred in freeze-dried whole eggs for all pesticides present except p,p'-DDE. Some reduction occurred in the amount of lindane in the freeze-dried yolk but dieldrin and p,p'-DDT values remained essentially the same. The "z" statistic was used to establish significant differences between mean pesticide concentrations in the liquid and freeze-dried whole eggs and egg yolks (Dixon and Massey, 1957).

As shown in Table 1, the reductions of lindane, dieldrin, p,p'-DDT and o,p'-DDT-DDD and total DDT in the freeze-dried whole egg and of lindane in the freeze-dried egg yolk were very highly significant. DDE concentrations increased in both the freeze-dried whole egg and egg yolk. Dechlorination of p,p'-DDT to form p,p'-DDE during freeze drying could account for this increase.

Lindane possesses the highest vapor pressure of the pesticides incorporated in this investigation (9.4×10^{-6} mm Hg at 20°C). It was also the most easily removed, as seen from a 79% reduction in whole eggs and a 49% reduction in egg yolks. Dieldrin and p,p'-DDT possess vapor pressures of 1.8×10^{-7} and 3.0×10^{-7} mm Hg at 25°C, respectively. Freeze drying removes only 37% of the dieldrin from whole eggs, whereas 57% of the p,p'-DDT was removed. The ratio of concentrations of dieldrin, p,p'-DDT and lindane in the whole egg was 3:2:1, which also may have influenced the ease of removal.

Langlois et al. (1964) have reported a 60% loss in DDT and a loss of 80% lindane by spray drying milk contaminated with approximately 25 ppm of each pesticide on a fat basis. Ruzicka et al. (1967) reported the same rank order of loss of pesticides in the roller drying of milk, but showed lower losses of 15% for dieldrin, 20% for p,p'-DDT and 25% for γ-BHC. These workers also reported a 15% loss of p,p'-DDE.

The greater ease in the removal of pesticides from whole eggs could be due to the lower concentrations of pesticide residues present on a liquid basis or to greater ease of volatilization from a less dense or less viscous substance. Dilution of the egg yolk material with the egg white proteins may result in a different crystal structure or a greater surface area being formed during freeze drying which could facilitate vaporization of the pesticides.

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JOHN S. GECAN, PARIS M. BRICKEY, WILLIAM V. EISENBERG and ANDREE ROAF
Division of Microbiology, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, D.C. 20204

INSECT PROBLEMS OF PECAN SHELLING PLANTS AND THEIR RELATION TO INSECTS AND INSECT PARTS IN PROCESSED PECANS

SUMMARY—Factory studies were conducted to determine the incidence of various types of insect contaminants in inshell and shelled pecans and their significance as related to processing methods. Pecans may become infested in the field with primary and/or secondary insect pests and in the shelling plant by storage insects. Analytical results showed raw stock sorting by air separation to be ineffective for removing insect-infested inshell nuts. Insects and their fragments are present in cracked infested nuts and are largely removed by subsequent air separation procedures. During processing, Curculio caryae *larvae are concentrated in the midget and small pecan pieces. Hand sorting under UV light was shown to be superior to other procedures for removal of curculio larvae from the finished product. Finished shelled pecan products contained relatively few insect fragments.*

INTRODUCTION

THIS STUDY WAS undertaken to determine the incidence of various types of insect contaminants in inshell and shelled pecans and their significance as related to processing methods.

Before harvesting, pecans are subject to attack by many insects. Primary field pests attacking the nut include the hickory shuckworm, *Laspeyresia caryana* (Fitch); the pecan nut casebearer, *Acrobasis caryae* (Grote); and the pecan weevil, *Curculio caryae* (Horn) (Osburn et al., 1966).

Larvae of both the hickory shuckworm and the pecan nut casebearer may tunnel into and destroy newly set nuts. Once the pecan shells have hardened, the larvae of succeeding generations may feed on or tunnel into the shucks. The shucks of these infested nuts frequently adhere to the shell, and larvae may get into the shelling plant with nuts from which the shucks have not been removed.

Pecan weevil damage is of two types. Nuts punctured before the shell hardens will drop prematurely and be worthless. Attack after the shell hardens results in destruction of the nut kernel by the developing curculio larvae. Upon maturing, the larvae bore out of the nut and enter the soil to complete their development. Once on the ground, the pecan with a larval exit hole is subject to attack by a wide variety of secondary field insects. These are considered in a later discussion.

EXPERIMENTAL

Collection of samples

Fifteen series of samples consisting of 352 subsamples were collected from 15 shelling plants. Successive flow samples were collected li.e., raw inshell pecans and various shelled fraccions including extra large, large, medium, small, and midget were taken in sequence). Subsamples were taken at points where insect removal or redistribution might occur. This method of collection was employed to determine the distribution of insect contaminants in the various pecan fractions.

Examination of samples

Each subsample of inshell pecans, consisting of the entire amount collected (10–15 lb), was examined for the presence of insects, mold, blanks, and shrivels by cracking each pecan and examining it with the naked eye. The presence of insects or mold was confirmed, when necessary, under 10× magnification.

Each subsample of shelled pecans, consisting of the entire amount collected (3–5 lb), was examined twice for curculio larvae by macroscopic pickout. The method consisted of spreading the sample, a tablespoonful at a time, on a black surface and examining it for larvae with the naked eye. Results for each subsample were expressed as number of larvae per pound in tabulating the results.

Microscopic insect contamination was determined by the flotation procedure (Vazquez and Gecan, 1968): A 100g sample was defatted twice with petroleum ether for 10 min on a steambath. Heavy filth (sand and oil, etc.) was isolated with chloroform and carbon tetrachloride. The organic solvents were removed by vacuum and isopropanol rinses. After sieving on a #230 sieve (U.S. Standard Sieve Series), the sample was transferred to a 2 liter trap flask with a mixture of 60% ethanol-calcium chloride. The mixture was acidified with HCl, boiled for 10 min, and extracted twice with light mineral oil.

RESULTS & DISCUSSION

Sorting operations for curculio removal

Due to the absence of field sorting, inshell pecans infested with field insects are included with nuts delivered to the shelling plant. These infested pecans are usually lighter, and attempts are made to eliminate them by air separation known as "pop removal." This operation is only partly successful in removing insects, and additional sorting procedures are necessary to deal with insects occurring in the shelled pecans.

Curculio larvae in the shelled pecans

Table 1—Relationship of percentage of curculio-infested inshell pecans to number of curculio larvae in various semi-finished (unsorted) and finished shelled products (thirteen shelling plants).

Sample no.	Origin of pecans (State)	Variety of pecans	Curculio-infested inshell pecans (after pop removal)	No. of curculio larvae/lb (avg of 3 1-lb subsamples)					
				Midget pieces		Small pieces		Medium pieces	
				Unsorted	Finished product	Unsorted	Finished product	Unsorted	Finished product
1	Miss.	NWS[b]	0.0	—[a]	—[a]	0	0	0	0
2	Ga.	NWS[b]	0.0	0	0	0	0	0	0
3	La.	NWS[b]	0.2	3	0	0	0	5	3
4	Tex. & Miss.	NWS[b]	0.2	2	0	2	2	1	0
5	La.	NWS[b]	0.4	1	0	1	0	0	0
6	Tex.	NWS[b]	0.4	3	0	0	0	0	0
7	—[a]	—[a]	1.1	35	—[a]	38	2	0	0
8	—[a]	—[a]	1.3	—[a]	—[a]	1	1	2	1
9	Tex.	NWS[b]	1.4	37	7	24	1	1	0
10	Ga., Okla., & Ala.	—[a]	1.5	—[a]	—[a]	55	14	—[a]	—[a]
11	—[a]	—[a]	1.9	19	1	14	1	4	1
12	—[a]	—[a]	3.2	176	8	232	1	1	0
13	Ga., La., & Okla.	—[a]	5.0	—[a]	0	—[a]	0	—[a]	2

[a]Data not available.
[b]Native Wild Seedling.

Table 2—Relationship of percentage of insect-infested inshell pecans to number of insect fragments in various semi-finished (unsorted) and finished (sorted) shelled products (six shelling plants).

Sample no.	% Insect-infested: Before pop removal	% Insect-infested: After pop removal	Finished halves	No. of insect fragments (avg of three 100g subsamples): Meal, Unsorted	Meal, Sorted	Midget pieces, Unsorted	Midget pieces, Sorted	Small pieces, Unsorted	Small pieces, Sorted	Medium pieces, Unsorted	Medium pieces, Sorted	Large pieces, Unsorted	Large pieces, Sorted	Waste shell
1	0.0	0.3	1.7	2.0	3.0	0.7	5.3	0.7	—[a]	0.3	0.5	3.0	1.0	34.6
2	0.0	0.3	0.7	7.3	—[a]	22.3	—[a]	4.7	13.7	2.7	5.0	2.0	2.0	195.0
3	0.0	1.0	0.7	—[a]	—[a]	—[a]	—[a]	4.0	4.0	0.7	1.0	—[a]	—[a]	61.0
4	0.3	—[a]	0.0	—[a]	—[a]	5.0	1.7	1.0	1.7	1.7	1.0	—[a]	0.3	14.9
5	1.0	1.0	0.3	1.3	—[a]	1.3	1.3	2.0	6.3	0.3	0.0	0.0	—[a]	135.0
6	1.0	1.1	—[a]	—[a]	—[a]	—[a]	12.3	5.0	2.0	1.3	0.0	—[a]	5.3	55.0

[a]Data not available.

may be removed by sorting procedures, such as electronic sorting, ordinary light hand-sorting, and ultraviolet light hand-sorting. In this study the first two techniques were employed primarily for removal of shell fragments, dark colored meats, and other extraneous materials, and the latter specifically for the elimination of curculio larvae. The data below show the average percent (range) of curculio larvae removed from pecan pieces by various sorting procedures.

Electronic sorting (ES) only	66% (13–100)
ES followed by ordinary light hand-sorting (OLHS)	89% (39–100)
ES and OLHS followed by hand-sorting under ultraviolet light	91% (63–100)

Curculio contamination

Table 1 shows the percent of curculio infested inshell pecans after "pop removal" and its relation to the number of curculio larvae per pound of various sized pieces before and after sorting procedures. The data for percent inshell curculio infestation before "pop removal" were not determined because, as discussed later, "pop removal" did not reduce the number of infested inshell pecans entering the processing stream.

Table 1 also shows that unsorted midget and small pecan pieces contain significantly greater numbers of curculio larvae than the medium sized pieces. This distribution of larvae among the three sizes of pecan pieces is directly related to larval size. The size of the larvae varies with different stages of larval maturity present in the infested pecans, and the relative number of different sized larvae are influenced by levels of inshell infestation (i.e., pecans with a heavy inshell infestation contain more small larvae per nut than those with a light infestation).

Insect contaminants, excluding curculio

Table 2 shows the percent insect-infested inshell pecans, excluding curculio, before and after "pop removal," and its relationship to the number of insect fragments in various inline subsamples. Although "pop removal" eliminated a considerable number of blank and shriveled nuts, it was relatively ineffective in removing insect-damaged pecans as shown by results in Table 2. This occurs because the weight of most insect-infested nuts does not significantly differ from that of sound nuts.

The results for waste shell show that forced-air shell removal is also very effective in removing insect fragments. However, insect fragment counts are apparently undiminished by subsequent sorting procedures, since the results show that the unsorted nutmeats and the respective finished sorted product contained essentially the same number of insect fragments.

Distribution of curculio during processing

Table 3 shows the theoretical uneven distribution of curculio larvae in certain pecan fractions as the pecans pass through the processing stream, starting with an initial theoretical contamination of one larva/lb in the inshell pecans. The figures in the "weight" column are the approximate yields of various types of nutmeats obtained during processing of 1000 lb of inshell pecans after "pop

Table 3—Theoretical distribution of curculio larvae in 1000 lb lot of unshelled pecans among various product fractions.[a]

Fraction	Weight, lb	No. of larvae	No. of larvae/lb
Unshelled pecans (100%)	1000	1000	1.0
Shell (62%)	620	0	0
Edible nutmeats (38%)	380	1000	2.6
(Processing loss) (3%)	12	0	0
Bulk halves[b] (53%)	201	0	0
Bulk pieces[b] (44%)	167	1000	6.0
Sized pieces fraction[b]			
Extra large (4%)	15	0	0
Large (14%)	57	0	0
Medium (12%)	46		
Small (5%)	19 (73 lb)	1000	13.7[c]
Midget (2%)	8		
Meal (2%)	8	0	0
Processing loss and other sizes (5%)	19	0	0

[a]Calculated from assumed contamination of one larva per lb of unshelled pecans, data on average percentage yields of product fractions, and distribution pattern during processing assuming no removal of any larvae.

[b]Percent yield is based on yield of edible nutmeats.

[c]This figure represents a one lb mixture of midget, small, and medium pieces. No figure is available for the individual size fractions because this would vary with the size range of the curculio larvae infesting the nuts.

Table 4—Types of insects found in the shelling plant, inshell, and shelled pecans.[a]

Shelling plant insects	Inshell pecan insects	Insect fragments in shelled pecans	Whole or equivalent insects in shelled pecans
Sap Beetles (A)	--------	Sap Beetles (L)	Sap Beetles (A, L)
Sawtoothed or Merchant Grain Beetles (A)	Sawtoothed or Merchant Grain Beetles (A, L)	Sawtoothed or Merchant Grain Beetles (A)	Sawtoothed or Merchant Grain Beetles (A,L)
Confused or Rust Red Flour Beetles (A)	Confused or Rust Red Flour Beetles (A, L)	Confused or Rust Red Flour Beetles (L)	Confused or Rust Red Flour Beetles (L)
Moths (Unidentified) (A)	--------	Moths (Unidentified) (A)	--------
	Pecan Weevil (Curculio) (L)	Pecan Weevil (Curculio) (L)	Pecan Weevil (Curculio) (L)
	Ants (A)	Ants (A)	Ants (A)
	Dermestid Beetle (A, L)	--------	--------
	Acorn Month (L)	Acorn Moth (L)	Acorn Moth (L)
	Silken Fungus Beetles (L)	Silken Fungus Beetles (L)	--------
	Pecan Nut Casebearer (L)	--------	Pecan Nut Casebearer (L)
	Spiders	--------	--------
		Beetles (Unidentified) (A)	--------
		Flies (A)	Flies (A)
		Cigarette Beetle (L)	--------
		Hickory Shuckworm (L)	Hickory Shuckworm (L)
		Indian Meal Moth (L)	Indian Meal Moth (L)
		Psocids	Psocids
			Spring Tails
			Hairy Fungus Beetles (L)

[a]Key: (A) = Adult; (L) = Larva.

removal." The data do not consider elimination of larvae during forced air shell removal operations, since their effect is insignificant.

The figures for "No. larvae/lb" in the various pecan fractions are determined from processing yields and analytical findings on laboratory samples of the various fractions. The data show that very ow levels of curculio infestation of inshell pecans result in very large numbers of larvae in the midget, small, and medium pecan pieces, which require removal by various sorting procedures.

dentification of specific insect types elated to source of contamination

Table 4 lists the various insects found in shelling plants and inshell pecans and the types found as whole insects or insect fragments in the shelled pecans. The type of contaminants, present as either whole insects or insect fragments, were stored product insects, pecan-infesting field insects, and miscellaneous field or storage secondary invaders. Those insects found in the shelling plant and in inshell pecans were, in most cases, also isolated from the finished products. The shelling plant insects in most cases gained access to the finished product either by direct contamitation or through infestations in the processing equipment. Some, however, were present in inshell pecans as secondry insects.

Table 5 lists the scientific name of each insect and its origin. This knowledge is useful for disclosing sources of insect infestation and determining routes of finished product contamination.

Mites

The majority of the mites found in the semiprocessed or finished pecan meats were members of the genus *Tyrophagus*. Most of these specimens were identified as *T. putrescentiae*, which is a very common stored product mite. Another frequent mite contaminant belonged to the family Orbatidae, commonly known

Table 5—Origin and scientific name of insect contaminants.

Primary field insects	
Hickory shuckworm	*Laspeyresia caryana*
Pecan nut casebearer	*Acrobasis caryae*
Pecan weevil (Curculio)	*Curculio caryae*
Secondary field insects	
Acorn moth	*Valentinia glandulella*
Hairy fungus beetles	*Mycetophagidae*
Sap beetles	*Nitidulidae*
Silken fungus beetles	*Cryptophagidae*
Storage insects	
Cigarette beetle	*Lasioderma serricorne*
Confused or rust red flour beetles	*Tribolium sp.*
Indian meal moth	*Plodia interpunctella*
Sawtoothed or merchant grain beetle	*Oryzaephilus sp.*
Secondary field and/or storage insects	
Ants	*Formicidae*
Dermestid beetle	*Trogoderma inclusum*
Incidental contaminants (field and/or storage)	
Flies	*Diptera*
Psocids	*Psocoptera*
Springtails	*Collembola*

as box or beetle mites. These are field mites that inhabit the upper layers of soils and are incidental contaminants of stored foods.

CONCLUSIONS

INSHELL PECAN insect infestation may result from primary or secondary invaders occurring in the orchard or in storage. Depending upon efficiency of sorting procedures and sanitary conditions during processing, insect contaminants may be present in the finished product as whole insects or insect fragments. Specific knowledge of the types of insect contaminants and their origins will aid in identifying sanitation problems and indicate means for their solution.

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JFS BASIC SCIENCE

G. M. SAPERS, S. F. OSMAN, C. J. DOOLEY and O. PANASIUK
USDA Eastern Utilization R&D Div., ARS, Philadelphia, Pennsylvania 19118

FLAVOR QUALITY OF EXPLOSION PUFFED DEHYDRATED POTATO. 3. Contribution of Pyrazines and Other Compounds to the Toasted Off-flavor

SUMMARY—Potato volatile concentrates were prepared from samples of explosion puffed and conventionally dehydrated potatoes by steam distillation, extraction of distillates with diethyl ether and solvent evaporation and were analyzed by GLC. Peak heights of ten components were associated with the intensity of a toasted off-flavor produced by the puffing process. Four of these and two additional minor components were found to have pyrazine-like aromas; two components had aromas characteristic of the thermal degradation of dry proline-glucose mixtures and two components had burnt aromas. 2-Methylpyrazine, 2,5-dimethylpyrazine, furfural, 5-methylfurfural, benzaldehyde, and phenylacetaldehyde were identified by mass spectrometry and retention time. An ethylmethylpyrazine, an ethyldimethylpyrazine, and trimethylpyrazine were tentatively identified by mass spectrometry. These results suggest that the toasted off-flavor is due to the presence of alkylpyrazines, compounds derived from proline, products of sugar pyrolysis, and products of Strecker degradation reactions.

INTRODUCTION

DEHYDRATED potato dice capable of more rapid reconstitution than a conventionally dried product may be prepared by heating the semi-dry (27% moisture) precooked dice with superheated steam under pressure (1 min at 65 psi) and suddenly releasing the pressure, resulting in an explosive expansion of the potato piece structure (Turkot et al., 1966). Explosion puffed dehydrated potatoes are sometimes subject to a characteristic flavor defect which is produced by the puffing process. Burnt and "aldehyde-like" elements of the off-flavor have been associated with the presence of 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal which can be determined by headspace vapor analysis (Sapers et al., 1970). However, an important element of the off-flavor which has been described as "toasted" cannot be attributed to these compounds (Sapers, 1970).

Roasted, nutty, popcorny and bready flavor notes in foods processed at high temperatures while in a dry state have been associated with alkylpyrazines and other heterocyclic nitrogen containing compounds (Hodge et al., 1969). A number of alkylpyrazines have been found in cocoa beans (van Praag et al., 1968), coffee (Stoffelsma et al., 1968; Stoll et al., 1967), peanuts (Mason et al., 1966) and soy products (Wilkens and Lin, 1970; Manley and Fagerson, 1970). Deck (1968) reported the isolation of eight

Table 1—Potato volatile concentrate components associated with the toasted flavor in explosion puffed dehydrated potatoes.

Peak no.	Retention Time (min)[a]	Peak height (cm) Puffed (Mod. toasted flavor)	Puffed (Sl. toasted flavor)	Conventional (No toasted flavor)	Peak aroma	Identity	Method of identification[b]
18	20.9–21.6	5.10	3.10	0.40	Toasted	2-Methylpyrazine	MS, RT
19	22.1–22.2	0.70	0.65	0.25	Pyrazine	–	–
21	24.4–24.6	3.70	1.85	Trace	Pyrazine	2,5-Dimethylpyrazine	MS, RT
22	24.8–25.0	3.90	1.90	Trace	Proline degrad.	–	–
26	30.0–30.2	1.05	0.55	Trace	Pyrazine	2,3-and/or 2,5-Methyl, ethylpyrazine[c]	MS
27	31.7–31.9	Trace	Trace	Trace	Pyrazine	Trimethylpyrazine[c]	MS
29	33.8–34.4	5.10	4.25	0.70	–	Ethyldimethylpyrazine[c]	MS
					–	Furfural	MS, RT
32	39.4–40.0	0.80	0.65	0.40	Burnt	Benzaldehyde	MS, RT
33	43.2	Trace	Trace	Trace	Pyrazine	–	–
34	43.5–44.2	0.80	0.60	0.35	Burnt, Proline degrad.	5-Methylfurfural	MS, RT
35	45.2–45.7	1.60	1.10	0.40	Burnt	–	–
37	48.2–48.8	8.40	6.65	2.65	Floral	Phenylacetaldehyde	MS, RT

[a]Carbowax 20M column programmed 5 min at 50°, 50–120° at 6°/min, 15 min at 120°, 120°–150° at 3°/min, 15 min at 150°.
[b]MS = mass spectrometry, RT = retention time.
[c]Tentative.

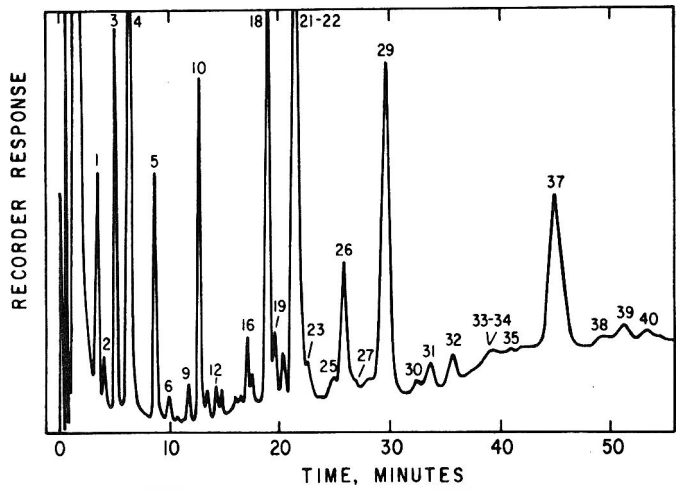

Fig. 1—Gas chromatogram of a potato volatile concentrate prepared from explosion puffed dehydrated potatoes.

alkyl-substituted pyrazines from potato chips.

Current studies have demonstrated that alkylpyrazines and other compounds associated with amino acid-reducing sugar reactions at high temperatures are present in explosion puffed dehydrated potatoes having a toasted flavor.

EXPERIMENTAL

Preparation of potato volatile concentrates

A 600g sample of dehydrated potato, ground to 20 mesh, was reconstituted with 2500 ml distilled water for 1 hr, mixed with 1000g Na_2SO_4 and 1000 ml 40% (w/v) Na_2SO_4 solution and then steam distilled at atmospheric pressure until approximately 500 ml of distillate had been collected in two equal fractions. Each distillate fraction was adjusted to pH 9 with NaOH solution, saturated with Na_2SO_4 and extracted with diethyl ether (5 × 25 ml). The ether extracts were dried with anhydrous Na_2SO_4, pooled and evaporated under nitrogen until the volume was reduced to 1 ml. Potato volatile concentrates were prepared from samples of explosion puffed potatoes having low and moderate toasted off-flavor levels as well as from conventionally dried potatoes in which the off-flavor was absent.

Analysis of potato volatile concentrate

Potato volatile concentrates were analyzed by GLC using a 9 ft 1/8-in stainless steel column containing 20% Carbowax 20M on 60/80 acid washed Chromosorb W at a helium flow rate of 20 ml/min. The column temperature was held at 50° for 5 min, increased to 120° at 6°/min, held at 120° for 15 min, increased to 150° at 3°/min and held at 150° for 15 min.

Tentative identifications were made by comparing retention times of unknown components with those of authentic compounds using isothermal conditions appropriate to the compound being examined; additional comparisons were made of retention time and peak symmetry after potato volatile concentrates were "spiked" with authentic compounds. Identifications were confirmed by mass spectrometry using the chromatographic system described above in tandem with a CEC 103 Mass Spectrometer with a porous stainless steel GC-MS interface. Peak 37 was trapped in a chilled capillary tube and identified by introducing it into a CEC Model 21-110B Mass Spectrometer using a direct inlet probe.

The aroma contribution of potato volatile concentrate components was established by carrying out gas chromatographic separations using the conditions and temperature program described above as well as appropriate isothermal conditions. Each analysis was performed in duplicate, once with the flame ionization detector operating to determine retention times, and once with the detector off to permit the investigators to record the aroma of the column effluent at the detector flame head as a function of time. Aromas of eluted peaks were evaluated independently (separate analyses of the same sample) by three co-workers involved in flavor research. Retention times generally were reproducible to within 0.1–0.7 min using the multilevel temperature program; however, data used to assign aromas to specific peaks showed no discrepency between the retention time and peak width of known "marker" compounds having characteristic aromas (n-hexanal and phenylacetaldehyde) and the time during which these aromas could be detected. It was then possible to associate aromas with specific chromatographic peaks.

Volatile products of proline degradation

Proline, a normal constituent of potatoes and an important precursor of a characteristic browned flavor (Hodge et al., 1969), was evaluated as a potential contributor to the toasted off-flavor in explosion puffed potatoes. Dry equimolar mixtures of proline plus glucose were heated under nitrogen at 90–165°C for 1 to 1-1/2 hr, during which time browning and gas evolution were observed. Volatile products of the reaction were condensed out of the nitrogen stream from the reaction flask using an acetone-CO_2 (s) cold trap. The proline-glucose condensate had the typical proline degradation aroma.

A similar aroma was generated when equimolar quantities of pyrrolidine and pyruvaldehyde were mixed at room temperature; odor formation occurred rapidly and was accompanied by extensive browning. This reaction was used by Kobayashi and Fujimaki (Hodge et al., 1969) to prepare N-acetonylpryrroline. The mixture was vacuum distilled and the distillate retained for GLC analysis.

These preparations and a potato volatile concentrate obtained from a sample of explosion puffed dehydrated potatoes were analyzed using the Carbowax 20M column at 145°C. The retention time of components having aromas resembling that of heated proline-glucose were determined by the same procedure used for the examination of potato volatile concentrates.

RESULTS & DISCUSSION

STEAM distillates (adjusted to pH 9) obtained from explosion puffed dehydrated potatoes were found to have a characteristic toasted aroma similar to that of the reconstituted product. Potato volatile concentrates also possessed this aroma along with some harsh and fruity notes. Similarly prepared distillates obtained from conventionally dried potatoes had an aroma suggestive of sulfur compounds rather than a toasted aroma.

A chromatogram obtained from the analysis of 5 μl of a potato volatile concentrate prepared from explosion puffed potatoes is shown on Figure 1. The chromatographic data summarized in Table 1 was obtained from the analysis at a lower attenuation of 1 μl of volatile concentrates prepared from potato samples varying in off-flavor intensity. Peaks 21–22, seen as one large peak in Figure 1 were partially resolved on chromatograms from which these data were derived. Likewise, peak 34 was more clearly defined at the lower attenuation used for these analyses. Chromatograms for puffed and conventionally dried potato samples were qualitatively similar although some major components of the puffed samples were present only as traces in the conventional samples. This is consistent with the flavor of the products, the conventional sample being very bland. Peak heights of at least 10 components could be related to the intensity of the off-flavor. Peaks 18 and 21, major components of the concentrate, were identified by mass spectrometry and retention time as being 2-methylpyrazine and 2,5-dimethylpyrazine, respectively. Peaks 26, 27, and 29, which were smaller and/or incompletely resolved, were tentatively identified by mass spectrometry as an ethylmethylpyrazine, trimethylpyrazine, and an ethyldimethylpyrazine. In addition, unknown compounds having pyrazine-like aromas were eluted from the column in the vicinity of peaks 19 and 33; these compounds could not be seen on chromatograms or detected by mass spectrometry, presumably because of their low concentration and probable admixture with other compounds which were present at higher concentrations. A number of alkylpyrazines were found by van Praag et al. (1968) and by Bondarovich et al. (1967) to have relative retention times on Carbowax 20M consistent with the appearance of pyrazine-like aromas in the present

investigation. Alkylpyrazines may be derived from reactions between sugars and amino acids, the former being the source of pyrazine carbon atoms and the latter determining the pyrazine structure and being the source of nitrogen (Koehler et al., 1969). All of the aromas associated with pyrazines resembled but were not identical to the toasted off-flavor in explosion puffed potatoes.

Two potato volatile concentrate components eluted from the column on the downsides of peaks 22 and 34 were found to have strong aromas similar to that of a dry proline-glucose mixture subjected to thermal degradation. Compounds having similar aromas and "aroma retention times" were found to be minor components of condensates obtained from thermally degraded proline-glucose and a vacuum distillate of the pyrrolidone-pyruvaldehyde reaction mixture. It seems likely that the unknown potato components having the proline degradation aroma are derived from this amino acid and may be related to the pyrrolines and pyrroles described by Hodge et al. (1969) and by Yoshikawa et al. (1965). This aroma also bears some similarity to the toasted off-flavor in puffed potatoes.

One of the most intense odors found in the column effluent was that of phenylacetaldehyde which appeared at a time coinciding with peak 37. Retention times of phenylacetaldehyde and peak 37 were identical. This compound, furfural and benzaldehyde recently have been identified in steam volatile oils obtained from fresh potatoes (Buttery et al., 1969). Furfural and 5-methylfurfural have been identified as products of glucose (Walter and Fagerson, 1968), and sucrose (Johnson et al., 1969) pyrolysis. Phenylacetaldehyde arises from the Strecker degradation of phenylalanine (Mason et al., 1969).

It can be concluded from this research and previously reported studies (Sapers, 1970) that the toasted off-flavor of explosion puffed dehydrated potatoes represents a complex of flavor notes associated with alkylpyrazines, products of proline degradation and to a lesser extent, Strecker degradation aldehydes and products of sugar pyrolysis.

Undoubtedly, additional potato components including some which contribute to the toasted off-flavor, might be detected and identified by the selection of other procedures for sample preparation and analysis. However, a more comprehensive study of off-flavor volatiles was considered to be unnecessary in view of the primary objective of the research program, namely, to eliminate the puffing off-flavor by inhibiting those reactions responsible for the formation of the offensive compounds. Research along these lines is in progress.

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T. PHILIP and F. J. FRANCIS
Department of Food Science and Technology, University of Massachusetts, Amherst, Massachusetts 01002

OXIDATION OF CAPSANTHIN

SUMMARY—Oxidation of capsanthin by molecular oxygen at 40°C in the solid state is discussed. The absence of an induction period in the oxygen absorption curve indicated that oxidation does not involve the typical autoxidation pattern. A number of keto-carotenoids such as capsanthone, 3-keto-kryptocapsone, and 3-keto-β-apo-8′-carotenal were isolated in the oxidation products. Oxidation of capsanthin involves primarily the oxidation of hydroxyl groups, followed by scission of the chain at the carbon-carbon bond α to the in-chain carbonyl group.

INTRODUCTION

CAPSANTHIN is the major carotenoid of capsicum spices and accounts for 35% of the total carotenoids in red peppers (Curl, 1962). The changes of color in paprika during processing and storage, with subsequent browning, is attributed to oxidative attack catalysed by light (Lease and Lease, 1956; 1962). Oil soluble antioxidants are known to retard the color loss of ground paprika on storage (Van Blaricom and Martin, 1951), indicating an autoxidation mechanism.

Bodea and Nicoara (1955) proposed that oxidation of β-carotene by oxygen is initiated at the 3 and 3′-position of the β-ionone group. This reaction mechanism, though similar to the formation of hydroxy-carotenoids in nature, is contrary to the generally accepted theory of autoxidation. Elahi (1967), on the other hand, oxidized β-carotene and obtained 4 and 4′-hydroxy carotenoids in agreement with the autoxidation mechanism proposed by Farmer (1942).

The present investigation was undertaken to study the mechanism of oxidation of capsanthin in the solid state in a model system.

MATERIALS & METHODS

Paprika

Paprika (Columbia GEM) was obtained from Cal-Compack Foods Inc., Santa Ana, California.

Thin layer chromatography

Thin layer plates (20 × 20 cm) were prepared by coating a slurry of silica gel G with water (1:2 w/v) with the aid of an applicator, dried at room temperature, and activated at 105°C for 30 min. For R_f value determination and preparative purposes an adsorbent thickness of 0.250 and 0.500 mm was used respectively.

Isolation and purification of capsanthin

Ground paprika (1 kg) was extracted successively with a mixture of acetone and petroleum ether, (1:1) until the extracts were colorless. The combined extracts (10 liters), after removal of solvent were dried in a desiccator. The dried pigments were saponified overnight with 20% KOH in methanol (50 ml). The saponified carotenoids were extracted with ethyl ether and the ether layer washed with distilled water, dried over anhydrous sodium sulfate, and the solvent evaporated (yield 16g of crude pigment).

The crude pigments were then partitioned between 95% methanol and petroleum ether (1 liter each solvent/g of pigment). The methanol layer containing capsanthin, after removal of solvent, was purified by thin layer chromatography on silica gel using a solvent system consisting of 3.5% ethanol, 10% benzene and 20% acetone in petroleum ether (max quantity of crude pigment that can be loaded onto a plate of 0.500 mm thickness = 50 mg). In this solvent system, hydrocarbons and monols move along with the solvent and the capsanthin band was scraped off, eluted with acetone and repurified in the same solvent system (yield 120 mg/gm of crude pigments).

Capsanthone and 3-ketokryptocapsone

A mixture of capsanthin (20 mg) and aluminum t-butoxide (0.8g) in acetone (20 ml) and benzene (20 ml) was refluxed for 36 hr, then cooled and shaken with N sulfuric acid. The organic layer was washed with distilled water, followed by saturated sodium bicarbonate solution and distilled water, dried over anhydrous sodium sulfate and the solvent removed. Capsanthone was purified by thin layer chromatography on silica gel G using a solvent system consisting of 3.0% ethanol, 10% benzene and 20% acetone in petroleum ether. This is essentially the method of Barber et al. (1961).

3-Ketokryptocapsone was obtained by refluxing a solution of capsanthone in acetone under a stream of oxygen and purifying by thin layer chromatography on silica gel G as in the case of capsanthone.

3-Keto-β-apo-8′-carotenal

A solution of capsanthin (20 mg) in benzene (15 ml) and 10% methanolic KOH (60 ml) was refluxed for 2 hr. The mixture was cooled, diluted with benzene (300 ml), washed consecutively with distilled water, 0.5N sulfuric acid, saturated solution of sodium bicarbonate and distilled water, then dried over anhydrous sodium sulfate and the solvent removed. 3-Keto-β-apo-8′-carotenal which presumably arose from the oxidation of β-citraurin, was purified by thin layer chromatography on silica gel G using a solvent system consisting of 2.0% ethanol, 10% benzene and 20% acetone in petroleum ether. This method was designed for β-citraurin (Cholnoky et al., 1963) but was used in this work to prepare 3-keto-β-apo-8′-carotenal. The identity of capsanthone, 3-keto-kryptocapsone, and 3-keto-β-apo-8′-carotenal was established by R_f values and visible and IR spectra.

Oxygen absorption by capsanthin

A Gilson Differential Respirometer was used for oxygen absorption studies. 8 micromoles of capsanthin were coated inside the flasks provided with the respirometer by first dissolving the appropriate amounts in methylene dichloride and removing the solvent under vacuum. The flasks were well greased, connected to the respirometer, and kept in position with the springs provided. The two arms of the flasks were filled with 1 ml each of 1% solution of 2,4-dinitrophenyl hydrazine in 75% sulfuric acid and 10N KOH respectively. It is assumed that these solutions have negligible vapor pressure. The flasks were lowered into the water-bath kept at 40°C and tested for leaks. After equilibration, the manometer readings were taken at appropriate intervals of time. A set of

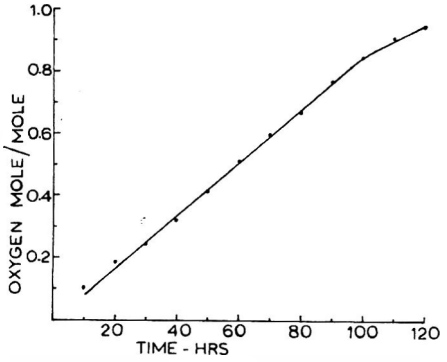

Fig. 1—Amount of oxygen absorbed by capsanthin at 40°C (mole/mole).

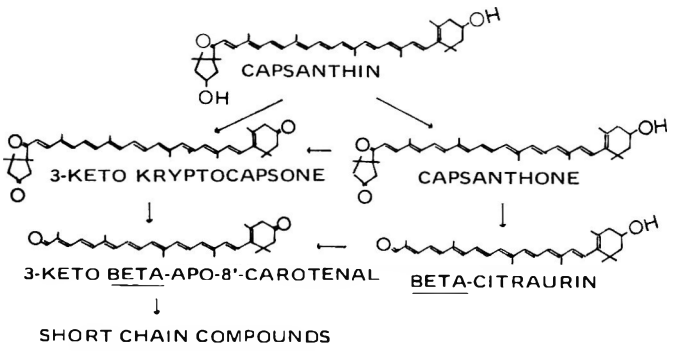

Fig. 2—A scheme for the oxidation of capsanthin by oxygen.

Table 1—R_f values, visible spectral maxima and infra-red absorption bands of major oxidation products of capsanthin by oxygen at 40°C.

Band no.	R_f value[a]	Visible light absorption maxima (nm) Hexane			Benzene			IR absorption bands[b] cm^{-1}			Identity[c]
1A	0.70	(445)	466	490	–	480	(510)	1740,	1710,	1660	3-keto kryptocapsone
1B	0.64	–	472	(495)	–	485	–	1740,	1660		capsanthone
2A	0.76	–	450	–	–	465	–	1740–1710,		1660	?
2B	0.71	–	465	495	–	477	–	1740,	1710,	1660	3-keto kryptocapsone
1	0.80	–	432	–	–	457	–	1710,	1660		3-keto-β-apo-8′-carotenal

[a]Silica gel G: 0.250 mm; solvent system: 3.5% ethanol, 10% benzene and 20% acetone in petroleum ether; temperature: 25°C.
[b]Infra-red bands in spectral grade chloroform using a 0.1 mm cell.
[c]Compounds were identified by comparison of their R_f value, visible spectra and infra-red spectra with authentic samples.

four flasks were maintained along with four blanks. The initial readings of the manometer showed irregularities and sample readings were corrected for variations in the blank readings. The blanks showed a maximum variation of 25 microliters on either side. The average values were used for the calculation.

Identification of oxidation products

Capsanthin (20 mg) was coated inside a glass coil and oxidized at 40°C in a stream of pre-warmed oxygen (90–110 ml/min) for different periods (0–2 hr). The oxidation products were dissolved in chloroform and separated by thin layer chromatography on silica gel G using a solvent system consisting of 3.5% ethanol, 10% benzene and 20% acetone in petroleum ether.

RESULTS

Oxygen absorption by capsanthin

The amounts of oxygen absorbed by capsanthin were corrected to standard temperature and pressure and plotted against time is shown in Figure 1. The rate of oxygen absorption by capsanthin reaches a peak corresponding to one mole of oxygen/mole of capsanthin, before the rate falls off (90 hr). The curve does not show an induction period typical of autoxidation indicating the absence of such a mechanism.

Identification of oxidation products

Three major bands and several minor bands of high R_f value were observed in the oxidation products of capsanthin. The major bands were found to consist of more than one component on rechromatography due to oxidation on the plate. The minor bands could not be isolated in sufficient quantities for their identification. However, their high R_f values and yellow color indicated that they have shorter chromophores than the major oxidation products.

Table 1 shows the R_f values, visible spectral maxima and infra-red absorption bands of major oxidation products. These compounds were identified by comparison of their R_f values, visible spectra and infra-red spectra with those of authentic samples.

The abundance of keto-carotenoids in the oxidation products and the rapid conversion of capsanthone into 3-keto kryptocapsone on thin layer plates indicated that the primary oxidation of capsanthin involved the oxidation of hydroxyl groups to keto groups. The lack of an induction period in the oxygen absorption curve (Fig. 1) supports this possibility. The presence of 3-keto-β-apo-8′-carotenal in the oxidation products and its rapid formation from 3-keto kryptocapsone on the thin layer plates indicates that chain scission occurs at the carbon-carbon bond α to the in-chain carbonyl group. The products of oxidation do not accumulate and the presence of several shorter chain compounds indicates that 3-keto kryptocapsone, once formed, is rapidly oxidized to short chain compounds. Small quantities of a compound which appeared to be β citraurin appeared on the plates but the quantity was too small for positive identification. This compound is normally produced by alkaline fission of capsanthin and would be expected to be an intermediate in this oxidation series. The oxidation of 3-keto-β-apo-8′-carotenal may proceed further to create possibly 3-keto-β-apo-10′-carotenal and 3-keto-β-apo-12′-carotenal but these would be hidden with the shorter chain compounds.

Based on these observations a scheme of oxidation of capsanthin is provided in Figure 2.

DISCUSSION

PAPRIKA is a complex food system and carotenoids are present along with other food components. The amount and type of carotenoids vary depending on the variety and source of paprika and De la Mar (1967) observed a loss of 96% of the carotenoids on exposure to sunlight. The greatest loss occurred in the hydrocarbon fraction. It is well established that carotenoids differ in their stability to oxidative deterioration. Lycopene is less stable than β-carotene and carotenoids of the spirilloxanthin series, lacking the 1,2-double bond, but containing an additional double bond in the 3,4-position are less stable than lycopene.

The oxidation of capsanthin by oxygen resulted in the conversion of hydroxyl groups to keto groups. This reaction is similar to the oxidation of astaxanthin to astacene by air. Formation of carbonyl groups is followed by chain scisson at the carbon-carbon bond α to the in-chain carbonyl group.

There is sufficient evidence that a free radical type of oxidation exists in the case of β-carotene (Elahi, 1967). However, this is not the only mechanism operating in the oxidation of carotenoids. Xanthophylls are more resistant to oxidative deterioration than hydrocarbons and this explains the reason for the preferential loss of hydrocarbons in carotenoid containing foods.

Paprika contains 10–12% triglycerides containing high amounts of unsaturated fatty acids and capsanthin is esterified as dilaurate in paprika (Philip and Francis, 1970). The presence of less stable carotenoids as well as the presence of highly autoxidisable fatty acids in paprika, may enhance the lability of capsanthin to oxidation. On the other hand, the existence of capsanthin as its dilaurate, may enhance its stability to oxidation.

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T. PHILIP, W. W. NAWAR and F. J. FRANCIS
Department of Food Sciences and Technology, University of Massachusetts, Amherst 01002

THE NATURE OF FATTY ACIDS AND CAPSANTHIN ESTERS IN PAPRIKA

SUMMARY—The triglycerides present in whole ground paprika and paprika pods were extracted and hydrolyzed. The fatty acids were methylated, separated by gas chromatography and identified by mass spectrometry. The whole paprika and pods, respectively, contained approximately 66 and 45% linoleic acid, 14 and 19% palmitic acid, 12 and 14% oleic acid and 5 and 17% linolenic acid. Small quantities of myristic and lauric acids and traces of capric, stearic and palmitoleic acids also were present. Capsanthin, which amounted to 35% of the total carotenoids, occurred as the dilaurate ester. It was isolated from paprika by thin-layer chromatography after interesterification of the triglycerides. Capsanthin dilaurate, synthesized in the laboratory, gave identical R_f value and infrared and visible spectra to those of the naturally occurring compounds.

INTRODUCTION

FRUIT xanthophylls are invariably esterified (Goodwin, 1965) and this probably modifies their susceptibility to oxidative changes. The nature of the pigments, the physical state in which they exist and their association with other components are important from the point of appearance as well as stability to oxidative deterioration (Ramakrishnan, 1967).

The color of paprika is due to carotenoids with capsanthin predominating to the extent of 35% of the total carotenoids (Curl, 1962). The change of color in paprika during processing and storage with subsequent browning is attributed to oxidative attack catalyzed by light (Lease and Lease, 1956). The present report is related to the nature of capsanthin esters and the fatty acids of paprika fat.

MATERIALS & METHODS

Paprika

Samples of ground paprika and paprika pods were obtained from Cal-Compack Foods, Inc., Santa Ana, California.

Extraction

Ground paprika (10 g) was extracted successively with a mixture of acetone and petroleum ether (1:1 v/v) until the extracts were colorless. The combined extracts (1 liter), after removal of solvents under vacuum, were dried in a desiccator overnight (yield 1 g).

Isolation of capsanthin

Capsanthin was isolated by the method of Philip and Francis (1970).

Fatty acid analysis

The method of Metcalfe et al. (1966) was used in this work. To 150 mg fat, 4 ml of 0.5 N methanolic sodium hydroxide was added and the mixture heated on a steam bath until the fatty material went into solution. After addition of 5 ml of BF_3-methanol mixture (14% w/v) the solution was boiled for 2 min. A saturated solution of sodium chloride was then added until 2 layers formed. The methyl ester layer was taken up in petroleum ether and concentrated. The methylated fatty acids were separated on a 6 ft by 1/8-in. diethylene glycol succinate column using an F & M gas chromatograph with a flame ionization detector. The esters were identified by comparing their retention data and mass spectrometric fragmentation with those of authentic compounds. Mass spectrometry was done with a Hitachi-Perkin Elmer Model RMU-6A medium resolution mass spectrometer.

Thin-layer chromatography

Silica gel G in the form of a slurry with distilled water (1:2 w/v) was uniformly applied to a thin-layer chromatographic plate (20 by 20 cm) to a thickness of 0.500 mm with the aid of an applicator, dried at room temperature for 30 min and activated at 105°C for 30 min. R_f values were determined on plates with an adsorbent thickness of 0.250 mm.

Spectrophotometry

Carotenoids in general and symmetrical carotenoids in particular do not give significant absorptions in the infrared region of the spectrum, due to the difficulty in obtaining a uniform coating of the pigment on KBr disc in sufficient quantities on a microscale. Too thick a coating often results in considerable reduction of the intensity of the light reaching the detector. This difficulty was overcome by spraying the KBr disc with an artists' airbrush, and as low as 5 mg was sufficient to obtain a uniform coating. The pigments isolated from paprika were dissolved in methylene chloride and coated uniformly on KBr disc (1 cm in diameter). The sprayed discs were used for infrared spectrophotometry with a Perkin-Elmer Model 337 IR Spectrophotometer. The visible spectra were obtained with a Perkin-Elmer UV-Vis-NIR Model 450 Spectrophotometer in spectral-grade hexane.

Separation of capsanthin esters from triglycerides

The high amounts of triglycerides in paprika (10%), as compared to total carotenoids (1%), make the separation of xanthophyll esters difficult by direct thin-layer chromatography, because the triglycerides dissolve and carry the pigments along with them under the solvent system studied. Also, short-chain triglycerides were found to have the same R_f values as those of the xanthophyll esters on silica gel G under the solvent system studied. However, under the conditions of methylation of fatty acids by the method of Luddy et al. (1968), it was observed that the xanthophyll esters do not interesterify. Therefore, methylation followed by thin-layer chromatography on silica gel G can be used to isolate the xanthophyll esters.

1 g of the dried extract from paprika containing triglycerides and the xanthophyll esters was mixed with 2 ml benzene and the mixture warmed to dissolve the fat. The solution was transferred to a screw-capped bottle (30 ml) and 10 ml of 0.4 N sodium methylate in anhydrous methanol added. The bottle was then immersed in a water bath maintained at 65°C with the screw cap securely applied, and shaken for 30 sec. The heating was continued for another 1.5 min without shaking. The flask was removed, cooled and the contents transferred to a separatory funnel containing 50 ml petroleum ether. The lower layer was drained after gently shaking and the petroleum ether layer repeatedly washed with distilled water. The washed petroleum ether layer was dried and evaporated. The possibility of free xanthophylls being methylated by this method was tested by methylating pure capsanthin under identical conditions and observing the changes in the adsorption affinity on thin-layer chromatographic separation, using silica gel G and a solvent system consisting of 10% acetone in petroleum ether.

The residue containing xanthophyll esters and methyl esters of fatty acids was fractionated by thin-layer chromatography using silica gel G and a solvent system containing 10% acetone in petroleum ether. In this solvent system, the methyl esters and hydrocarbons moved along with the solvent front and free xanthophylls having 2 or more hydroxyl groups do not move at all. The separated bands are essentially xanthophyll esters. 8 separated bands were observed and after noting their R_f value, they were scraped off from thin-layer plates and eluted with acetone. After removal of solvent, each band was hydrolyzed with 10% KOH in methanol at room temperature for 2 hr. The hydrolyzed pigments were extracted with ethyl ether, washed with distilled water and the solvent removed. Based on the R_f value and visible spectra of the hydrolyzed pigments, 3 bands corresponding to capsanthin esters were recognized. The major capsanthin ester was isolated in larger quantities for further investigation. The 2 minor bands could not be isolated in sufficient quantities without undue labor and materials.

Silylation

The major capsanthin ester band (1-5 mg) was dissolved in N,N-dimethylformamide and an excess of bis-(trimethyl silyl) trifluoroacetamide (0.5 ml) added. The reaction was carried out in a 5-ml screw-capped bottle. After flushing the bottle with nitrogen, the reaction mixture was warmed to 50°C for 5 min. The solvent was removed under a stream of nitrogen and the residue subjected to thin-layer chromatographic separation on silica gel G using a solvent system consisting of 10% acetone in petroleum ether. Absence of additional bands indicated that free hydroxyl groups were absent in the capsanthin ester.

Preparation of capsanthin dilaurate

Capsanthin (20 mg) isolated from paprika was dissolved in an excess of pyridine (20 ml) and the solution transferred to a separatory funnel. Lauroyl chloride (100 mg) was added

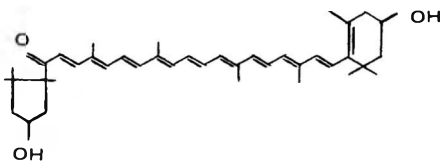
dropwise and the solution shaken vigorously f 5 min. Petroleum ether (200 ml) was then added and, after shaking, the mixture was washed with dilute HCl (1 + 2), distilled water and the solvent evaporated. The residue, after drying, was subjected to a preliminary purification on a column containing a mixture of Sea Sorb 43 and Celite (1:1), the ester fraction being eluted with petroleum ether containing 10% acetone. Capsanthin dilaurate was further purified by thin-layer chromatography on silica gel G, using 10% acetone in petroleum ether as the developing solvent.

RESULTS

Fatty acid distribution

Columbia GEM paprika and pods contain 10 and 5% ether extractives, respectively. The fatty acid distribution of the whole ground paprika and paprika pods is shown in Table 1. The paprika and pods contained, respectively, 66 and 45% linoleic acid, 14 and 19% palmitic acid, 5 and 17% linolenic acid and 12 and 14% oleic acid. Small quantities of myristic and lauric acids and traces of capric, stearic and palmitoleic acids also were present.

Structure of capsanthin



Identification of capsanthin esters

The major capsanthin ester band was hydrolyzed and the fatty acid methylated by the method of Metcalfe et al. (1966). The methyl ester was identified by gas chromatography and mass spectrometry as methyl laurate. The infrared spectra of capsanthin ester (Fig. 1) showed very strong absorption corresponding to the stretching vibration of the ester carbonyl (140 cm^{-1}). Carbonyl stretching vibration of the carbonyl group of capsanthin appears as a weak band at about 1670 cm^{-1}, typical of conjugated carbonyl compounds of high molecular weight (Bellamy, 1954). The infrared spectrum of the ester did not show significant absorption due to hydroxyl vibration as compared to capsanthin. Silylation of the ester, followed by thin-layer chromatography on silica gel G did not show any additional bands, indicating the absence of free hydroxyl groups. This evidence indicates that both hydroxyl groups were esterified in capsanthin. The R_f value, visible spectrum and infrared spectrum of the ester were identical with those of the synthesized capsanthin dilaurate, thus confirming its identity.

The 2 minor bands which gave capsanthin on hydrolysis were tentatively identified as monoesters, based on their R_f value and visible spectra. Presumably, the monoesters arose by the hydrolysis of the diester during the experiment.

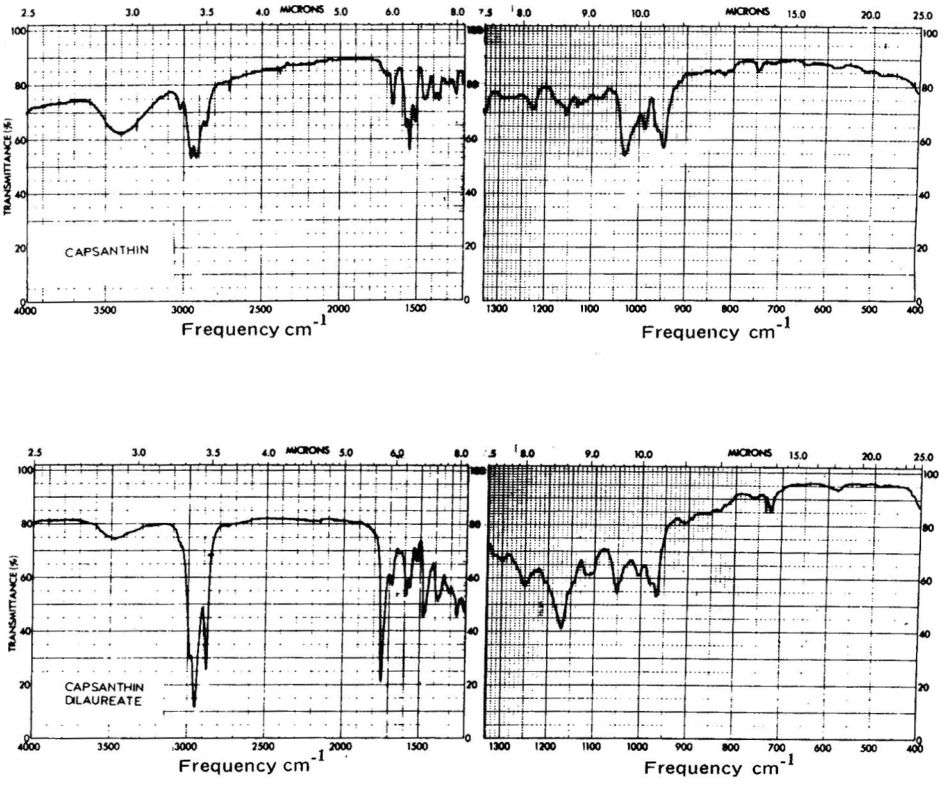


Fig. 1—Infrared absorption curves of capsanthin (above) and capsanthin dilaurate (below).

R_f values

Table 2 shows the R_f values of capsanthin, capsanthin dilaurate and capsanthin monoesters isolated from paprika. In the solvent system, consisting of 3.5% ethanol, 10% benzene, 20% acetone and 67.5% petroleum ether, capsanthin has an R_f value of 0.53, whereas the capsanthin dilaurate moves along with the solvent front. However, in a solvent system consisting of 10% acetone in petroleum ether, capsanthin does not move, whereas the dilaurate has an R_f value of 0.52.

Visible spectra

Figure 2 shows the visible spectra of capsanthin and capsanthin dilaurate isolated from paprika. Esterification does not significantly change the shape and maximum of the spectrum of capsanthin.

DISCUSSION

THE TRIGLYCERIDES of paprika contain high amounts of linoleic acid as well as significant amounts of linolenic acid, both susceptible to autoxidation. The

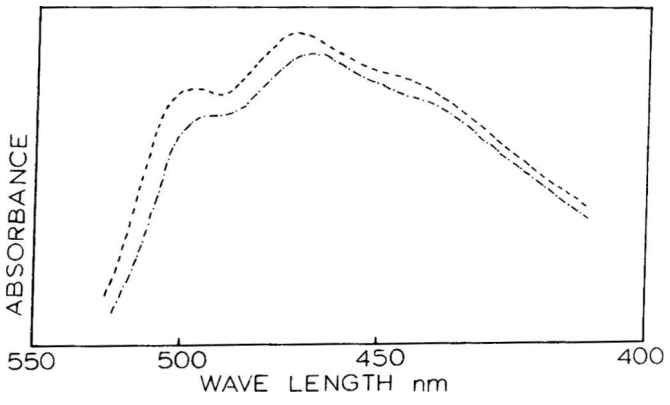


Fig. 2—Visible spectra of capsanthin (—. —. —) and capsanthin dilaurate (— — —) in hexane.

Table 1—Fatty acid distribution of the triglycerides of whole ground paprika and paprika pods (Columbia GEM).

Fatty acid	Whole ground paprika (%)	Paprika pods (%)
Capric	Traces	Traces
Lauric	1	1
Myristic	2	4
Palmitic	14	19
Palmitoleic	Traces	Traces
Stearic	Traces	Traces
Oleic	12	14
Linoleic	66	45
Linolenic	5	17

Table 2—R_f values of capsanthin, capsanthin dilaurate and capsanthin monoesters on silica gel G (0.250-mm thickness).

Carotenoid	R_f value	Solvent system
Capsanthin	0.53	Ethanol, benzene, acetone and petroleum ether in the ratio 3.5:10:20:66.5 v/v
Capsanthin dilaurate	0.52	10% Acetone in petroleum ether
Capsanthin monoesters	0.21 0.20	10% Acetone in petroleum ether

presence of these fatty acids, though common in seed fats, decreases the stability of carotenoids in paprika. Since the seeds contribute a large proportion of fat, removal of seeds prior to grinding paprika should enhance the pigment stability.

Capsanthin, the major carotenoid of paprika, is present naturally as its dilaurate ester and this enhances its epiphasic properties and solubility in neutral fat, with which it is associated. Esterification reduces the adsorption properties of capsanthin, but does not significantly change the hue. Though esterification may confer stability to capsanthin, the presence of triglycerides containing high amounts of unsaturated fatty acids may be an important factor contributing to the fading of paprika during processing and storage.

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TIBOR FULEKI
Horticultural Products Laboratory, Horticultural Research Institute of Ontario
Vineland Station, Ontario, Canada[1]
and Canada Department of Agriculture, Research Station, Kentville, Nova Scotia

ANTHOCYANINS IN RED ONION, Allium cepa

SUMMARY—Seven cyanidin and one peonidin glycosides were found in the bulbs of the Ruby and Southport Red Globe varieties of red onion. The major anthocyanin and the one present in second largest quantity were identified on the basis of their chromatographic and spectral properties as cyanidin 3-glucoside and cyanidin 3-diglucoside, respectively. The cyanidin 3-diglucoside was not found identical with either the 3-sophoroside or the 3-gentiobioside of cyanidin, therefore, this pigment is not only a "new" compound but it also represents a "new" glycosidic class for anthocyanins. The peonidin monoside present only in minor quantity was identified as peonidin 3-glucoside.

INTRODUCTION

THE COLOR OF the red onion is due to the presence of anthocyanins. An early survey (Robinson and Robinson, 1932) identified the anthocyanin in red onion as cyanidin-pentoseglycoside and indicated that most pentoseglycosides are rutinosides. Fouassin (1956) employing paper chromatography found three cyanidin glycosides (one monoside, one diglycoside). Brandwein (1965) identified peonidin 3-arabinoside in the outer scale-leaves of the Southport Red Globe variety. Previous work by the author (Fuleki, 1969) showed the presence of eight anthocyanins in red onion (Ruby variety) but none of them could be tentatively identified as peonidin 3-arabinoside. The work reported herewith was undertaken to identify the major anthocyanin of red onion.

EXPERIMENTAL

Onions

The Ruby and Southport Red Globe varieties used for the isolation of pure onion anthocyanins, were grown on the experimental plots of the Canada Department of Agriculture Research Station, Kentville, N.S., the Muck Research Station of the Horticultural Research Institute of Ontario, Bradford, Ont. and Jos. Harris Co. Inc., Rochester, N.Y.

Paper chromatographic equipment

The separation and purification of individual anthocyanins were carried out on Whatman No. 3 MM paper in Model A-300 Chromatocab (Research Equipment Corp., Oakland, Calif.) and Panglas Model 500 Chromatanks (Shandon Sci. Co. Inc., Sewickley, Pa.). The R_f, R_{Cy} and R_G values were determined by a different system which employed Whatman No. 1 paper and Kurz-Miramon all-glass chromatography apparatus (Kensco, Oakland, Calif.).

The solvent systems used for paper chromatography were the following:

BAW 1-butanol-glacial acetic acid-water (4:1:5), upper phase, freshly prepared.

BBFW 1-butanol-benzene-formic acid-water (100:19:10:25), aged three days, upper phase (Fuleki and Francis, 1967a).

BBPW 1-butanol-benzene-pyridine-water (5:1:3:3)

BFW 1-butanol-formic acid-water (100:25:60), upper phase (Fuleki, 1969).

BH 1-butanol-2N hydrochloric acid (1:1), upper phase. The chromatogram was equilibrated over the aqueous phase for 24 hr.

Forestal glacial acetic acid-conc. hydrochloric acid-water (30:3:10).

Formic formic acid-conc. hydrochloric acid-water (5:2:3)

1% HCl conc. hydrochloric acid-water (3:97).

HAc-HCl glacial acetic acid-conc. hydrochloric acid-water (15:3:82).

15% HAc glacial acetic acid-water (15:85).

MAW methanol-glacial acetic acid-water (90:5:5).

PEAW pyridine-ethyl acetate-water (5:12:4)

Phenol phenol-water (4:1,w/v).

The chromogenic reagents used for the location of sugars were:

AHP 0.91 ml aniline and 1.66g o-phthalic acid dissolved in a mixture of 48 ml 1-butanol, 48 ml ethyl ether and 4 ml water. The sugar chromatograms were dipped into this reagent, air-dried and heated in an oven at 105°C for 2 min.

$AgNO_3$ 12g silver nitrate was dissolved in 25 ml water and the volume was made up to 1,000 ml with acetone. The sugar chromatograms were dipped into this reagent, air-dried, sprayed with 0.5N NaOH in ethanol and dried. To reduce the background color, the chromatograms were dipped into a dilute sodium thiosulfate solution, washed in water and dried.

Authentic pigments

The preparation of authentic pigments from the sources listed in Table 1 followed the standard chromatographic procedure.

Extraction of onion pigments

The dry outer scale-leaves were removed from the bulbs and soaked in 3% conc. HCl in MeOH overnight at room temperature. The extract was decanted and the process was repeated with MeOH-1.5N HCl (85:15) and 3% conc. HCl in MeOH. The extraction of the pigments from the fleshy inner scale-leaves was essentially the same, except that the flesh was macerated in a Waring Blendor containing 3% conc. HCl in MeOH. The extract was removed from the slurry by vacuum filtration after an overnight leaching period. The extraction was repeated at least twice with 3% conc. HCl in MeOH. The extracts were concentrated in a rotary evaporator under reduced pressure. Since the same pigments were present in the dry outer and the fleshy inner scale-leaves (Fuleki,

Table 1—Sources for authentic pigments.

Pigment	Abbreviation	Source	Reference
Cyanidin 3-glucoside	Cy 3-Gl	rhubarb petioles	Gallop, 1965
Cyanidin 3-rutinoside	Cy 3-Ru		
Cyanidin 3,5-glucoside	Cy 3,5-Gl	Fluka A.G., Buchs, Switzerland	
Cyanidin 3-sophoroside	Cy 3-Sop	raspberry	Harborne and Hall, 1964
Cyanidin 3-gentiobioside	Cy 3-Gent	*Primula sinensis* var. Dazzler, leaves	Harborne and Sherratt, 1961
Cyanidin 3-galactoside	Cy 3-Ga	cranberry	Zapsalis and Francis, 1965
Cyanidin 3-arabinoside	Cy 3-Ar		
Peonidin 3-galactoside	Pn 3-Ga		
Peonidin 3-arabinoside	Pn 3-Ar		
Cyanidin	Cy		
Peonidin	Pn		
Peonidin 3-glucoside	Pn 3-Gl	cranberry	Fuleki and Francis, 1967b

[1] Request for reprints should be sent to this address.

1969), the concentrated extract of both tissues were used for the preparation of pure anthocyanins.

Isolation of pure onion pigments

The pigment extract was streaked on 46 × 57 cm sheets of Whatman No. 3 MM paper and developed by descending chromatography with BFW. The onion anthocyanins were resolved on extended development (up to 65 hr) into eight bands (Fuleki, 1969). The numbering of the anthocyanin bands was begun with the fastest moving pigment. To achieve a maximum separation the development was continued until the fastest moving pigment which was going to be isolated (No. 2) reached the area within 2-3 cm from the bottom edge of the paper. The developed chromatograms were air dried and the bands were cut out. The pigment was eluted with MAW and the solvent was evaporated at room temperature and under atmospheric pressure. The pigments were further purified by repeated chromatography with BFW (3 times) and 15% HAc (once) in that order.

Identification of the onion anthocyanins

The identification of the anthocyanins followed in general the chromatographic and spectroscopic procedure described by Harborne (1967).

R_f, R_{Cy} and R_G value determinations

These values were established by spotting the samples along with authentic markers on Whatman No. 1 paper and developing it by descending chromatography with the specified solvent system. R_{Cy} values (chromatographic mobility relative to Cy 3-Gl) were determined for the pigments in solvent systems where the chromatograms had to be overdeveloped because of the slow mobility of the pigments.

Spectral measurements

The visible and UV spectra were obtained on the purified pigment dissolved in methanolic 0.03% conc. HCl with a Beckman DB recording spectrophotometer.

Acid hydrolysis

The anthocyanin was hydrolysed by heating approx. 2 ml purified anthocyanin solution with 2 ml 2N HCl in a water bath for 60 min. After cooling the anthocyanidin formed was extracted with 1-pentanol. The mineral acid was removed from the aqueous sugar solution remaining after the extraction of aglycone by repeatedly extracting it with 2-3 ml portions of 10% di-n-octylmethylamine in chloroform (Smith and Page, 1948). The sugar solution was washed twice with chloroform to remove residual amine. Both the aglycone and the sugar solutions were concentrated by evaporation.

Controlled acid hydrolysis

The hydrolysis was carried out as described above except that small aliquots were withdrawn after 1, 2, 4, 8, 16, 32 and 60 min of heating. The samples were applied along with authentic anthocyanins directly on Whatman No. 1 papers and they were developed in four solvent systems (BAW, BFW, BH and HAc-HCl).

RESULTS & DISCUSSION

Pigment separation

Chromatograms of the red onion extract developed with BFW, BBFW or BAW yielded eight anthocyanin bands. Since most of the anthocyanins were present in relatively small quantities (Fuleki, 1969) only the major onion anthocyanin (No. 4) could be isolated in sufficient quantity for a complete identification. The anthocyanin present in second largest quantity (No. 6) proved to be a "new" pigment; therefore, a larger than usual quantity would have been required for a complete characterization. Small quantities of most of the minor and trace anthocyanins (No. 2, 3, 7 and 8) were also isolated and purified to some extent, to allow a partial identification.

Table 2–Chromatographic data for onion anthocyanins.[a]

Pigment	Solvent: BBFW R^b_{Cy} × 100	BFW R^b_{Cy} × 100	BAW R^b_{Cy} × 100	BH R_f × 100	1%HCl R_f × 100	HAc HCl R_f × 100	Identification
No 2	–	–	–	33(2)	8(5)	26(2)	Cy monoside
No 3	149	136	43	29(5)	10(4)	34	Pn 3-Gl
No 4	104	109	39	24	8	28	Cy 3-Gl
No 6	40	59	34	16	9	32	Cy 3-diGl
No 7	30	34(5)	25	9(5)	16	41	Cy-glycoside
No 8	18(4)	–	14(3)	–	–	–	Cy-glycoside
Standards:							
Cy 3-Gl	100	100	39	24	7	27	
Cy 3-Ga	89	96	38	22	9	29	
Cy 3-Ar	198	162(5)	44	36	8(5)	28	
Cy 3-Ru	71	78(5)	36	27	18(4)	44	
Cy 3-Sop	40	42(5)	38	23	44	65	
Cy 3-Gent	41(1)	49(1)	29(2)	12(1)	19(3)	42	
Cy 3,5-Gl	24	26(5)	31	9	16	41	
Pn 3-Gl	148	141	43	27	9(4)	34(5)	
Pn 3-Ga	130	122	41	25	11(4)	36(5)	
Pn 3-Ar	255	209(4)	46	37	9	33	

[a]Values are averages of at least six determinations. In cases where less than six determinations were carried out, the number is given in brackets.

[b]R_{Cy} = chromatographic mobility relative to Cy 3-Gl.

Table 3–Spectral data for onion anthocyanins.

Pigment	λ_{max} in MeOH-HCl (nm) UV	vis.	$E_{440}/E_{vis.\,max}$	$E_{UV\,max}/E_{vis.\,max}$	$E_{acyl\,pk}/E_{vis.\,max}$
No. 4	280	523	24	58	10
No. 6	279	522	25	63	13
No. 7	276	519	19	90	24
Cy 3-Gl[a]	274	523	24	60	<20
Cy 3-Sop[a]	282	523	23	58	–
Cy 3-Gent[a]	–	521	29	–	–
Cy 3,5-Gl[a]	273	524	12	44	<20

[a]Literature values (Harborne, 1963, 1967; and Harborne and Sherratt, 1961).

Pigment identification

An earlier communication (Fuleki, 1969) tentatively identified the anthocyanins in bands No. 1, 2 and 4 as cyanidin monosides; in band No. 5, 6 and 7 as cyanidin biosides; in band No. 8 as cyanidin diglycoside; and in the No. 3 band as peonidin monoside. The identifications were based on chromatographic mobility, color and chromogenic reactions.

The chromatographic data presented in Table 2 support the above general identifications. Two of the onion anthocyanins could be more accurately identified on the basis of their chromatographic mobility. The No. 3 and 4 anthocyanins had identical R_f values with that of authentic Pn 3-Gl and Cy 3-Gl respectively. The R_f values for the No. 6 onion anthocyanin were in the range expected of a cyanidin bioside, but it did not correspond with any of the authentic pigments.

The spectral values (Table 3) gave additional evidence for the identification suggested by the chromatographic data. The $E_{440}/E_{vis.max.}$ and $E_{UV\,max.}/E_{vis.max.}$

Table 4—Chromatographic data for the aglycons of onion anthocyanins.

Aglycon	Solvent		
	BAW	Formic	Forestal
	$R_f \times 100$		
No. 2	–	21	50
No. 3	–	28	59
No. 4	73	23	53
No. 6	70	22	51
No. 8	–	20	52
Markers			
Cyanidin	68	21	52
Peonidin	70	26	58

Table 5—Chromatographic data for the sugar moieties of onion anthocyanins.

Sugar	Solvent			
	BBPW	Phenol	BAW	PEAW
	$R_G \times 100$			
No. 3	101	98	–	–
No. 4	93	96	100	100
No. 6	97	100	101	–
Markers				
Glucose	100	100	100	100
Galactose	86	113	92	96
Rhamnose	183	63	179	–
Arabinose	119	141	124	–
Xylose	141	121	147	–

ratios indicated that the 5-hydroxyl group on the benzoprylium nucleus was free and the $E_{acyl\ peak}/E_{vis.max.}$ showed that the sugar was non-acylated in the No. 4, 6 and 7 onion anthocyanins.

Further evidence on the identity of the onion pigments was obtained by chromatographing the anthocyanidins and sugars produced on acid hydrolysis. The chromatographic data on the aglycones (Table 4) showed that all but the No. 3 anthocyanin are cyanidin glycosides. The anthocyanidin of the No. 3 anthocyanin gave R_f values similar to that of authentic peonidin.

A considerably larger quantity of anthocyanin is needed to identify the sugar moiety than for the readily visible aglycone. Consequently the sugars of the most abundant onion anthocyanins only could be identified. As the results in Table 5 show, the No. 3, 4 and 6 pigments yielded only glucose.

The results indicated that the No. 3, 4 and 6 onion anthocyanins were Pn 3-Gl, Cy 3-Gl and cyanidin 3-diglucoside (Cy 3-diGl) respectively. To verify this identification controlled acid hydrolysis was carried out on the No. 3, 4 and 6 pigments. As it was expected the No. 3 and 4 pigments did not give any and the No. 6 anthocyanin gave only one intermediary product. The intermediate anthocyanin had identical R_f values with that of authentic Cy 3-Gl (Table 6).

Table 6—Products of controlled acid hydrolysis of the No. 6 onion anthocyanin.

Pigment	Solvent			
	BAW	BFW	BH	HAc HCl
	$R_f \times 100$			
No. 6	33	7	27	32
Intermediate	37	13	36	32
Anthocyanidin	53	67	79	12
Markers				
Cy 3-Gl	37	13	39	32
Cy 3-Ga	36	12	36	32

No. 4 onion anthocyanin. The major anthocyanin of red onion was identified as Cy 3-Gl. This identification was based on chromatographic data on the pure pigment, the aglycone and sugar moiety. The spectral measurements, the results of controlled acid hydrolysis as well as the colors produced with chromogenic reagents (Fuleki, 1969) also supported this conclusion.

It was mentioned in an earlier communication (Fuleki, 1969) that the only complete identification on the onion anthocyanins appears to be erroneous. Brandwein (1965) working primarily on the identification of onion flavonoids, identified the anthocyanin in the Southport Red Globe variety as Pn 3-Ar. The presence of only one anthocyanin was recognized by Brandwein (1965); therefore, it is safe to assume that he was dealing with the major onion anthocyanin.

The anthocyanin composition of a number of red onion varieties were examined to see if varietal differences would account for the discrepancy between Brandwein's and our identification. The results showed no apparent qualitative or visually noticeable quantitative difference. The R_f values for the only peonidin glycoside (No. 3) present in small quantities in all examined varieties, were consistently different from that of authentic Pn 3-Ar (Table 2).

No. 6 onion anthocyanin. The anthocyanin occurring in second largest quantity in onion was identified as Cy 3-diGl. This identification was supported by the chromatographic data obtained on the pure anthocyanin and its degradation products. The spectral values and color reactions also supported this identification. Controlled acid hydrolysis gave further proof that the anthocyanin in the No. 6 band is Cy 3-diGl.

Two kinds of anthocyanidin 3-diglucosides are known to occur in nature: one with sophorose (β,1–2 linkage) and the other with gentiobiose (β,1–6 linkage). The R_f values obtained from the same chromatograms for the No. 6 pigment and for authentic Cy 3-Sop and Cy 3-Gent (Table 2) showed that the No. 6 onion anthocyanin is not identical with either of the known 3-diglucosides. This indicates that the No. 6 anthocyanin is a "new" compound which also represents a "new" glycosidic class for anthocyanins.

No. 3 onion anthocyanin. The only peonidin glycoside found in onion was identified as Pn 3-Gl. This identification is based on the results of R_f and R_G value determinations on the pure pigment and on the anthocyanidin and sugar produced by acid hydrolysis. This pigment was present in even smaller quantity than the No. 6 anthocyanin.

No. 2, 7 and 8 onion anthocyanins. These pigments were isolated in very small quantities. The R_f values obtained on the anthocyanins and their aglycons as well as the spectral data obtained on the No. 7 anthocyanin allowed the partial identification of these pigments. The No. 2 onion anthocyanin was identified as cyanidin monoside, the No. 7 and 8 anthocyanins as cyanidin diglycosides.

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M. G. MOSHONAS and E. D. LUND
USDA, Southern Utilization R&D Div., ARS
U.S. Fruit & Vegetable Products Lab., Winter Haven, Florida 33880

A GAS CHROMATOGRAPHIC PROCEDURE FOR ANALYSIS OF AQUEOUS ORANGE ESSENCE

SUMMARY—A procedure for direct GLC analysis of orange essence was developed. The procedure uses a Porapak precolumn to separate the water, methanol, acetaldehyde and ethanol from the remainder of the volatile organic constituents of the essence. Determination of the organic material in the essence can be obtained by this method from a 25 µl sample of aqueous essence. The method allows a quantitative estimation of acetaldehyde, methanol and ethanol as well as the smaller amounts of other organic compounds in the essence.

INTRODUCTION

ORANGE ESSENCE, a concentrated aqueous distillate from orange juice, is being used increasingly by citrus processors as an additive for flavor enhancement of concentrated orange juice. Analysis of the essence by extraction with an organic solvent followed by gas-liquid chromatography (Wolford et al., 1962; Wolford and Attaway, 1967; Schultz et al., 1967) has shown the organic portion of the essence consists principally of ethanol with smaller amounts of other volatile organic compounds. Over 100 compounds were found to be present in the essence ranging in volatility from acetaldehyde to the sesquiterpene hydrocarbon valencene. Ethyl butyrate, limonene, and linalool were found to be the three most abundant constituents after ethanol.

The difficulties inherent in solvent extraction methods are discussed in several recent reviews on the isolation and analysis of volatile organic flavoring compound (Forss, 1969; Weurman, 1969). Incomplete extraction and loss of volatiles during concentration are a source of error in the application of solvent extraction methods as pointed out by these authors. A procedure is desirable which will avoid these difficulties, as well as the related problem of handling and transferring small quantities of volatile compounds. This system should separate the organic fraction from water, concentrate the organic fraction, give a GLC analysis and eliminate the necessity of transfer of the sample.

The recent commercial availability of porous polymer beads with their unique properties as packing materials for gas chromatography of volatile organic compounds has made it possible to construct a unified system for essence analysis as described above.

EXPERIMENTAL

Apparatus

A gas chromatograph (F&M Model 810) equipped with a flame ionization detector was modified as shown in Figure 1. The apparatus consists of a 3 ft × 1/8 in column packed with 50–80 mesh Porapak Q coupled to a 10 ft by 1/8 in column packed with 20% Carbowax 20M on 60/80 Gas-Chrom by means of a low internal volume 3-way valve. The effluent from the Porapak column either passes directly to the flame ionization detector or is diverted by the valve, through a trap located outside of the oven and then through the Carbowax column before passing into the detector. The trap consists of eighteen 1 cm loops of 1/16 in I. D. stainless steel tubing coiled as shown in Figure 1, with the depth of the coiled portion approximately 2 in. The carrier gas line was equipped with a similar trap of 1/8 in tubing, cooled with liquid nitrogen.

Function of the Porapak

Porapak (Waters Associates, Inc., Framingham, Mass.), a column packing composed of porous polymer beads, allows very rapid elution of water with a minimum of tailing. Because the polymer beads do not contain any liquid coating, large samples of water can be injected without decomposition and bleeding of the column. Table 1 indicates the retention time of water and the five most volatile compounds which have thus far been identified in orange essence on a Porapak column.

In order to eliminate any bleeding of the Porapak, which may introduce spurious peaks into the GLC trace, the packing had to be extracted overnight with acetone in a Soxhlet extractor and conditioned at 250°C for 16 hr before use. The small amount of bleeding remaining after this treatment was not sufficient to interfere with the GLC trace with essences of 100-fold or greater concentration.

Operation

A 25 µl sample of essence is injected and the water, methanol, acetaldehyde, and ethanol allowed to pass through the Porapak column and directly into the flame ionization detector. The column is held at 80°C and the helium flow rate at 33 ml/min during this period. As soon as the ethanol peak has passed through the column, the 3-way valve is turned, the column heated to 175°C and the remainder of the sample is eluted from the Porapak column into the liquid nitrogen cooled trap. After 1 hr at 175°C, the column temperature is reduced to 100°C, the cooling bath removed from the trap and the trap heated to 250° in 5 sec to inject the condensed sample into the Carbowax column. The Carbowax column is then temperature programmed from 100°C–220°C at 2°C/min and a trace obtained for the remainder of the essence components.

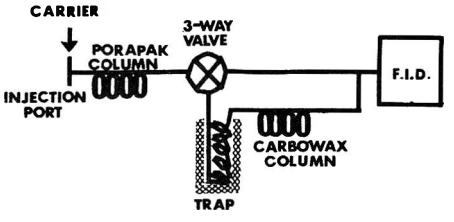

Fig. 1—Apparatus for direct GLC analysis of aqueous orange essence.

Trap efficiency

Trap efficiency was determined by injection of 2.0 µl samples of 0.1% aqueous solution of a number of compounds representative of typical volatile essence constituents. For the purpose of the efficiency test, the apparatus was modi-

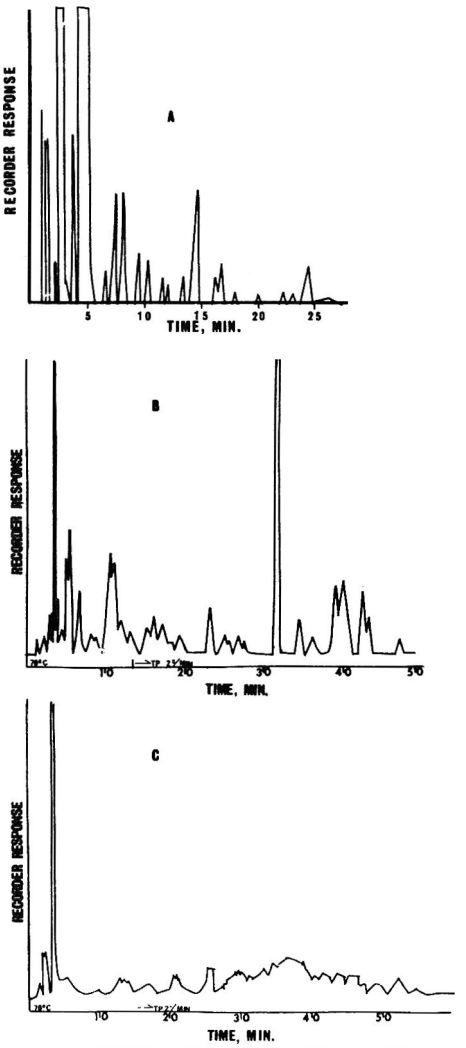

Fig. 2—Gas chromatograms of orange essence showing:

(A) Aqueous essence using the modified gas chromatograph; (B) Extracted, non-aqueous essence; and (C) Aqueous essence using modified gas chromatograph with no trapping of compounds.

fied by removing the Carbowax column and replacing it with an empty piece of 1/8-in tubing. In each case, two samples were run. The first sample was allowed to pass directly into the detector from the Porapak column and the second sample was diverted into the liquid nitrogen cooled trap, the Porapak column heated to 200°C for 2 hr, then the cooling bath removed and the trap heated to 250°C in 5 sec, driving the condensed sample into the detector. The carrier gas flow rate was 33 ml/min. The efficiency of the trap was calculated by dividing the peak area for the second sample by the peak area calculated for the first sample and multiplying by 100. Table 2 indicates the percent efficiencies for the various compounds.

Total organics analysis

Employing the same modified system used to determine trap efficiencies allows a convenient procedure for determination of the percentage of combined organic compounds in the essence exclusive of methanol, acetaldehyde, and ethanol. The essence sample is injected and the water, methanol, acetaldehyde and ethanol allowed to pass through the Porapak column and directly to the detector at 80°C and a helium flow rate of 33 ml/min as before. The 3-way valve is turned, the remainder of the sample is eluted into the liquid nitrogen-cooled trap by heating the column to 175°C for 1 hr, the cooling bath is removed, and the trap heated with a heat gun to 250°C in 5 sec. The mixture is volatilized from the trap into the detector in the form of a slug of vapor which is recorded by the detector as a few sharp peaks.

RESULTS & DISCUSSION

GLC TRACES shown in Figure 2 represent the analysis of a typical commercial orange essence using this apparatus. This essence is considered by the manufacturers to be approximately 100-fold in concentration relative to juice, and has a COD value (Dougherty, 1968) of 54,500.

Trace 2A is obtained by injecting 25 μl of essence and operating the apparatus as described, with the temperature programmed as shown in the figure.

Trace 2B represents the result obtained by GLC analysis of anhydrous essence. This trace was obtained from 0.1 μl of anhydrous essence by detaching the Carbowax column from the trap and attaching it directly to the injection port in place of the Porapak column. The carrier gas flow rate was the same as for the upper trace. Temperature programming was carried out as shown in the figure. The anhydrous essence was obtained by the solvent extraction method of Wolford et al. (1962). A 1400 ml sample of the essence was saturated with sodium sulfate, extracted with three 400 ml portions of distilled methylene chloride and concentrated by distillation through a Vigreux column to give 490 mg anhydrous essence, essentially free of ethanol.

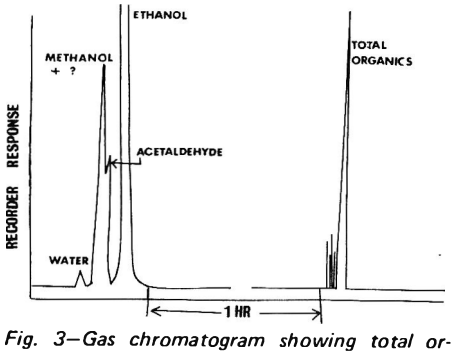

Fig. 3–Gas chromatogram showing total organics in aqueous orange essence.

Trace 2C was obtained by injection of 25 μl of aqueous essence and operation of the apparatus without including the trapping step. After the ethanol had passed through the Porapak column, the remainder of the sample was diverted through the trap which was placed in the oven and thus allowed to pass directly into the Carbowax column. The column was then temperature programmed as shown in Figure 2.

The marked contrast between the shallow broad peaks of the lower trace and the sharp peaks in the upper trace demonstrates the necessity for a concentration step in the procedure. The sharpness of the peaks in the upper trace is similar to that in the middle trace, showing that the resolution obtainable is as good as that for a conventional GLC analysis, using an anhydrous essence sample.

The resolution obtainable and the efficiency of the trap are related, since the larger the trap, the greater will be the trapping efficiency because of the larger surface area, while the resolution will be poorer as a result of the larger volume of the injected sample. As a result, it was necessary to obtain the data on trap efficiencies described in the Experimental section. As shown in Table 2, the efficiencies are all very good, essentially 100% within the limits of experimental error. The lower ethanol value, 0.01%, at 96% is possibly indicative of a slight drop in efficiency at lower concentrations.

Figure 3 shows a GLC trace obtained from 2 μl of commercial essence using the set-up described for total organics analysis. The first small peak is water, which always appears as a small peak even though the detector is very insensitive to water. The large peak after water is a mixture of methanol and an unknown; the smaller peak appearing as a shoulder on the back side of the methanol peak, acetaldehyde; and the next large peak ethanol, according to mass spectral analysis. After 1 hr, the total organics peak appears as shown in the figure. Less than 10% of this peak is due to bleed from the Porapak. The percentage of ethanol was determined by injecting samples of known concentrations of ethanol in water, operating the apparatus as for total organics analysis, and plotting the peak areas as a function of ethanol concentration. From the resultant linear plot the percentage of ethanol in the essence was determined to be 10.4%. The percentage of ethanol was calculated to be 10.0%, from the COD of the essence and assuming the organic material to be essentially 100% ethanol. The closeness of these two values indicates a good correlation between the two methods.

Table 1–Retention times of volatile orange essence constituents on Porapak Q.[a]

Compound	Retention time (min)
Water	1.2
Methanol	3.3
Acetaldehyde	4.2
Ethanol	7.2
Acetone	12.3
Ethyl formate	15.0

[a]Conditions: temperature 110°C; He flow – 10 ml/min; column – 1/8 in. × 5 ft packed with 50/80 Porapak Q.

Table 2–Trap efficiency.

Compound	Efficiency (%)
Cis-3-hexene-1-ol	100
Isoamyl alcohol	98
Acetone	100
Ethyl butyrate	97
Ethanol – 0.1%	98
Ethanol – 0.01%	96

The new method produces a considerably different qualitative picture from the anhydrous essence. Reference to Figure 2 reveals that, if one takes into account the fact that the anhydrous essence sample was programmed more rapidly than the aqueous essence sample, thus compressing the trace, the two traces are basically similar with several noticeable exceptions. The qualitative differences between the two traces can be explained either by a loss of material during the concentration of the solvent or by incomplete extraction of certain components of the essence mixture.

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Mention of brand names is for identification only and does not imply recommendation by the USDA.

P. ISSENBERG, M. R. KORNREICH and A. O. LUSTRE
Dept. of Nutrition & Food Science, Massachusetts Institute of Technology, Cambridge, Mass. 02139

RECOVERY OF PHENOLIC WOOD SMOKE COMPONENTS FROM SMOKED FOODS AND MODEL SYSTEMS

SUMMARY—Procedures for extraction, isolation, and concentration of wood smoke phenols from commercial summer sausage, laboratory smoked pork belly, and model systems were evaluated during development of methods for quantitative determination of individual phenolic compounds in smoked food products. Extraction of summer sausage with 50% aqueous ethanol, followed by thorough washing of the residue resulted in 84% recovery of added ^{14}C-phenol. Additional losses occurred during isolation and concentration of a phenolic fraction, resulting in an overall recovery of 59% of the added phenol. Concentrates were prepared from 5% NaOH extracts and compared by gas chromatographic analysis with those prepared by aqueous ethanol extraction. A greater variety of substituted methoxy and dimethoxy phenols was found in the concentrates prepared from alkaline extracts. Recoveries of phenols from summer sausage, pork belly, and model systems simulating wet tissue and fat are compared.

INTRODUCTION

PRESERVATION of foods by exposure to wood smoke is probably the oldest form of food processing. In some countries, smoking is still employed primarily as a preservation method. In the United States, smoke is used to produce typical color and flavor, and refrigeration provides preservation. In lightly smoked products, it is difficult to determine the antimicrobial or antioxidant significance of smoke components. In view of current concern about chemicals added to foods, it is important that the exact nature of smoke components consumed in foods be known. The safety of smoked foods has been established, as well as the safety of any food product can be, by their long history of use. Knowledge of the composition of smoked products can provide necessary background for evaluation of new chemicals which may be proposed as food ingredients. Such studies may also reveal the presence of compounds with potential commercial value as antioxidants and antimicrobial agents.

Many wood smoke components have been identified, but little attention has been given to occurrence of these components in smoked food products. Malanoski et al. (1968) determined specific polynuclear aromatic hydrocarbons in a variety of smoked meat and fish products, but most investigators have measured only classes of smoke components in foods. Bratzler et al. (1969) determined total carbonyls, total acids, and total phenols in bologna smoked in the laboratory, and correlated the radial distribution of these classes with smoke flavor. Phenol content provided a high ($r = 0.81$) correlation with smoke flavor. Acid and carbonyl levels in the smoked product were not greatly different from those found in the unsmoked emulsion. Phenols were the only class of compound present in the smoked meat which were entirely absent from unsmoked meat. Concentrations ranging from 0.12 mg/100g at the center to 3.70 mg/100g at the surface were measured.

Most investigators have measured total phenols by the method of Tucker (1942) or by the 4-aminoantipyrine method (Emerson, 1943). Both methods rely on coupling with the phenol, in the position *para* to the hydroxyl group, to form a colored derivative which is determined spectrophotometrically. Phenols with substituents in the *para* position usually do not react. *Para*-substituted phenols are present in hardwood sawdust smoke (Fiddler et al., 1966; Lustre and Issenberg, 1969) and may account for about half of the phenolic fractions recovered from smoked meat products (Lustre and Issenberg, 1970). Clearly, available methods may not provide realistic estimates of total phenols in smoked foods. Even an accurate determination of total phenols, however, would be of limited value in understanding the chemistry of the smoking process. The phenolic fraction of wood smoke is complex. It contains at least 30 components, some of which have carbonyl as well as phenolic functional groups. Fiddler et al. (1970) have reported evidence that dihydroxyphenols are present in a commercial liquid smoke preparation. Even among the relatively simple compounds, guaiacol, 4-methylguaiacol, and 2,6-dimethoxyphenol, large differences in odor thresholds have been observed (Wasserman, 1966). It is likely that structural differences between components of the phenolic fraction result in major differences in the components' interactions with meat components, their antioxidant and antimicrobial activities, and in their contribution to flavor.

Reliable methods for determining specific phenols in smoked food products are required. This paper reports an initial investigation of the feasibility of extending the methods previously employed for identifying phenolic components of smoked foods (Lustre and Issenberg, 1970) to quantitative determination of these compounds.

EXPERIMENTAL

Isolation of phenols from water and oil solutions

In this series of experiments, solutions containing known quantities of selected phenols, previously identified as major components of smoke condensates and foods, were treated by the methods employed for isolating a phenolic fraction. These studies were undertaken to determine the partition behavior of the phenols and reproducibility of the results. The water and oil models had been used to study partition of phenolic components between smoke vapor and liquid model systems (Kornreich, 1969).

A stock solution was prepared containing 0.1g each of guaiacol, methylguaiacol, phenol, 2,6-dimethoxyphenol (syringol), and 2-hydroxy-3-methylcyclopent-2-en-l-one (Cyclotene) in 10 ml acetonitrile. Cyclotene was included because it was found in phenolic fractions isolated from wood smoke condensates by fractionation based on acidity (Lustre and Issenberg, 1969). We wished to determine whether it was found in this fraction because of its acidity, or occurred as a contaminant resulting from its presence in large quantities in the condensates. A 0.5-ml portion of the stock solution, containing 5 mg of each component, was added to 50 ml 5% sodium hydroxide solution or to 5 ml of triolein (Nutritional Biochemicals Corp., Cleveland, Ohio).

The method for isolating phenols from these solutions was based on the procedure of Braus et al. (1952). The aqueous alkaline solution, containing sodium phenolate salts, was extracted with one 200-ml and two 50-ml portions of diethyl ether. The ether extract is referred to as the neutral fraction. The aqueous basic fraction was cooled in an ice water bath, neutralized by saturation with carbon dioxide, and extracted with two 200-ml and three 100-ml portions of ether. The ether extract, termed the phenol fraction, should have contained phenols and other weakly acidic compounds. The aqueous phase is the acid fraction.

The phenol fraction was dried with sodium sulfate and filtered into a round-bottomed flask. The filter paper was washed with three 50-ml portions of ether which were then combined with the filtrate. The solution was concentrated in a rotary evaporator and transferred to a 15-ml graduated centrifuge tube. It was concentrated further in a stream of prepurified nitrogen to a volume of 1.5 ml.

The 5 ml of triolein, containing 5 mg of each component, was dissolved in 100 ml of ether and shaken with 50 ml of 5% aqueous NaOH solution. The emulsion which formed was centrifuged to separate the phases. The ether layer was extracted with a second 50-ml

portion of 5% NaOH solution. The alkaline aqueous phases were combined and washed with 50 ml of ether to remove any remaining triolein. They then received the same treatment as the corresponding phase from the water models for isolation and concentration of the phenolic fraction.

Phenols isolated from the model solutions were determined by gas chromatographic analysis of their trimethylsilyl ether (TMS) derivatives. In order to minimize quantitative uncertainties that arise from solvent evaporation during concentration and transfer, 5 mg of 3,5-dimethylphenol (0.5 ml of 1% solution in acetonitrile) was added as an internal standard just before the ether extracts were dried with sodium sulfate. The internal standard was used only in studies of the model systems. Phenol fractions were concentrated to 0.5 ml in a stream of prepurified nitrogen and a 50-μl portion was transferred to a Microflex reaction tube (Kontes Glass Co., Vineland, N.J.). Fifty microliters of bis-(trimethylsilyl)-trifluoroacetamide (Regisil, Regis Chemical Co., Chicago, Ill.) was added, and the tube was sealed with a silicone rubber septum and screw cap. The reaction mixture was shaken for 30 sec and allowed to stand for 20 min. Preliminary studies showed that this procedure resulted in complete conversion of phenols from smoke condensates to TMS derivatives. Sample volumes of 0.1 μl were analyzed by gas chromatography on a 2.3-mm I.D. × 5.2-m column packed with 2% OV-17 and 0.1% Igepal CO-880 on Chromosorb G (100/120 mesh). Helium flow rate was 25 cc/min. Column temperature was held at 100°C for 5 min and then programmed at 2°C/min to 250°C. An F & M model 810 gas chromatograph with flame ionization detector was used. These recovery studies were replicated five times to determine reproducibility.

In a separate experiment, ^{14}C-labeled phenol was employed to determine at which steps in the procedure significant losses occurred. Approximately 4 μc of uniformly labeled ^{14}C-phenol (5.2 mc/mmole, Tracerlab, Waltham, Mass.) was combined with 0.5 ml of the stock phenol solution and was then diluted to 25 ml with distilled water. A concentrated phenol fraction was isolated from this solution by the methods described above. Samples of all fractions were taken and radioactivity was measured in a liquid scintillation counter (Packard model 2211, Packard Instrument Co., Downers Grove, Ill.). The composition of the scintillator solution (Bruno and Christian, 1961) was 1 part xylene, 3 parts methylcellusolve, 3 parts dioxane, 8% naphthalene, 1% PPO (2,5-diphenyloxazole) and 0.5% POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene].

Isolation of phenols from meat samples

In earlier studies 5% NaOH was used to extract phenols from model systems, smoke condensates (Lustre and Issenberg, 1969), and smoked meat samples (Lustre and Issenberg, 1970). This initial extraction yielded concentrates whose components could be identified. Preliminary experiments, in which a mixture of phenols was added to unsmoked pork belly, indicated that recoveries were low but uniform for most smoke phenols when 5% NaOH was used.

Tucker (1942) used 50% aqueous ethanol to extract phenols from smoked meat products prior to colorimetric determination of total phenols. We investigated this solvent system's effectiveness in extracting phenol from pork belly.

Uncured fresh pork belly (50g) was cut into 2-mm square pieces and homogenized in a Waring Blendor with 200 ml of 50% aqueous ethanol. To this mixture was added 1 ml of a solution containing 50 mg of uniformly labeled ^{14}C-phenol (1 μc). The mixture was homogenized again for 10 min and transferred to two 250-ml centrifuge bottles. The blender was washed three times with 30 ml of 50% aqueous ethanol, and the washings were added to the centrifuge bottles. The mixtures were centrifuged at 0°C and 1800 rpm (700 × g) for 10 min, causing protein to precipitate as a thick cake at the bottom and fat to separate as an upper layer. The aqueous alcoholic solution was decanted and filtered through S & S (Schleicher & Schuell, Keene, N.H.) 595 filter paper in a Buchner funnel.

Fat and protein remaining in the centrifuge bottle were suspended in 5 ml of 50% aqueous ethanol and sonified for 2 min (Biosonik III, Bronwill Scientific Co., Rochester, N.Y.). An additional 50 ml of solvent was added and the mixture was thoroughly stirred. Sonification disintegrated the protein particles and appeared to soften the fat. The mixture was centrifuged at 0°C and 1800 rpm (700 × g), and the aqueous ethanol phase was decanted and added to the original supernatant. Contents of the centrifuge bottles were washed a total of five times. All washings were combined, after measurement of radioactivity, with the original supernatant. To precipitate suspended protein, 50 ml of 40% trichloroacetic acid (TCA) was added to the combined extracts. The mixture was stored at 4°C for 2–4 hr and then filtered through S & S 595 paper in a Buchner funnel.

A phenol fraction was isolated from the aqueous ethanol extract by fractionation based on acidity (Lustre and Issenberg, 1970). The procedure was similar to that used in isolating phenols from water and oil models. Phenol fractions were concentrated to a final volume of 10 ml.

Radioactivity was measured in all fractions during isolation and concentration of the phenol fraction. One-milliliter portions of ether solutions were added to 10 ml of scintillator fluid in counting vials. For aqueous solutions, 1 ml was added to 1 ml of Hyamine (Packard Instrument Co., Downers Grove, Ill.). The Hyamine was then neutralized with concentrated hydrochloric acid with phenolphthalein as indicator, and the mixture was counted in 10–15 ml of scintillator solution. Solid samples, mostly proteins, were solubilized with an equal volume of alcoholic potassium hydroxide. After 1 to 5 days, depending on the nature of the solid sample, the mixture was treated and counted as an aqueous solution.

Samples of a commercially prepared smoked summer sausage ("Beef Stick," Hickory Farms, Toledo, Ohio) were treated with aqueous ethanol and with 5% NaOH solution (Lustre and Issenberg, 1970) to compare extraction of smoke phenols. 50g samples, representing the outer 3 mm of surface, were examined. Phenol fractions were prepared, concentrated to volumes of 0.5 ml, and examined by gas chromatography on a 2.3-mm I.D. × 1.83-m column packed with 5% Carbowax 20M-TPA on 100/120 mesh Chromosorb W. The helium flow rate was 20 cc/min. Column temperature was held at 100°C for 10 min and then programmed at 4°C/min to 220°C. A Varian-Aerograph model 1200 chromatograph (Varian-Aerograph, Walnut Creek, Calif.) with flame ionization detector was used.

Table 1—Recovery of phenols from aqueous and triolein solutions.

Component	Recovery[a] From water %	Recovery[a] From triolein %
Phenol	81 ± 8	89 ± 4
Cyclotene	87 ± 9	83 ± 4
Guaiacol	80 ± 5	80 ± 5
Methylguaiacol	77 ± 4	84 ± 1
Syringol	99 ± 13	92 ± 2

[a]Mean value ± standard deviation, based on 5 replicate samples.

Table 2—Recovery of ^{14}C-phenol from aqueous solution.

Sample	Added phenol %
Original aqueous solution	100
After removal of neutral fraction	90
After removal of acid fraction	90
After drying with Na_2SO_4	83
After concentration to 15 ml	72

RESULTS & DISCUSSION

Recovery of phenols from aqueous and triolein solutions

Recoveries of phenol, Cyclotene, guaiacol, methyl guaiacol, and syringol from solution in water and triolein are shown in Table 1. For most components, recovery was between 80 and 90%. These recoveries indicate that the method is suitable for studies of absorption of smoke components by simple model systems. Reproducibility is adequate for measurement of fairly large differences in concentrations. The losses were probably the result of incomplete extraction of the test compounds. The internal standard, 3,5-dimethylphenol, was added before concentration of ether extracts in order to minimize the influence of subsequent losses on apparent recovery. All quantitative chromatographic measurements were made by measuring peak heights relative to the internal standard and then referring to a calibration curve. (This procedure minimizes volumetric errors but is likely to result in overestimation of absolute recovery.) Loss of phenols by evaporation would not be apparent if the internal standard evaporated to the same extent. This fact may partially account for the high recovery of 2,6-dimethoxyphenol, the least volatile component of the test mixture.

Using radioactive phenol, we made a more detailed examination of sources of loss; the results appear in Table 2. Ten

Table 3—Recovery of ^{14}C-phenol from pork belly extracted with 50% aqueous ethanol.

Sample	Added phenol %
Meat containing ^{14}C-phenol	100
Aqueous ethanol extract	40
First washing of fat and protein	27
Second washing of fat and protein	13
Third washing of fat and protein	3
Fourth washing of fat and protein	1
Fifth washing of fat and protein	0.4
Fat and protein after 5 washings	13
Total phenol recoveries in extracts	84

Table 4—Recovery of ^{14}C-phenol during isolation of phenol fraction from pork belly extract.

Sample	Added phenol %
Aqueous ethanol extract	100[a]
After removal of neutral fraction	78
After removal of acid fraction	80
After concentration to 50 ml	82
After concentration to 10 ml	70

[a]Represents 84% of phenol added to pork belly sample.

percent of the phenol present in the aqueous basic solution was lost to the neutral fraction during ether extraction. This loss may have resulted from incomplete formation of phenolate salts or from partial solubility of salts in the water-saturated ether solvent. The latter explanation is more likely since essentially complete conversion to phenolates can be expected in 5% NaOH solution. Negligible loss occurred during removal of the acid fraction. The loss associated with drying of the phenol fraction probably results from adsorption of phenol on the filter paper and vaporization during transfer operations. Further loss, due to volatility of phenol, occurred during concentration to a volume of 15 ml in the rotary evaporator. Because an internal standard was absent, the overall recovery (72%) observed in this experiment is a more realistic estimate than that shown in Table 1.

Recovery of phenols from meat samples

The difficulty in completely extracting phenol added to pork belly is indicated by the results presented in Table 3. Even five washings of the fat and protein with 50% aqueous ethanol failed to recover 13% of the added ^{14}C-phenol. For routine recovery of phenol, three washings would probably be sufficient. A large quantity of phenol, 50 mg/50g of tissue, was used in this experiment to simplify the analysis. It is not known whether similar recovery would be attained with smaller concentrations. The mechanism of retention is unknown; reaction with protein and simple physical partition effects are possible.

Additional losses occur during isolation of the phenol fraction from the pork belly extract (Table 4). Loss of phenol to the neutral fraction was greater than was observed in studies of simple aqueous solutions (Table 2). The high ethanol concentration in this extract probably increased solubility of the phenolate salt in ether. There was negligible loss of phenol during concentration of the ether solution from 800 ml to 15 ml in a rotary evaporator, but approximately 10% of the initial amount was lost when the volume was reduced to 10 ml. Overall recovery of phenol from the pork belly sample was $0.84 \times 0.70 \times 100 = 59\%$.

Recovery of phenolic compounds other than phenol was not determined, and examination of smoked summer sausage demonstrated the difficulty in applying results obtained for one test compound to the variety of compounds present in smoked food products. The sample extracted with 5% NaOH yielded a concentrate containing 13 phenolic components, whereas only phenol and guaiacol were present in the concentrate prepared from the aqueous ethanol extract. The aqueous ethanol solvent system is apparently inappropriate for less polar substituted phenols. Serious bias may be introduced by the extracting solvent. 2,6-Dimethoxyphenol was the major component of phenolic fractions isolated from smoke condensates (Lustre and Issenberg, 1969) and from smoked meat samples (Lustre and Issenberg, 1970) extracted with 5% NaOH; this component was not detectable in aqueous ethanol extracts.

Procedures for extraction and preparation of concentrates require improvement if they are to be used for quantitative studies of interactions of phenols with meat components. Some steps may be improved easily. Use of a solvent in which water is less soluble would probably reduce loss of phenols to a neutral fraction. Losses incurred during solvent removal can be minimized by rectification with some increase in time required for concentration. The incomplete extraction of phenols from the meat, which may be evidence of true interaction between the phenol and protein, is a more difficult problem. All of these factors require further investigation.

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J. G. SCHMIDT and F. C. PARRISH JR.
Departments of Animal Science and Food Technology, Iowa State University, Ames, Iowa 50010

MOLECULAR PROPERTIES OF POSTMORTEM MUSCLE. 10. Effect of Internal Temperature and Carcass Maturity on Structure of Bovine Longissimus

SUMMARY—A study was carried out with light and electron microscopic techniques to discover the effect of heating and carcass maturity upon the connective tissue and myofibrillar proteins of longissimus from veal (5–6 months), A (12–20 months) and D (54–60 months) maturities. Longissimus was heated to internal temperatures of 1) 50°C; 2) 60°C; 3) 70°C; 4) 80°C and 5) 90°C. Light microscopy indicated that connective tissue fibers increased in size and degree of aggregation with carcass maturity. Also, the more aggregated fibers from D maturity were more heat resistant than the less aggregated fibers of veal and A maturity. Endomysial connective tissue shrinkage was initiated at 50°C and it was completed at approximately 70°C. On the other hand, perimysial connective tissue shrinkage required internal temperatures of 70°C and higher before any significant fiber changes were observed. However, if endomysial and perimysial connective tissue fibers showed similar degrees of aggregation, they appeared to react similarly to heat regardless of maturity group. Electron and phase contrast microscopy showed that myofibrillar proteins compressed and sarcomeres shortened at 50°C. Heating to 60°C caused loss of M-line structure, initiation of disintegration and coagulation of thin and thick filaments and further myofibrillar protein shrinkage. Heating to 70 and 80°C caused more disintegration of thin filaments and coagulation of thick filaments. At 90°C, an amorphous structure resulted, but regardless of the internal temperature the principal banding features of the sarcomere could be identified. These changes are discussed in relationship to changes in meat tenderness.

INTRODUCTION

HEATING causes many structural and chemical alterations in muscle tissue that significantly affect meat tenderness. The extrusion of myofluids (Hamm, 1966) and the degradation of the 2 major structural components of muscle, the collagenous and myofibrillar proteins (Ritchey et al., 1963; Hostetler and Landmann, 1968), have been the predominant changes noted during heating. Degree of internal heating significantly affects these components and the resultant tenderness. Machlik and Draudt (1963) and Schmidt et al. (1970) found that heating to an internal temperature of 60°C resulted in maximal beef tenderness. This finding is probably related to the shrinkage and partial solubilization of bovine muscle collagen initiated at 60°C (Goll et al., 1964). As the internal temperature of longissimus from A maturity is increased above 60°C, tenderness decreases (Schmidt et al., 1970), presumably because temperatures above 60°C cause drying, hardening and coagulating of the myofibrillar proteins (Hostetler and Landmann, 1968). Apparently, myofibrillar protein coagulation more than offsets the tenderization effect of further collagen solubilization at these higher temperatures. On a molecular level, toughness due to drying and coagulating of myofibrillar proteins may originate from the loss of water of hydration surrounding thick and thin filaments.

Since heating and maturity effects on structural components have not been completely shown, our study was carried out to systematically study the morphological changes of the structural proteins of postmortem muscle during cooking. Therefore, this paper reports the effect of different internal temperatures and maturity on bovine longissimus structure observed with the light, phase-contrast and electron microscopes.

MATERIALS & METHODS

Light microscopy

7-day postmortem bovine longissimus was removed from 4 veal (3–6 months), 4 A (12–20 months) and 4 D (54–60 months) maturity carcasses. Steaks 3.1 cm in thickness were cut from the 8th to 13th thoracic vertebrae region, randomly grouped and then broiled to one of the following internal temperatures: 1) 50°C; 2) 60°C; 3) 70°C; 4) 80°C and 5) 90°C. Internal temperatures were measured with a thermometer inserted in the geometric center of the steak. Immediately after cooking to the designated internal temperature, a small sample was removed from the steak's geometric center and immersed in buffered 10% formalin solution. After a 24-hr fixation period, the tissue was diced into small cubes, dehydrated in dioxane and embedded in paraffin. The paraffin blocks were trimmed and sectioned at 10 μ on a Spencer rotary microtome. These sections were mounted on a glass slide, stained with either Verhoeff's elastic stain (Galigher and Kozloff, 1964) or Gridley's (1951) reticulum stain then mounted in Permount. Brightfield and phase-contrast microscopy was done with a Zeiss Photomicroscope. All light, phase-contrast and electron micrographs were printed with a point source Durst enlarger.

Phase-contrast and electron microscopy

Bovine longissimus samples from the 8th to 13th thoracic vertebrae region of 4 veal, 4 A and 4 D maturity carcasses were removed according to a modified biopsy technique of Price et al. (1965) for phase microscopy. Only samples from A maturity were observed by electron microscopy. At-death muscle tissue samples, taken only from A maturity carcasses, were immediately immersed in 1.15–4.0% glutaraldehyde fixative solutions buffered at pH 7.4 with either 0.13 M Sorenson's phosphate or 0.1 M cacodylate buffer. Tissue slices were also removed from the homologous muscle in the carcass after 7 days of aging from all 3 maturity groups. These slices were placed in water-tight plastic bags and heated in a water bath to one of the following internal temperatures: 1) 50°C; 2) 60°C; 3) 70°C; 4) 80°C and 5) 90°C. After the tissue slices had reached the proper internal temperature, they were fixed in 1.15–4.0% glutaraldehyde solutions buffered at pH 7.4 with a 0.13 M Sorenson's phosphate solution for 3 hr. The glutaraldehyde was then removed and the fixed tissue was washed with either 0.13 M Sorenson's phosphate or 0.1 M cacodylate buffer, pH 7.4. Sucrose was added to adjust the osmolarity of the wash solution to that of the fixative solution. Tissue was dissected while immersed in the wash solution. Next, the tissue cubes were transferred to a phosphate or cacodylate buffered 1% osmium tetroxide solution containing added sucrose for 1 hr. Dehydration in graded acetone was followed by infiltration and embedding in a modified Araldite-Epon mixture as reported by Anderson and Ellis (1965). The cubes were polymerized at 60°C for 56 hr and sectioned.

Thick sections for phase-contrast microscopy were cut with a glass knife at 0.75μ or less on an LKB ultramicrotome. Sections were transferred with a platinum loop to a glass slide and mounted in Permount.

Thin sections for electron microscopy were cut with either a glass knife or a diamond knife on the LKB ultramicrotome. Sections of approximately 75 mμ or less were mounted on either carbon-coated or uncoated grids. These grids were stained with 2% uranyl acetate in 50% methanol for 15 min, rinsed, dried and poststained with lead citrate for 2 min. All electron micrographs were taken with a RCA EMU-4 instrument operated at 50 KV.

RESULTS & DISCUSSION

Light microscopy

Light microscopy observations of 7 day postmortem longissimus preparations exhibited irregularly shaped muscle fibers, large perimysial and smaller innate endomysial connective tissue fibers (Fig 1). Sections from all maturity groups contained many collagenous and reticular fibers, but only a few elastic fibers. Collagenous and reticular fibers appeared within the endomysial and perimysial spaces. Elastic fibers were irregularly dispersed and were most frequently observed in and adjacent to blood vessels

Figures 1–6 are brightfield micrographs of cross-sectional (CS), 7-day postmortem, carcass maturity D, bovine longissimus heated to the designated internal temperature (°C).

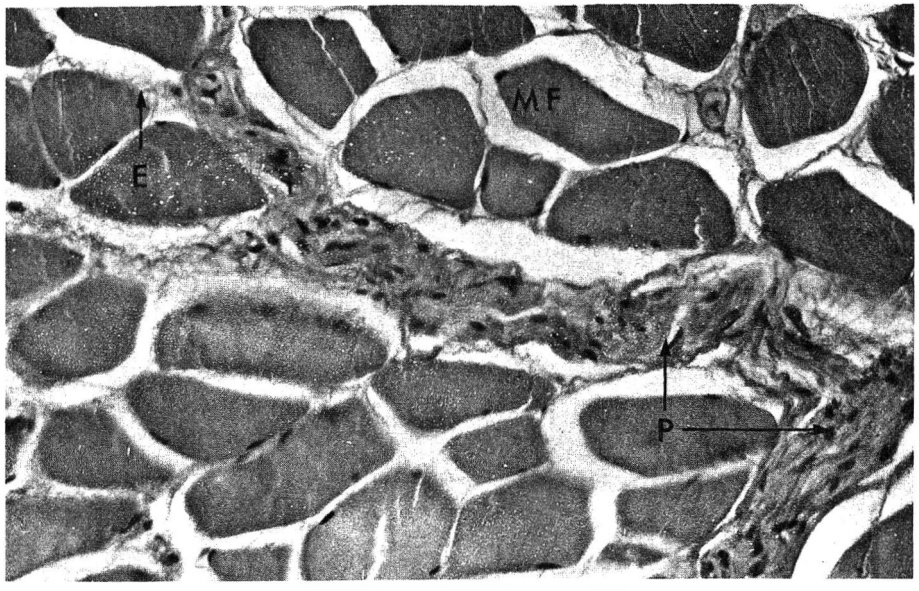

Fig. 1–Irregularly shaped muscle fibers (MF), smaller endomysial (E) and larger more aggregated perimysial (P) collagenous fibers of uncooked longissimus. ×353.

Fig. 2–Longissimus heated to 50°C. ×353.

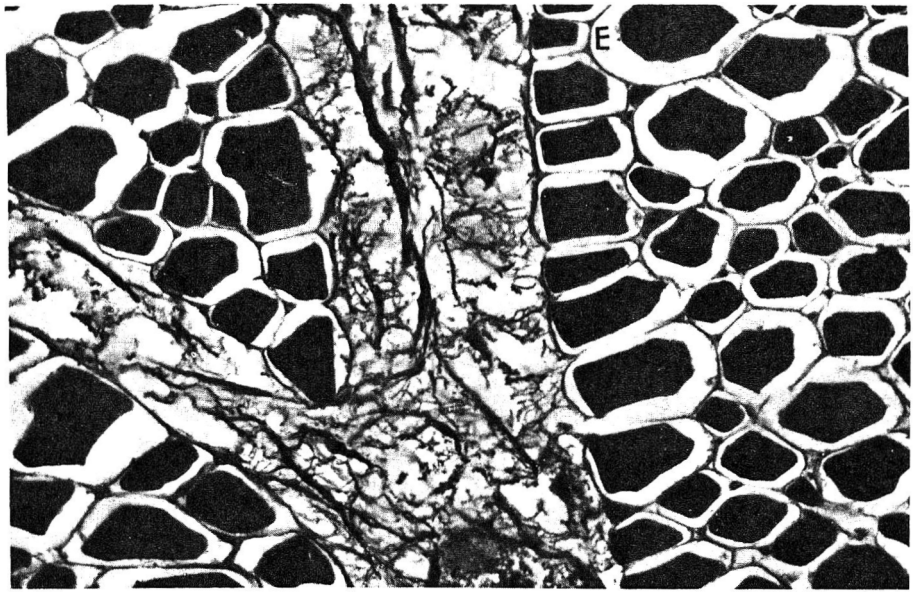

Fig. 3–Longissimus heated to 60°C. Endomysial (E) collagenous fibers are noticeably compressed and shrunken. ×353.

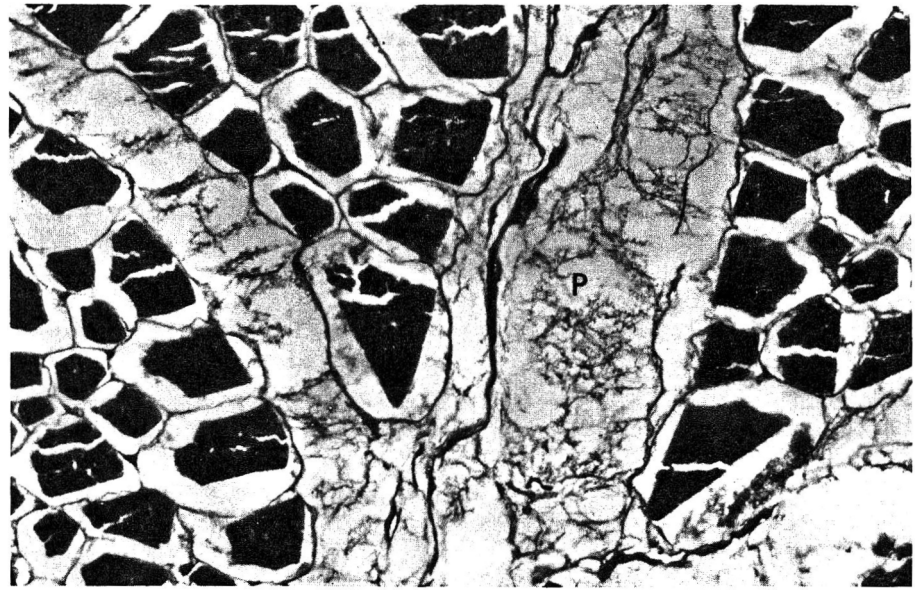

Fig. 4–Longissimus heated to 70°C. Perimysial (P) collagenous fibers are compressed and shrunken. ×353.

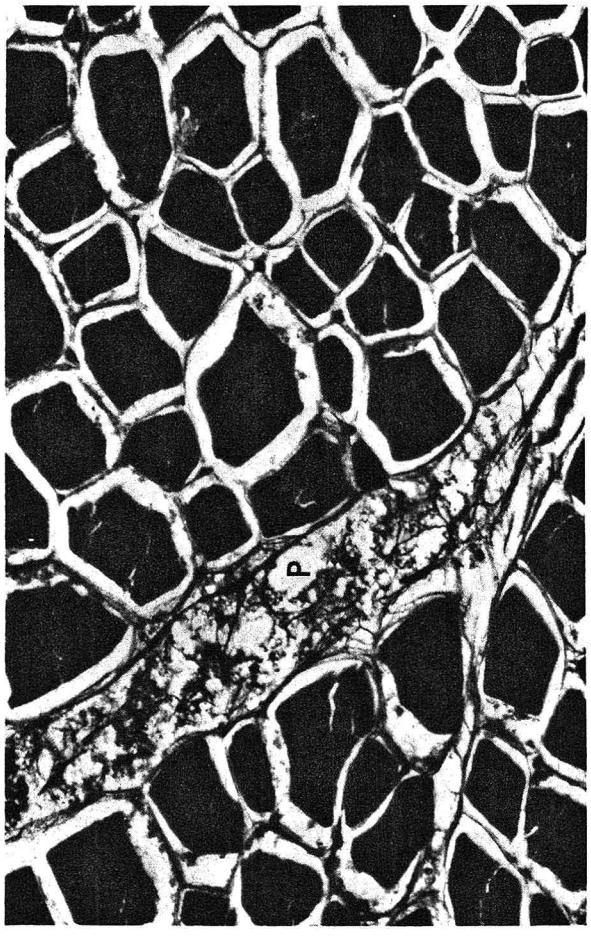

Fig. 6—Longissimus heated to 90°C. Muscle fibers remain intact, whereas connective tissue fibers (P) are fragmented and solubilized. ×353.

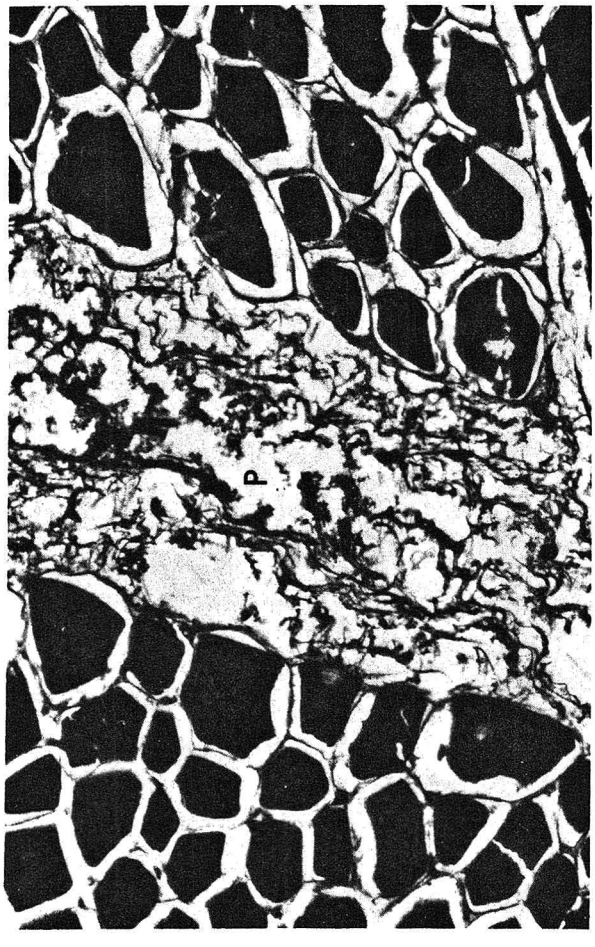

Fig. 5—Longissimus heated to 80°C. Perimysial (P) fibers are fragmented. ×353.

and nerve ganglia. Heating to an internal temperature of 50°C resulted in decreased muscle fiber diameters (Fig. 2). An internal temperature of 60°C caused further muscle fiber and endomysial and perimysial connective tissue shrinkage (Fig. 3). At internal temperatures of 70°C and above (80 and 90°C) a progressive increase in connective tissue shrinkage and fragmentation was evident (Fig. 4, 5, and 6). Thus, connective tissue shrinkage begins at approximately 50°C and continues until fragmentation eventually occurs at the higher temperatures.

Light microscopy observations also showed that the size and degree of aggregation of connective tissue fibers increased with carcass maturity. Moreover, the effect of heat on connective tissue fibers was age dependent, since the more highly aggregated fibers of D maturity were more resistant to heat than the less aggregated fibers of veal and A maturity. This observation may partially explain why longissimus from D maturity usually required higher internal temperatures (70°C) than veal or A maturity (50–60°C) to reach maximal tenderness (Schmidt et al., 1970). Thus, to the extent that tenderness is affected by connective tissue, meat from older animals would have to be heated to a higher internal temperature or for longer periods of cooking time than meat from younger animals, to produce the same increase in tenderness. Also, heating affected the fascicular endomysium before any noticeable shrinkage occurred in perimysium for all maturities. Elastic fibers were relatively heat resistant.

Phase-contrast microscopy

Sections observed with phase-contrast microscopy indicated that cellular changes were similar for the 3 maturities; therefore, only sections of longissimus from maturity A are reported. Observation of longitudinal sections of uncooked, at-death muscle fibers showed the relaxed banding pattern typical of skeletal muscle (Fig. 7a). A cross section (Fig. 7b) of unheated at-death muscle demonstrated contiguous, irregularly shaped muscle fibers containing well-defined myofibrils and nuclei lying adjacent and contiguous to the fiber membrane. Extracellular stellate cells can also be observed. In comparison, myofibrils of unheated muscle aged at 2°C for 7 days were not as sharply defined or continuously in-register; however, Z lines, shortened I bands and A bands were evident (Fig. 8a). Cross sections of unheated muscle aged at 2°C for 7 days showed that the structural features were relatively unchanged, with the exception that some shrinkage of muscle fibers had occurred (Fig. 8b). Heating 7-day post-mortem muscle to 50°C caused fiber shrinkage and further loss of structural integrity within the myofibril; however, banding patterns remained clear (Fig. 9a). At 50°C, endomysial connective tis-

Fig. 7a—13b—Phase-contrast micrographs of longitudinal (LS) and cross-sectional (CS), at-death (AD) and 7-day postmortem (PM), maturity A, bovine longissimus heated to the designated internal temperature (°C).

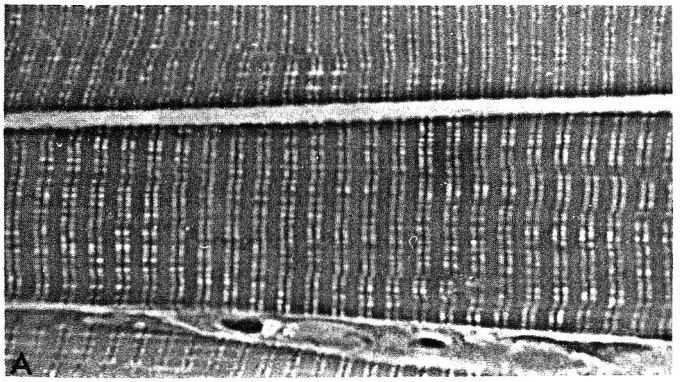

Fig. 7a—the extreme order and regularity of in-register Z lines, I bands and A bands of unheated at-death longissimus (LS) .×1,200.

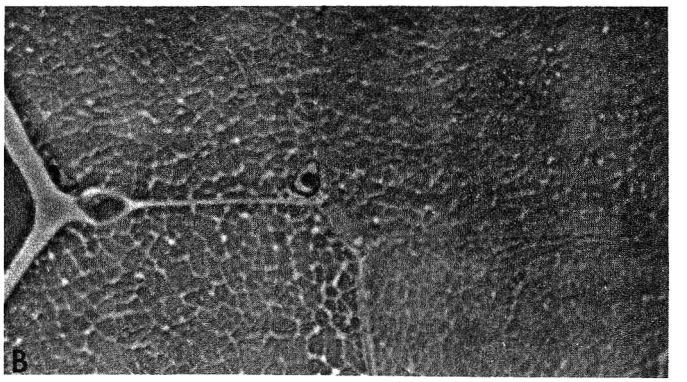

Fig. 7b—Unheated longissimus (AD, CS). ×1,200.

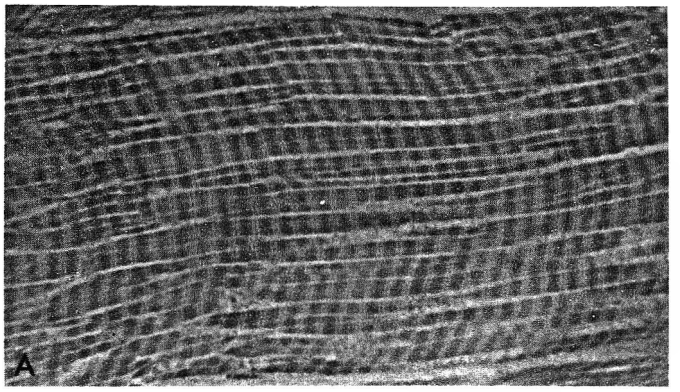

Fig. 8a—Unheated longissimus (PM, LS). ×1,200.

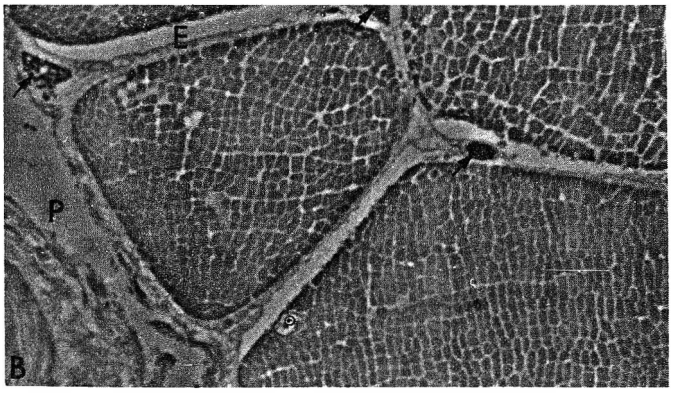

Fig. 8b—Unheated longissimus (PM, CS). Endomysial (E) connective tissue fibers are continuous with perimysial (P) fibers and stellate cells (arrows) are frequently observed in the endomysium. ×1,200.

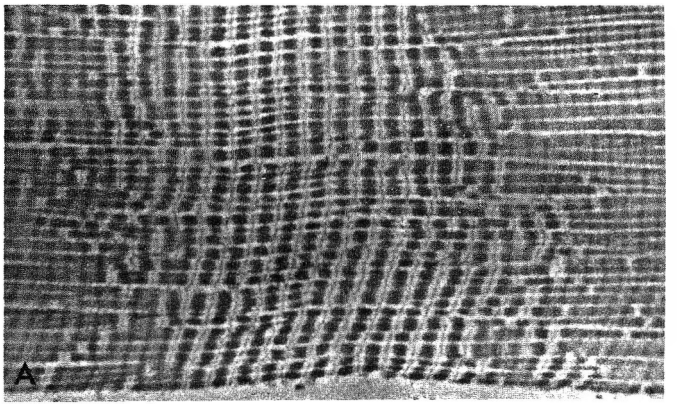

Fig. 9a—Longissimus (PM, LS) heated to 50°C. ×1,200.

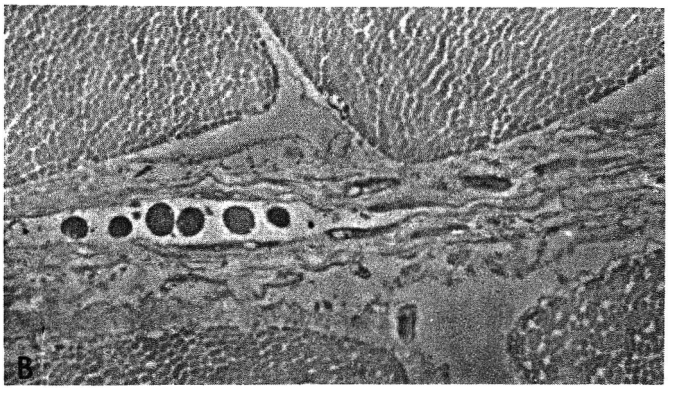

Fig. 9b—Longissimus (PM, CS) heated to 50°C. ×1,200.

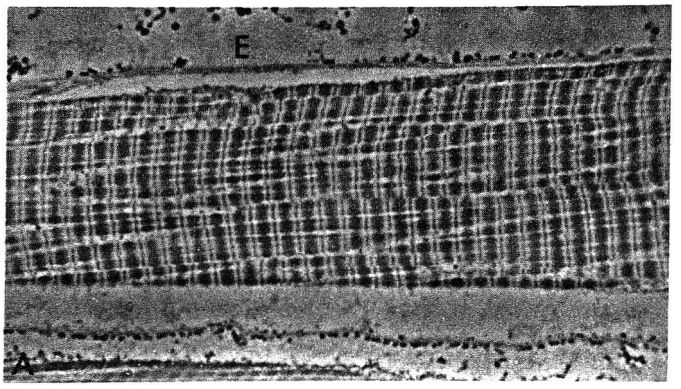

Fig. 10a—Longissimus (PM, LS) heated to 60°C. ×1,200.

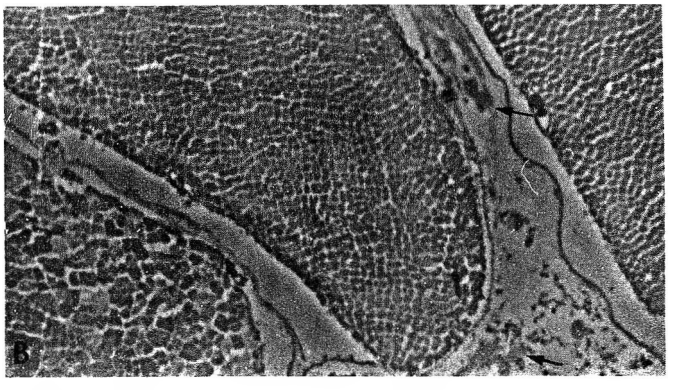

Fig. 10b—Longissimus (PM, CS) heated to 60°C. Note that the perimysial (P) fibers have shrunk. ×1,200.

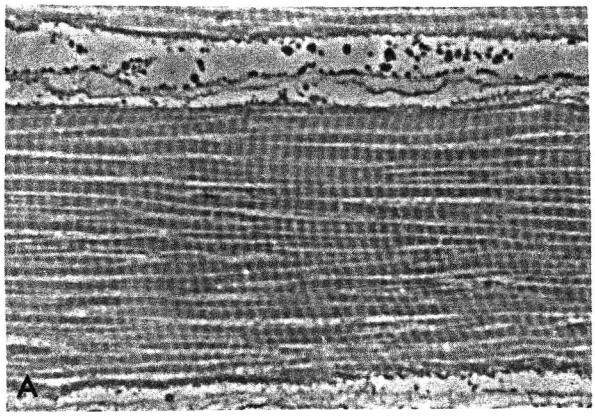

Fig. 11a–Longissimus (PM, LS) heated to 70°C. ×1,200.

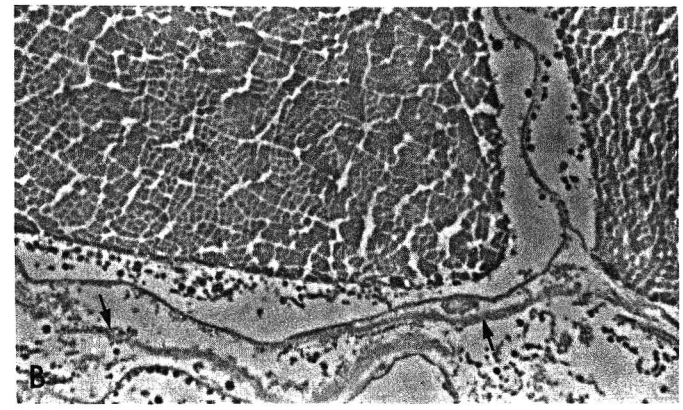

Fig. 11b–Longissimus (PM, CS) heated to 70°C. Note perimysial fiber shrinkage (arrows). ×1,200.

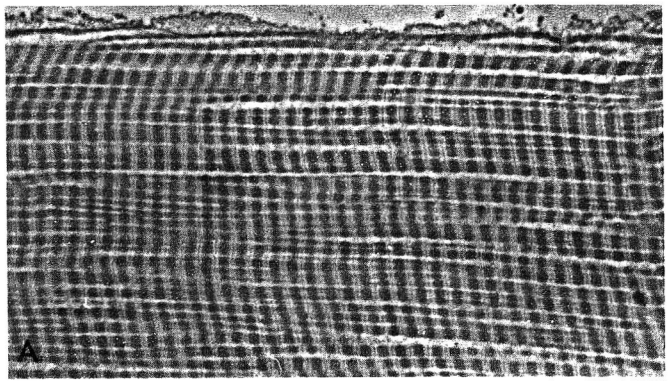

Fig. 12a–Longissimus (PM, LS) heated to 80°C. ×1,200.

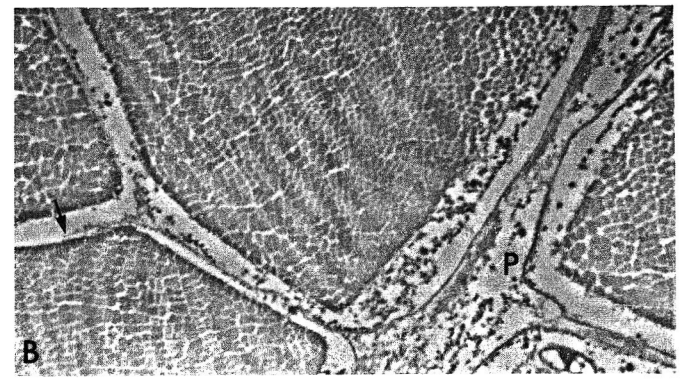

Fig. 12b–Longissimus (PM, CS) heated to 80°C. Endomysial fibers (arrows) are frequently fragmented at the high temperatures.

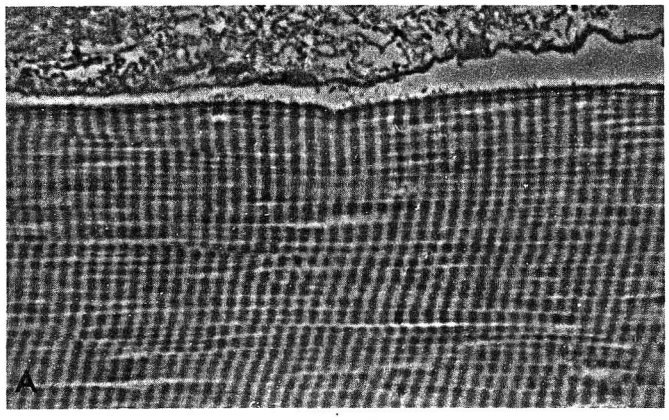

Fig. 13a–Longissimus (PM, LS) heated to 90°C. ×1,200.

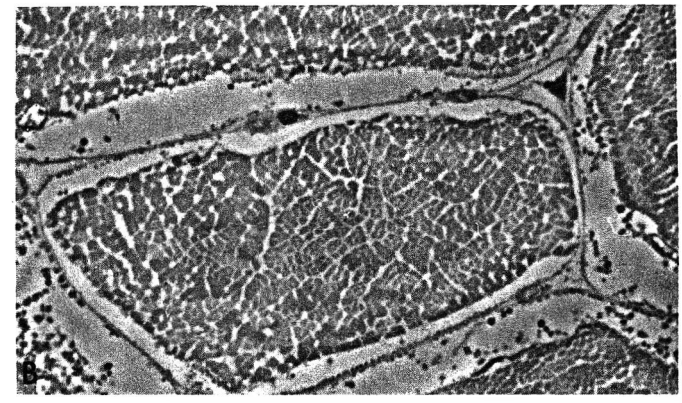

Fig. 13b–Longissimus (PM, CS) heated to 90°C. ×1,200.

sue fibers were compressed and diminished in size, suggesting that some thermal shrinkage of these fibers had occurred. The larger perimysial connective tissue fibers, on the other hand, appeared thermally unaffected at 50°C (Fig. 9b). Temperatures of 60°C caused further muscle fiber shrinkage, although Z lines, I bands and A bands remained intact (Fig. 10a). Also, severe shrinkage, compression and fragmentation of endomysial connective tissue fibers, and initiation of shrinkage of the more aggregated perimysial fibers occurred (Fig. 10b). Temperatures of 70°C or above caused further myofibril degradation; however, the conventional banding patterns of myofibrils remained at all internal temperatures studied (Figs. 11a, 12a, 13a). Cross sections of muscle heated to 70, 80 and 90°C showed that myofibrillar shrinkage, indicated by wider endomysial spaces, was severe at high internal temperature. Endomysial connective tissue shrinkage was maximal at 70°C, but perimysial fiber shrinkage increased progressively with higher internal temperatures (Figs. 11b, 12b, 13b).

These phase-contrast microscopy results indicate that morphological features of muscle cells, such as myofibrils, nuclei and extracellular stellate cells remained relatively intact at all internal temperatures; however, cellular shrinkage due to extrusion of myofluids, and endomysial connective tissue shrinkage began at 50°C. Perimysial connective tissue shrinkage occurred at 70°C and above (80 and 90°C). Thus, the major structural change associated with improved meat tenderness

Figures 14a–20b are electron micrographs of longitudinal (LS) and cross-sectional (CS), at-death (AD) and 7-day postmortem (PM), maturity A, bovine longissimus heated to the designated internal temperatures (°C).

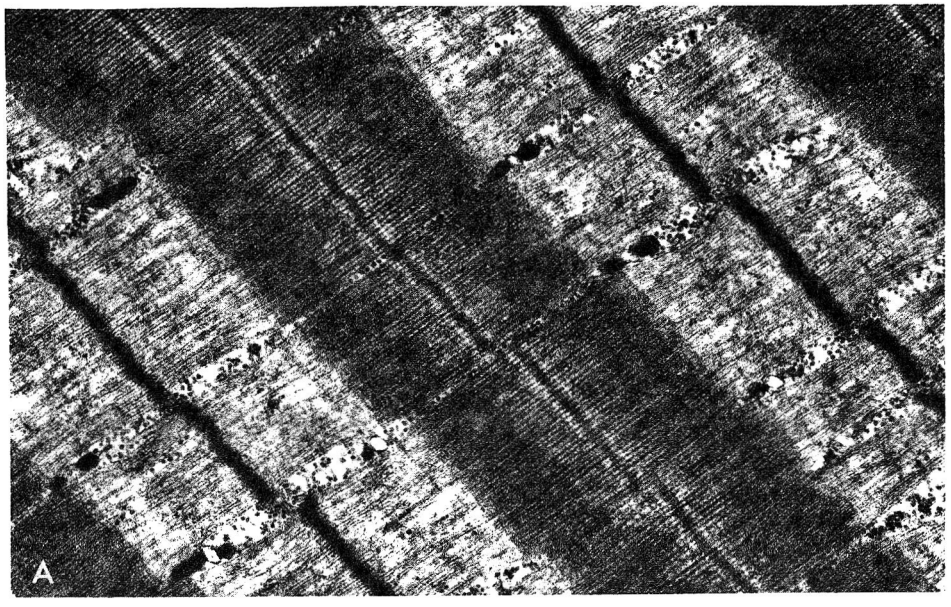

Fig. 14a–Unheated at-death longissimus (LS). ×14,918.

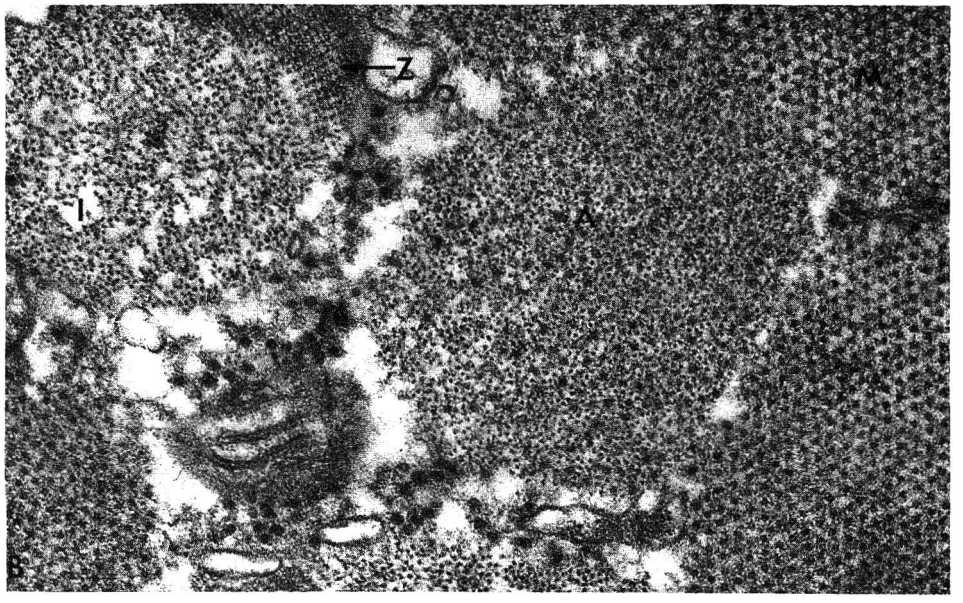

Fig. 14b–Unheated longissimus (AD, CS). Note the innate I-band thin filaments (I), Z line network (Z), A band thick and thin filaments (A) and the M line cross bridges. ×55,650.

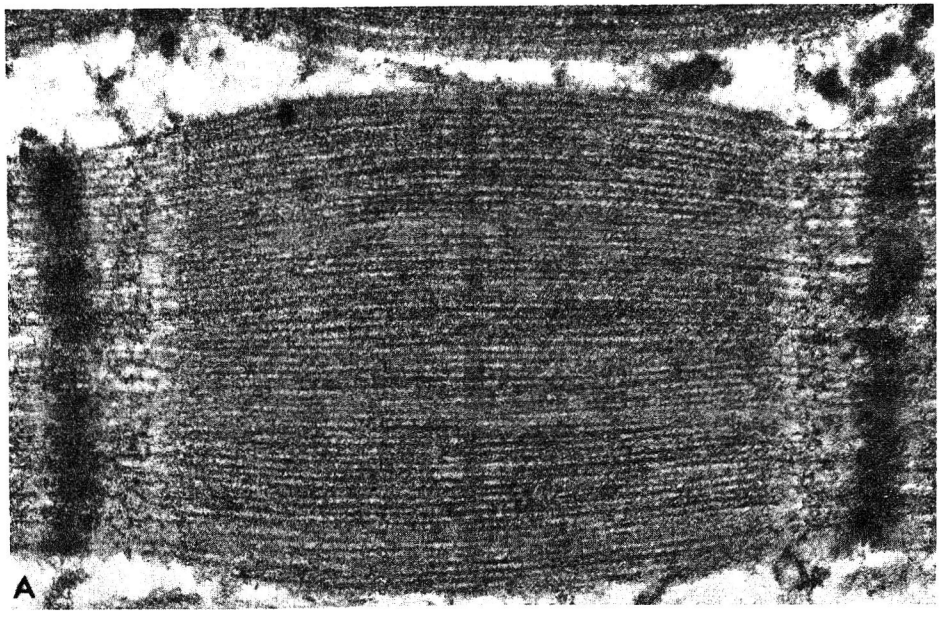

Fig. 15a–Unheated longissimus (PM, LS). ×56,763.

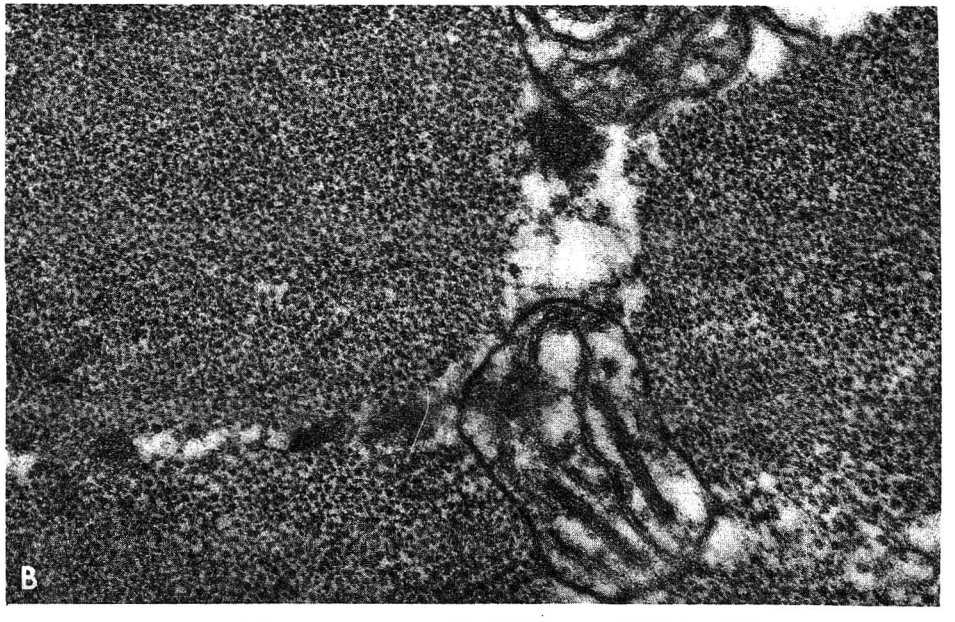

Fig. 15b–Unheated longissimus (PM, CS). ×58,432.

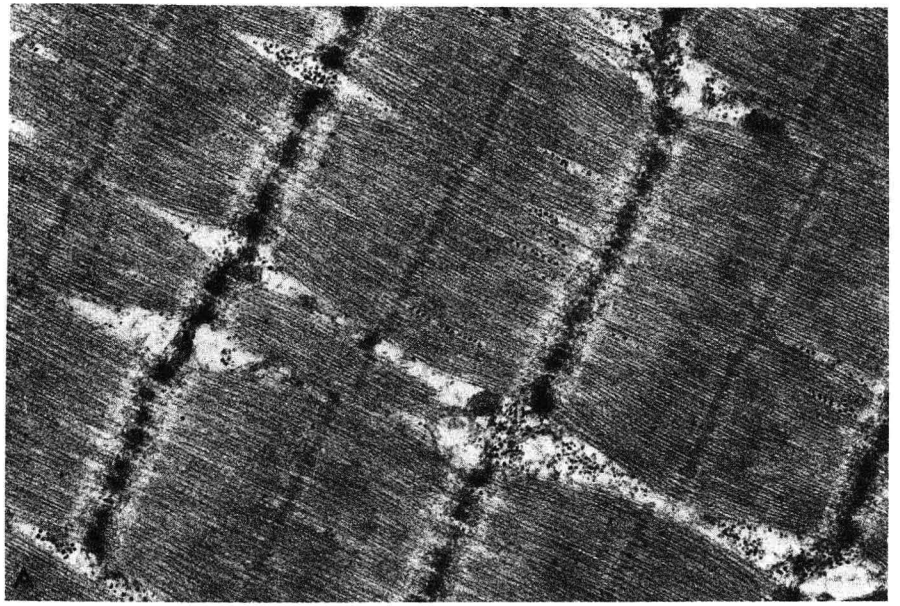

Fig. 16a—Longissimus (PM, LS) heated to 50°C. ×24,616.

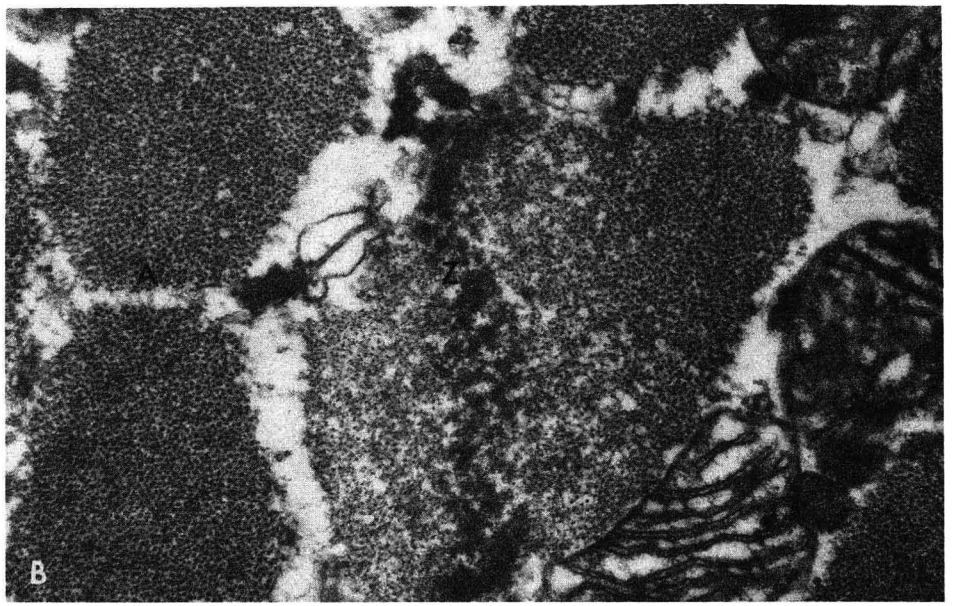

Fig. 16b—Longissimus (PM, CS) heated to 50°C. The Z line (Z) is predominantly diffused, whereas the A band (A) thick filaments are structurally unaffected by heating to 50°C. ×44,578.

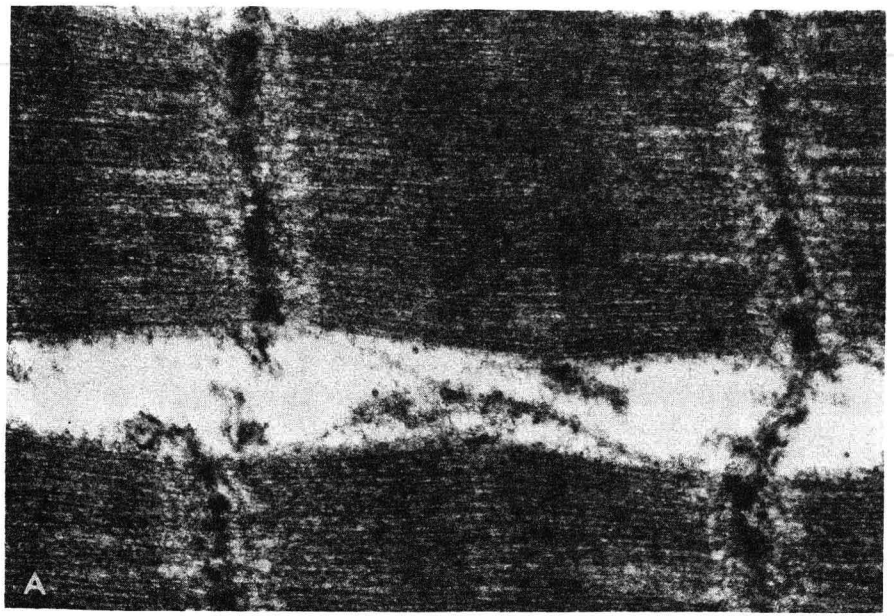

Fig. 17a—Longissimus (PM, LS) heated to 60°C. A band coagulation is initiated and the M line is absent at 60°C. ×46,735.

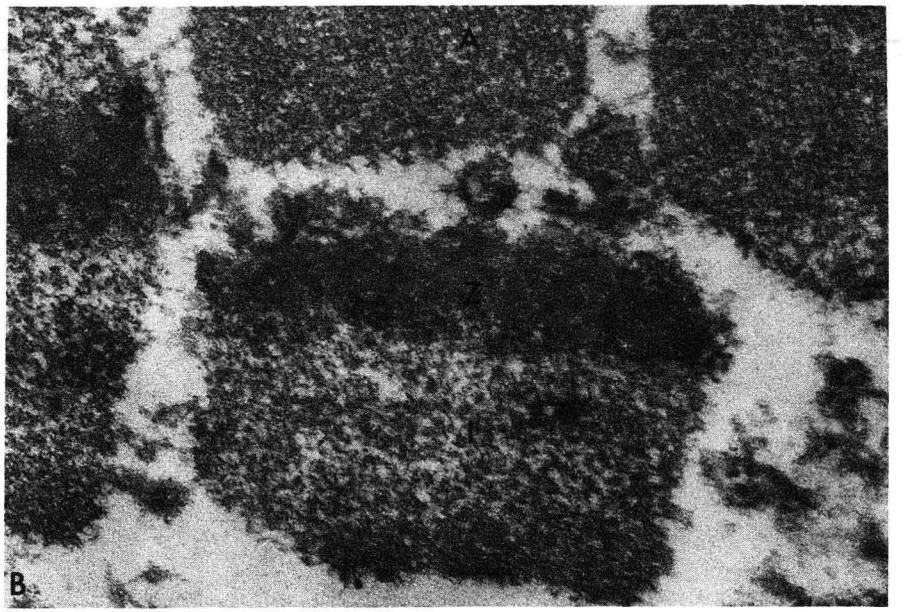

Fig. 17b—Longissimus (PM, CS) heated to 60°C. At 60°C the A band (A) and I band (I) filaments are degraded and the Z line (Z) is diffused. ×60,658.

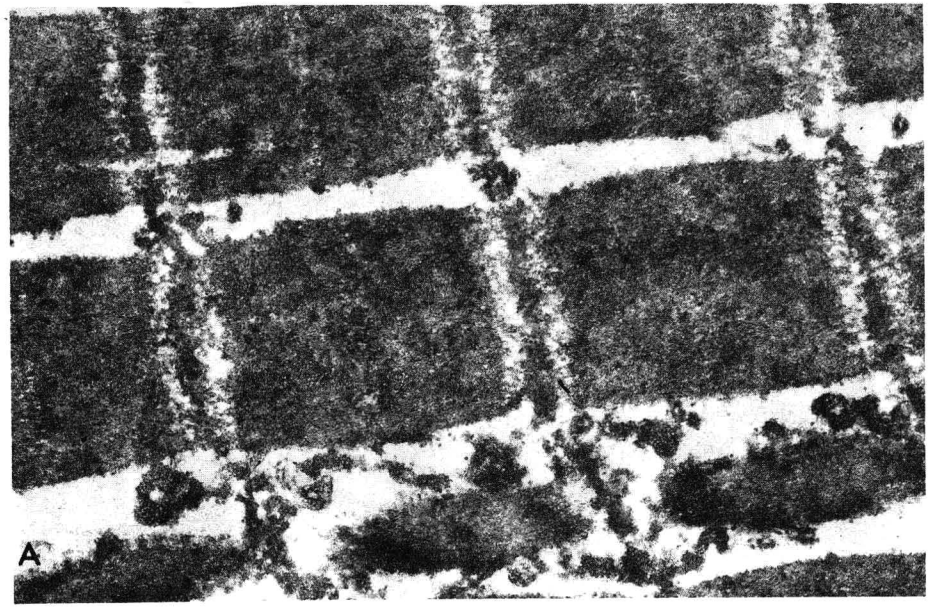

Fig. 18a—Longissimus (PM, LS) heated to 70°C. ×28,173.

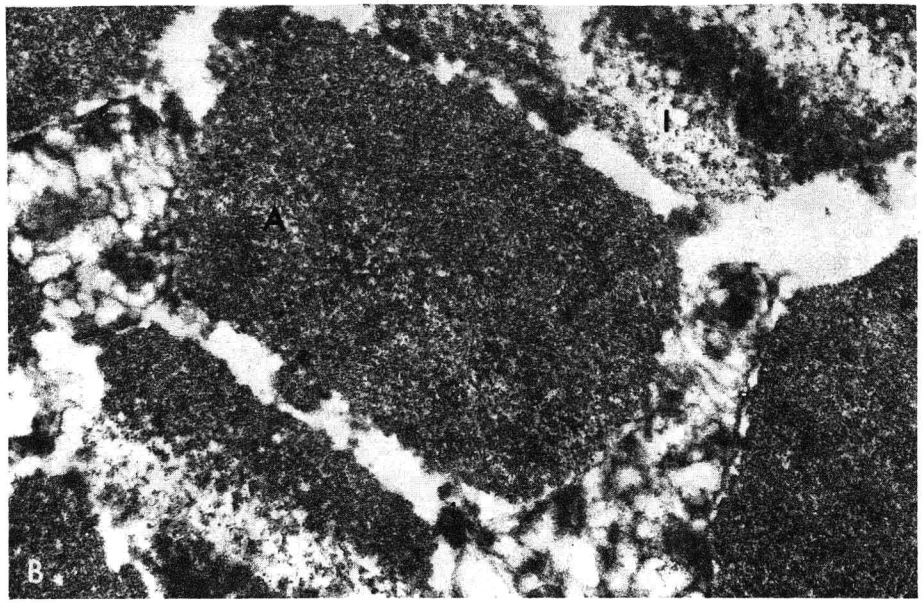

Fig. 18b—Longissimus (PM, CS) heated to 70°C. Note that A band filaments (A) are coagulated, and the I band filaments (I) are disintegrated. ×42,133.

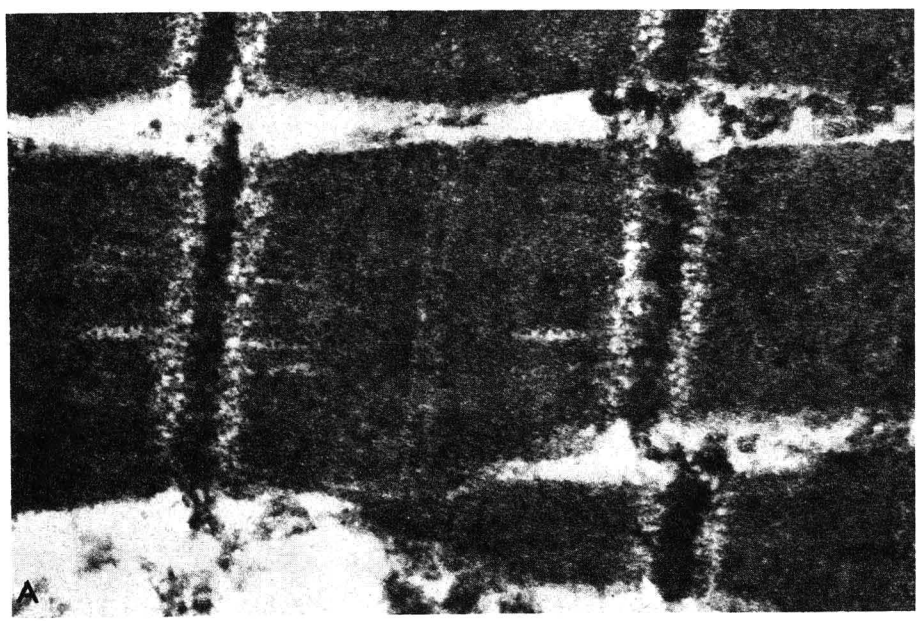

Fig. 19a—Longissimus (PM, LS) heated to 80°C. ×43,859.

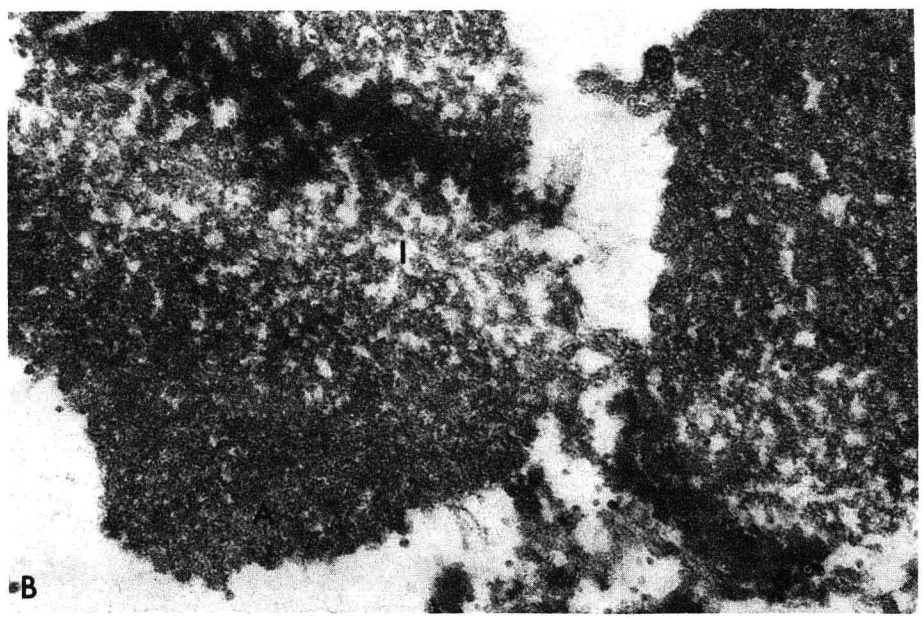

Fig. 19b—Longissimus (PM, CS) heated to 80°C. A band (A) filaments are primarily coagulated and I band (I) filaments are disintegrated. ×55,633.

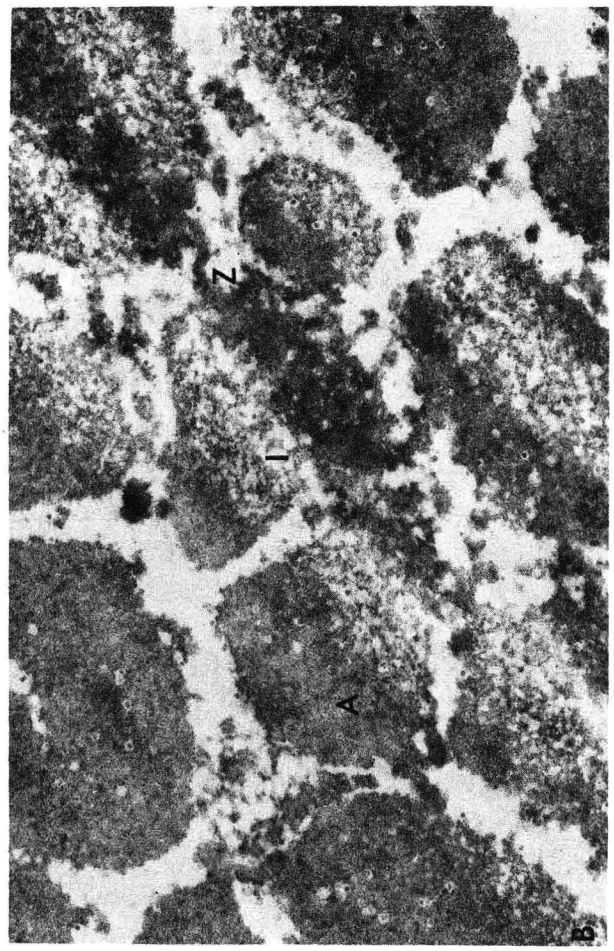

Fig. 20b–Longissimus (PM) (CS) heated to 90°C. A band (A), I band (I) and Z line (Z) regions are structureless. ×30,510.

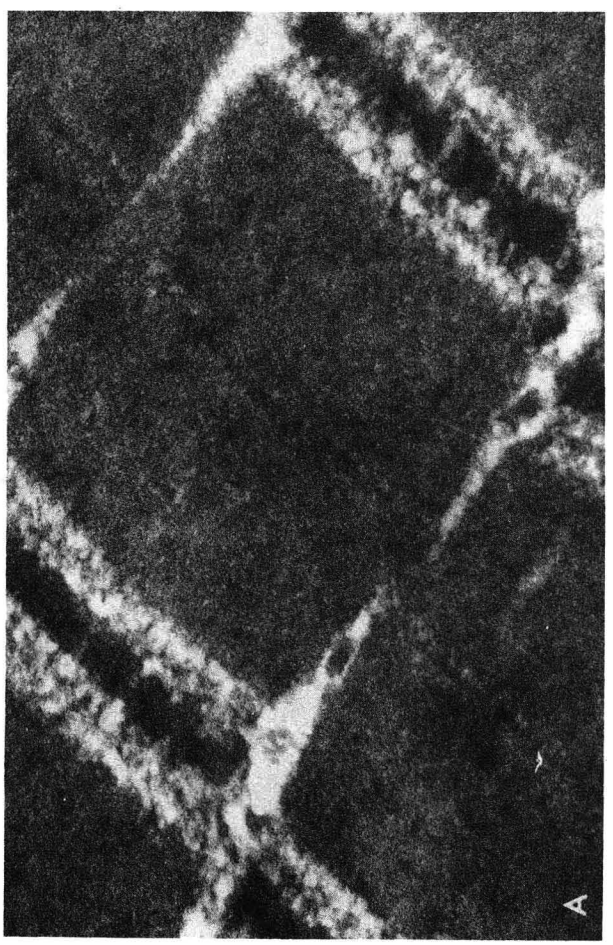

Fig. 20a–Longissimus (PM, LS) heated to 90°C. A band is amorphous, but the banding patterns persist. ×51,383.

observed with phase-contrast is a change in connective tissue and this change is manifested by decreased shear force (Schmidt et al., 1970).

Electron microscopy

Typical banding patterns, thick and thin filaments and other structural features of longitudinal and transverse sections were observed from at-death longissimus (Fig. 14a and 14b). The structural features to be emphasized are wide I bands containing normal Z lines and innate A bands containing H zones and intact M lines. After aging at 2°C for 7 days post-mortem, much of the structural integrity of at-death tissue was lost. Sarcomeres were shorter, as evidenced by shortened I bands and the absence of H zones (Fig. 15a); Z lines appeared diffuse; however, M lines and thick and thin filaments appeared relatively unaffected by aging (Fig. 15 a and b). Heating 7-day postmortem longissimus to 50°C caused further shortening of sarcomeres and extensive degradation of Z line structure; however, M lines and thick and thin filaments remained essentially the same as uncooked post-mortem muscle (Fig. 16 a and b). Increasing the internal temperature to 60°C initiated coagulation of thick filaments, disintegration of thin filaments and loss of M-line structure; however, little further change occurred to Z lines (Fig. 17 a and b). Coagulation of thick filaments may be the reason previous investigators (Aronson, 1966; Hostetler and Landmann, 1968) noted a decrease in the birefringence of muscle proteins at internal temperatures of 60°C and above. Temperatures of 70°C caused marked disintegration of thin filaments and further coagulation of thick filaments (Figs. 18 a and b). At 80°C (Figs. 19 a and b) and 90°C (Figs. 20 a and b) the filamentous structural integrity was almost gone, but the banding patterns remained amorphous.

From the electron microscopy studies, the following conclusions are made: 1) Z-line integrity is lost during postmortem aging; 2) heating muscle causes progressive myofibrillar shrinkage and degradation; 3) heating muscle to 60°C causes major myofibrillar structural alterations including loss of M-line structure, initiation of thin filament disintegration and thick filament coagulation; 4) heating muscle to 70°C and above causes progressive and severe thin filament disintegration and thick filament coagulation and finally 5) the conventional banding patterns of muscle were observed at all internal temperatures.

The composite results of light, phase-contrast and electron microscopy indicate that the effect of increasing internal temperatures on the structural components of muscle is significantly related to tenderness and shear resistance changes in bovine longissimus. Light and phase-contrast micrographs showed that increasing

internal temperatures caused a progressive connective tissue fiber shrinkage and fragmentation. Thus, heating should increase the tenderness of the connective tissue components of meat. But, progressive heating also caused an increase in myofluid extrusion that eventually resulted in a compressed and hardened structure. At an internal temperature of 60°C and above, myofibrillar proteins coagulated and hardened. The tenderness of meat should initially increase to a maximum because of connective tissue fiber fragmentation. With further heating, a decrease or no change in tenderness results because of the hardening and drying effects by fluid loss and myofibrillar protein coagulation. These latter effects would probably offset any favorable tenderness effect of further connective tissue fragmentation by increased heating.

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A. W. KHAN
Division of Biology, National Research Council of Canada, Ottawa, Canada

EFFECT OF TEMPERATURE DURING POST-MORTEM GLYCOLYSIS AND DEPHOSPHORYLATION OF HIGH ENERGY PHOSPHATES ON POULTRY MEAT TENDERNESS

SUMMARY—Tests made on pectoralis major muscles having post-slaughter pH values ranging between 6.1–7.0, indicated that holding poultry meat at 30 and 37°C during the onset of rigor mortis caused toughness. This toughening effect of high temperature appeared to occur when the pH level of the meat dropped from a value of about 6.3 to its ultimate low value and the adenosine triphosphate content dropped below 40% of its initial concentration. Holding temperatures at 10, 15 and 25°C during the onset of rigor mortis, or cooling to 15°C before the pH value dropped to about 6.3 produced more tender meat. After completion of post-mortem glycolysis and dephosphorylation of high energy phosphates, high temperature had no deleterious effect on tenderness. These results indicate that dephosphorylation of adenosine triphosphate at high temperature affects the mode or extent of stiffening of the muscular tissue and prevents tenderization.

INTRODUCTION

THE MOST APPARENT physical change during post-mortem aging of meat is the stiffening that occurs as the meat passes into rigor mortis. This physical change accompanies dephosphorylation of adenosine triphosphate and the breakdown of glycogen. The importance of these changes in determining meat quality has been shown in studies on post-mortem tenderization of poultry (de Fremery and Pool, 1963) and beef (Briskey, 1963; Marsh, 1954). Recent work in this laboratory showed that glycolysis occurring immediately before and during slaughtering and bleeding of poultry caused a low post-slaughter pH as well as toughness (Khan and Nakamura, 1970). The meat temperature is at or near the body temperature of the birds (37–40°C) during slaughtering, bleeding, plucking and evisceration of poultry. Temperatures in this range (37–40°C) have been shown to cause a marked increase in the rates of breakdown of glycogen and high energy phosphates (de Fremery and Pool, 1960; Marsh, 1954), and thus increase the rate of onset of rigor mortis. Although the rapid rate of onset of rigor mortis has been shown to cause toughness (de Fremery and Pool, 1960), the damaging temperature range and technique for minimizing the damaging effects are not known.

This paper reports tenderness changes occurring in poultry meat having pH values immediately after slaughter in the ranges 6.1–6.3 and 6.7–7.0, and subsequently aged at temperatures between 10 and 37°C during the period of post-mortem glycolysis and dephosphorylation of high energy phosphates. The range of aging temperatures suitable for producing tender meat and a technique to minimize toughness in meat of low post-slaughter pH are also described.

Table 1—Effect of holding a temperature during post-mortem glycolysis and dephosphorylation on the tenderness (shear force value) of poultry breast meat. (Values are averages of three birds. Average standard deviation, 0.3. Ultimate pH, 5.7–5.8.)

Post slaughter pH	Sample	Holding temp (°C)	Holding time[a] (hr)	Post-mortem shear force value (Kg) 24 hr	48 hr
	Control	15	7	1.7	1.6
	Test	10	7	1.6	1.5
	Control	15	7	1.7	1.7
	Test	25	7	1.9	1.8
6.7–7.0	Control	15	6	1.7	1.6
	Test	30	5	2.6	2.3
	Control	15	7	1.6	1.4
	Test	37	5	3.4	3.2
	Control	15	2	2.2	1.8
	Test	25	2	2.4	2.0
6.1–6.3	Control	15	2	2.2	2.0
	Test	30	1	3.2	3.2
	Control	15	2	1.8	1.7
	Test	37	1	4.7	4.6

[a]Time required for labile phosphate content of the muscle to decrease to less than 15% of the total soluble phosphate content.

EXPERIMENTAL

PECTORALIS major muscles were obtained from male chickens (Ottawa Meat Control Strain, eviscerated weight 2.0–3.0 Kg) hatched and reared in the laboratory under similar environmental and nutritional conditions. To obtain muscles of high post-slaughter pH (6.7–7.0), well rested birds were restrained in metal funnels during slaughtering and bleeding to minimize voluntary as well as involuntary struggling. To obtain meat of low post-slaughter pH (6.1–6.3), the birds were hung by the legs as in commercial practice and allowed to struggle freely during slaughtering and bleeding. After slaughtering (by cutting the jugular vein and carotenoid arteries) and bleeding, both pectoralis major muscles were excised immediately. Each muscle was provided with a thermocouple to monitor the temperature during aging and then vacuum packaged in a Cry-o-vac bag. To obviate the effect of bird-to-bird variability, one of the two muscles was used as a control sample, and the other as a test sample. Control samples were cooled rapidly to 15°C (cooling time 10–12 min) by immersion in a glycol bath at 5°C and then aged in crushed ice. Test samples were kept at the desired temperature (10–37°C) until the completion of the post-mortem glycolysis and dephosphorylation, and then aged in crushed ice.

The rate of dephosphorylation of high energy phosphates was determined by measuring the total soluble phosphate and phosphate ester content of the muscle tissue by differential acid hydrolysis according to Griswold et al. (1951). The adenosine triphosphate (ATP) content was determined spectrophotometrically using a mixture of glyceroaldehyde phosphate dehydrogenase and phosphoglyceric phosphokinase (Kornberg, 1955). The time taken after slaughter for the labile phosphate content to drop to less than 15% of the total soluble phosphate is reported as the time of onset of rigor mortis.

The rate of glycolysis was determined by measuring pH at suitable intervals beginning soon after the cessation of bleeding. The pH measurements were made in samples (1g) homogenized with neutralized iodoacetate solution (5 ml, 5 mM). In some samples lactic acid determinations were also made using lactic dehydrogenase (Olson, 1962).

Tenderness of the cooked meat was determined by shear force measurements made with a texture press system (Food Technology Corporation, Reston, Virginia, U.S.A.) equipped with a meat shear cell. Samples from the control and test materials were taken from a similar location in each muscle and were tested after 24 hr and 48 hr of aging. To reduce uncontrolled distortion of the excised muscle during

Table 2–Effect of pH level during holding at 37°C on poultry meat tenderness.[a]

State of muscle	pH[b] Before holding at 37°C	pH[b] After holding at 37°C	Post-mortem shear force value (Kg)[b] 24 hr	48 hr
During rigor	6.7	6.5	2.3	1.8
During rigor	6.6	6.4	2.0	1.9
During rigor	6.5	6.3	2.5	2.1
During rigor	6.2	5.9	5.0	4.4
After the completion of rigor	5.8	5.8	1.8	1.6

[a]Samples were cooled rapidly to 15°C and held at that temperature until the desired pH was reached, then raised to 37°C for 2 hr and subsequently cooled to 0°C for aging.

[b]Values are averages for four birds; average standard deviation 0.3.

Table 3–Effect of fast and slow cooling on the tenderness of breast meat having post-slaughter pH value of 6.3–6.4. (Value are averages of three birds. Average standard deviation 0.3.)

Cooling time to 15°C (min)	pH After cooling	pH Ultimate	Post-mortem shear force value (Kg) 24 hr	48 hr
10	6.2–6.3	5.8	3.3	3.0
120	5.8–6.0	5.8	5.4	4.8

cooking, the samples were clamped in a special mold designed according to de Fremery and Pool (1960) and cooked to an internal temperature of 82–85°C as described by Khan and Lentz (1965). Strips 1 cm^2 in cross-section were cut and 10–16 determinations were made on each sample.

RESULTS & DISCUSSION

HOLDING SAMPLES at 30 or 37°C during post-mortem glycolysis and dephosphorylation of high energy phosphates caused toughness, while holding at 10, 15 or 25°C did not affect tenderness (Table 1). At the high temperature, loss of tenderness was greater for meat of low post-slaughter pH than for meat of high post-slaughter pH in spite of a 4 hr difference in holding time. These findings are in agreement with those reported by Joseph (1968), indicating that low pH while the meat is at body temperature causes toughness. Since the shear force values of the control samples were always lower than that of the test samples, no attempts were made to find the significance level of the results.

The damaging effect of high temperature on tenderness occurred only over that period of rigor mortis during which the pH of the meat dropped from 6.2–6.3 to a value of about 5.9 (Table 2). After reduction in pH ceased and the onset of rigor mortis was complete, high temperature (37°C) had no deleterious effect on tenderness.

Lowering the temperature from 37–40° to 15°C before the pH had dropped to a value of about 6.2–6.3 appeared to minimize the loss of tenderness (Table 3). Meat from birds slaughtered under commercial conditions usually has a post-slaughter pH in the range of 6.1–6.3, and the onset of rigor mortis occurs before the carcasses are cooled. Since in meat having a post-slaughter pH of 6.8–7.0, the onset of rigor mortis occurs about 5–7 hr post-mortem, cooling before pH drops to 6.2–6.3 could easily be achieved. This could have practical significance since meat of high post-slaughter pH can be obtained by minimizing physical activity in birds immediately before slaughter and by minimizing struggle during slaughtering and bleeding (Khan and Nakamura, 1970).

Typical curves obtained for glycolysis and for dephosphorylation of ATP in meat of high post-slaughter pH are shown in Figure 1. A decrease in pH to a value of 6.2–6.3 coincided with the dephosphorylation-phase during which the ATP level of the muscle tissue fell to below 40–50% of its initial level. During this period, the shear force value of meat is generally low and remains more or less constant. The rapid stiffening of the muscular tissue does not begin until the ATP content of the muscle has declined to about 30% of its initial concentration. Thereafter the ATP content of the meat decreases to a minimum value and toughening occurs (de Fremery and Pool, 1960). It appears therefore that a high temperature during the final phase of dephosphorylation of ATP and glycogen breakdown affects the mode or extent of stiffening of the muscular tissue and tenderness.

In muscle the phenomena of post-mortem glycolysis, dephosphorylation of ATP and stiffening are closely related. Post-mortem glycolysis causes a net gain of three molecules of adenosine triphosphate for each 6-carbon unit of the glycogen that is converted to lactic acid and this helps in maintaining the ATP level in the muscle. Inhibition of glycolysis by low pH (Newbold and Scopes, 1967) and dephosphorylation of ATP lead to muscular stiffening (Perry, 1956). The effects of post-mortem dephosphorylation of ATP, and the mode or extent of stiffening on tenderness are not currently understood, but the present study indicates that the harmful effects of these changes can be minimized by ensuring that the final phases of dephosphorylation and glycolysis occur at temperatures below 25°C.

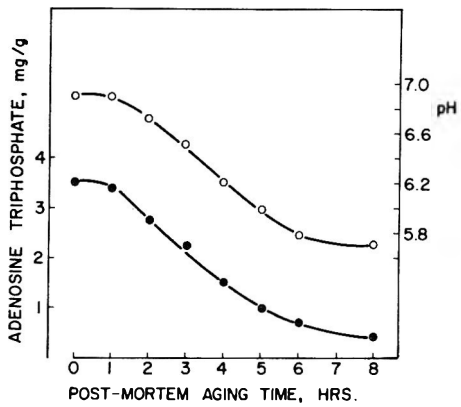

Fig. 1–Changes in the adenosine triphosphate content (•) and pH (o) of breast muscle during 8 hr post-mortem aging at 15°C.

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D. R. LANDES,[1] L. E. DAWSON and J. F. PRICE
Department of Food Science, Michigan State University, East Lansing, Mich. 48823

PROTEIN EXTRACTABILITY OF TURKEY BREAST MUSCLE EXHIBITING DIFFERENT RATES OF POST-MORTEM GLYCOLYSIS

SUMMARY—Changes in pH and in protein extractability were determined in turkey breast muscles from anesthetized and nonanesthetized birds from 0–72 hr post-mortem. Shear values were measured on cooked meat after 72 hr post-mortem aging. Total extractable-, total soluble fibrillar protein-, soluble actomyosin-, sarcoplasmic protein-, nonprotein-, and unextracted alkali soluble protein-nitrogen values were determined. These values remained fairly constant during the first hour post-mortem in muscles from anesthetized birds, but began to change immediately in muscles from control birds. Total extractable nitrogen, total soluble fibrillar protein nitrogen and soluble actomyosin nitrogen extracted from muscles of control birds increased during post-mortem aging. These fractions in muscles from other species either remain the same during rigor development or decrease. The muscles of anesthetized birds were more tender (measured by shear value) than those from the control birds.

INTRODUCTION

THE STUDY OF MEAT tenderness covers the transition of muscle from the living state to a food material. During this period extensive biochemical and physical changes occur. deFremery (1963, 1966a, 1966b) reported that many biochemical changes take place in chicken muscle when subjected to different processing conditions. Khan (1962) developed a muscle protein fractionation procedure, and he and his associates studied changes in extractability of proteins of chicken muscles during the aging and storage process (Khan, 1968; Khan et al., 1963; Khan and van den Berg, 1964a, 1964b; Khan and Lentz, 1965). From these and other studies it appears that tenderness is closely related to biochemical and physical changes that occur during the conversion of chicken muscle to meat.

This investigation was initiated to determine some of the biochemical and physical changes in breast muscle of turkeys treated to produce slow and fast rates of post-mortem glycolysis. The relationship of these changes with ultimate tenderness was also investigated.

MATERIALS & METHODS

Experimental animals

Twenty mature, Broad Breasted White, female turkeys were obtained from the Michigan State University Poultry Farm. The birds were processed in five groups of four birds. Two birds in each group were given injections of sodium pentobarbital (20 mg/kg body weight) in the brachial vein of the wing just before slaughter. The other two birds of each group received no pre-slaughter treatment. The birds were slaughtered by bleeding, scalded for 55 sec at 57°C in a Rotomatic scalder and the feathers were removed by an automatic rubber fingered picking machine. After evisceration, the birds were placed in Cryovac bags and held in slush ice throughout a 72 hr sampling period. After this sampling period the bags were evacuated and the birds were frozen at −23°C for storage until cooking and tenderness evaluation.

Muscle sampling procedure

Approximately 10g samples were taken from the pectoralis major muscle of each bird at 0, ¼, ½, 1, 3, 6, 12, 24, 48 and 72 hr post-mortem. The samples were taken immediately to a 2°C cold room for extraction and fractionation of the muscle proteins and determination of muscle pH. The zero time samples were taken as quickly as possible after bleeding.

Muscle pH determination

Within 3 min from the time each sample was taken, approximately 3g of muscle tissue was homogenized for 30 sec in 75 ml of 0.001M sodium iodoacetate solution in a Waring Blendor. The pH of each homogenate was determined using a Corning pH meter, Model 10.

Protein extraction and fractionation

Within 2.5 min from the time each sample was taken 5 ± 0.29g of muscle tissue were weighed and placed in a semi-micro Waring Blendor jar that contained enough KCl-phosphate buffer (0.8M KCl, 0.01M KF, Γ/2 1.0, pH 7.5) to fill the constricted portion of the jar. A 50 ml volumetric flask was held on top of the constricted portion of the jar to prevent the formation of a vortex as the sample was blended for 30 sec at high speed. The mixture was transferred to a 100 ml, graduated, conical centrifuge tube and the jar was washed with KCl-phosphate buffer solution. The washings were added to the centrifuge tube until a volume of 100 ml was reached. The sample was then extracted and fractionated as described in Figure 1.

Nitrogen determination

Duplicate nitrogen determinations were made in all of the fractions labeled in Figure 1 by the micro-Kjeldahl method described by the American Instrument Company (1961). Titration of the liberated nitrogen was accomplished using a Spectro-Electro Model SE automatic titrator (E. H. Sargent Co.).

Estimation of protein fractions

The following nitrogen-containing fractions were estimated from data collected using the extraction, fractionation and analysis procedures described above, and as shown in Figure 1.

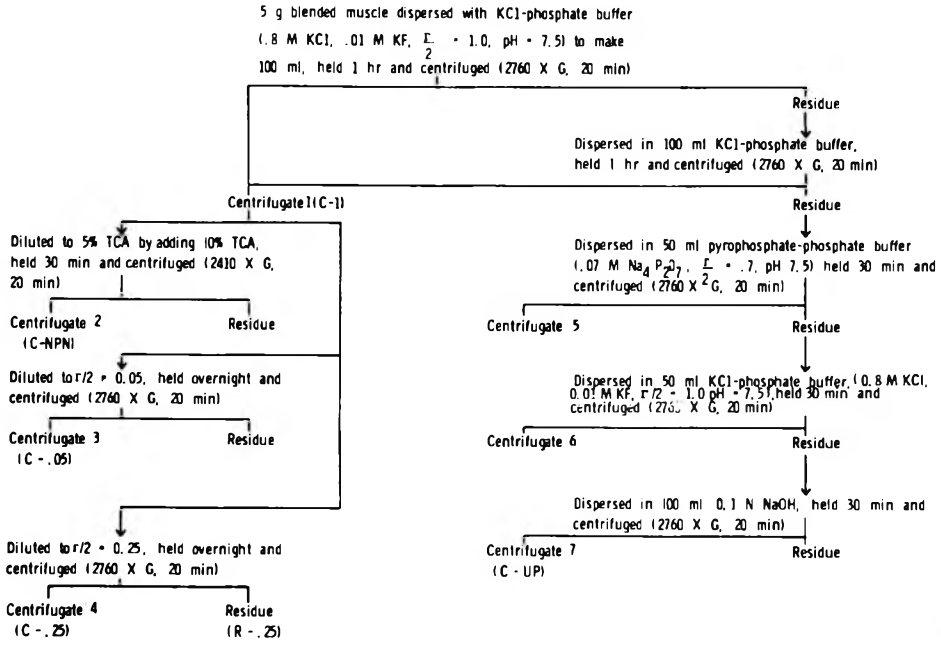

Fig. 1—Outline of the fractionation procedure for turkey muscle proteins.

[1] Present address: Department of Food Science, University of Georgia, Experiment, Ga. 30212.

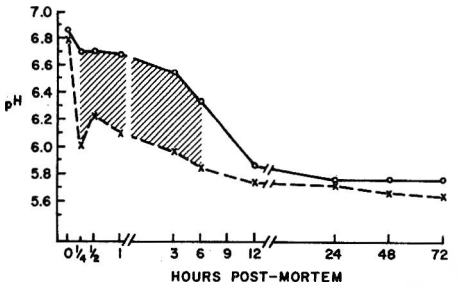

Fig. 2–Changes in pH of turkey breast muscle during 72 hr post-mortem. Solid line represents average pH for 10 anesthetized turkeys; dashed line for 10 control turkeys. Hatched area shows significant difference between control and treatment.

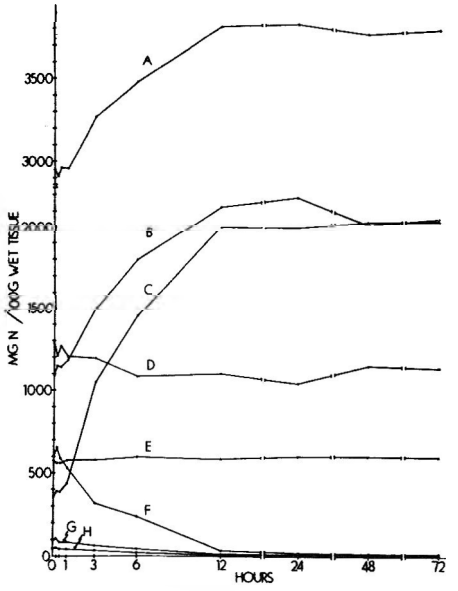

Fig. 3–Extractability of protein fractions from turkey breast muscle, 0–72 hr post-mortem, of birds given ante-mortem injections of pentobarbital. (See text for identification of curves.)

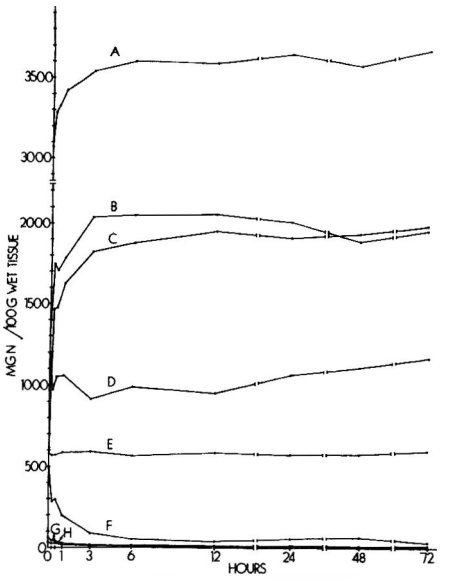

Fig. 4–Extractability of protein fractions from turkey breast muscle, 0–72 hr post-mortem, of birds with no ante-mortem treatment. (See text for identification of curves.)

A. Total extractable nitrogen (C-1)

B. Total soluble fibrillar protein nitrogen (C–1 minus C–.05)

C. Soluble actomyosin nitrogen (R–.25)

D. Sarcoplasmic protein nitrogen (C–.05 minus C–NPN)

E. Non-protein nitrogen (C–NPN)

F. Unextracted, alkali soluble protein nitrogen (C–UP)

G.

H. Centrifugates 5 and 6 (nitrogen fractions in original residue solubilized by pyrophosphate and salt solutions)

Tenderness evaluation

The birds were taken from frozen storage, halved and allowed to thaw overnight at 2°C. One-half of each bird was placed in a stainless steel pan and covered with aluminum foil. Thermocouples were inserted through the foil into the center of the thickest part of the breast muscle. The birds were cooked to an internal temperature of 85°C in a circulating air oven set at 149°C.

The cooked birds were cooled overnight at 2°C before sampling for tenderness. Samples were prepared for shear analyses using the method described by Dodge and Stadelman (1960) except for sample size. An Allo-Kramer shear press equipped with an electronic recorder was used to shear the samples. The shear press was equipped with a 1360.8 kg ring and a down-stroke of 15 sec was used.

RESULTS

Post-mortem pH changes

The changes in pH of turkey breast muscle are shown in Figure 2. The pH of the muscle from control birds dropped rapidly during the first 15 min post-mortem, then declined slowly through 72 hr except for a slight increase from ¼–½ hr. The pH of muscle from anesthetized birds behaved quite differently. It dropped very little through the first 6 hr post-mortem, and was significantly higher than the pH of muscle from control birds from ¼–6 hr post-mortem.

The pH of breast muscle of control birds 1 hr post-mortem (6.22) was lower than pH of chicken breast muscle at 1 hr post-mortem (6.8) as reported by deFremery (1963); however, pH of chicken breast muscle at 6 hr post-mortem (5.95) was comparable in both studies. In contrast, the rapid decline in pH of muscle in control birds during the first ¼ hr is comparable to the pH decline observed in the muscle of broiler chickens processed in a commercial processing plant as observed by Shrimpton (1960). No reports were found in the literature dealing with rate of pH decline in birds anesthetized before slaughter. However, the pH decline observed in the anesthetized birds indicated that the rate of post-mortem glycolysis was decreased when compared with the control birds. This is consistent with the results reported by deFremery (1966b).

Post-mortem changes in protein extractability

Changes in extractability of the eight protein fractions from turkey breast muscle during post-mortem aging are shown in Figure 3 for control birds and Figure 4 for the anesthetized birds. Protein extractability changes were more rapid in muscles from control birds. Fraction E (nonprotein nitrogen) remained constant throughout the aging period in muscle from both groups of birds, and was not significantly different between groups. Sarcoplasmic protein nitrogen, fraction D, varied some through 6 hr post-mortem and was significantly greater in muscles from anesthetized birds than in muscles from control birds. Changes during aging were not significant.

Fractions A, B and C, total extractable nitrogen, total soluble fibrillar protein nitrogen and soluble actomyosin nitrogen increased rapidly in muscles from control birds then leveled off at about 3 hr post-mortem. In muscles from anesthetized birds, these fractions changed little during the first hour, increased gradually reaching a peak about 12 hr post-mortem, and then leveled off.

Values that were observed in extractability of nonprotein nitrogen and sarcoplasmic protein nitrogen were in general agreement with previous results reported. Khan and van den Berg (1964b) for example, found little change in the nonprotein nitrogen and sarcoplasmic protein nitrogen in chicken breast muscle over a 2-day storage period or in chicken leg muscle over a 6-day storage period. In contrast, Maier and Fischer (1966) found that extractability of water soluble protein from chicken breast muscle was less at 24 hr than at 30 min post-mortem.

Aberle and Merkel (1966) working with beef found that soluble nonprotein nitrogen increased significantly during post-mortem aging of the longissimus dorsi and semitendinosus muscles, and the sarcoplasmic protein fractions remained constant in the longissimus dorsi muscle but decreased in the semitendinosus muscle. On the other hand, Kronman and Winterbottom (1960) and Goll et al. (1964) found that extractability of the sarcoplasmic fraction from beef muscle decreased with aging.

The continuous increase in total extractable nitrogen, total soluble fibrillar protein nitrogen and soluble actomyosin nitrogen from the muscle of the control

birds during early post-mortem periods appeared to be in general disagreement with previous findings in other species. Khan and van den Berg (1964b), Khan (1968) and Sayre (1968) working with chickens, Davey and Gilbert (1968) and Chaudry et al. (1969) working with beef and rabbit and Sayre and Briskey (1963) working with pigs found that myofibrillar protein extractability from muscle remained fairly constant or decreased during onset of rigor. Extractability of the myofibrillar protein fractions from the anesthetized birds is in general agreement with the findings from other species. In contrast, Maxon and Marion (1969) have also reported that extractability of the myofibrillar fraction of turkey breast muscle increased steadily up to 48 hr post-mortem. An explanation for this species difference was not evident from the results of this study.

Maximum levels of extractability of total extractable nitrogen, total soluble fibrillar protein nitrogen and soluble actomyosin nitrogen from muscle were greater in the anesthetized birds although initial levels were lower than the extractability levels for these fractions in the control birds. This may be related to the suggestion of Bendall and Wismer-Pederson (1962) that sarcoplasmic proteins may be denatured and precipitated on the myofibrillar proteins thus decreasing the extractability of these fractions. This could have occurred in the muscles used in this study to a small extent, with a smaller decrease in protein extractability from muscle in the anesthetized birds because of a slower pH decline.

The residue from the original KCl-phosphate buffer extraction was reextracted using the procedures shown in Figure 1. This was done to further examine this residue for other nitrogen fractions or incomplete initial fractionation. The three fractions obtained were designated as centrifugate 5 from pyrophosphate-phosphate buffer extraction, centrifugate 6 from KCl phosphate buffer extraction of the residue from previous step, and remaining centrifugate 7 which followed NaOH extraction. Further identification of the fractions was not made.

According to Hasselbach and Schneider (1951) a buffer containing pyrophosphate will dissociate actomyosin into actin and myosin. Assuming that some myofibrillar protein may have remained in the original residue, this procedure should result in a breakdown into products identified here as centrifugates 5 and 6. Levels of extractability of these fractions from muscles were fairly constant during the first hour post-mortem in the anesthetized birds, then they began to decline similar to the declining level of extractability in muscle of the control birds after zero time.

Tenderness evaluation

The tenderness level of all birds was estimated by shear values and it was found that the anesthetized birds were significantly more tender than the control birds ($P \leqslant .01$). The mean shear force for ten anesthetized birds was 6.93 kg/g of cooked muscle, and for ten control birds was 15.34 kg.

This is in agreement with the work of deFremery (1965) who found that chickens anesthetized with pentobarbital prior to slaughter were significantly more tender (measured by shear value) than birds that were allowed to struggle freely during slaughter.

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ROBERT T-I MA, P. B. ADDIS and E. ALLEN
Departments of Food Science and Industries and Animal Science, University of Minnesota, St. Paul, Minnesota 55101

RESPONSE TO ELECTRICAL STIMULATION AND POST-MORTEM CHANGES IN TURKEY PECTORALIS MAJOR MUSCLE

SUMMARY—Muscle samples were obtained from 20 turkey hens 5 min postexsanguination for the determination of sarcomere length, response to electrical stimulation, and time-course of rigor mortis. Additional samples were obtained from the opposite (undisturbed) pectoralis major muscle at 1, 2, 6 and 24 hr post-mortem for the determination of sarcomere length. Birds were divided into three groups based on the time to complete rigor mortis: (1) slow, > 125 min; (2) fast, < 50 min; and (3) intermediate, 50–125 min. Birds that displayed a long time course of rigor mortis had a low threshold of response (9v) and a long duration of response (106 sec) to electrical stimulation. Conversely, muscles which become inextensible within 50 min displayed a high threshold (123v) and low duration (8 sec). Muscles with intermediate rates of rigor had intermediate threshold (58v) and duration (50 sec). Increase in sarcomere length occurred within 1 hr post-mortem in the fast-rigor group, 2 hr in the intermediate-rigor group and 6 hr in slow-rigor birds. A similar experiment conducted on 24 toms indicated that postexsanguination struggling resulted in a decreased time course of rigor, elevated threshold and shortened duration of response to electrical stimulation. Strugglers displayed a more rapid ($P < .01$) post-mortem increase in sarcomere length than non-strugglers. The results suggested that in some presently-used commercial turkey processes, some of the carcasses are frozen with muscles in a pre-rigor, short-sarcomere state.

INTRODUCTION

IN RECENT YEARS there has occurred a diversification of the post-mortem chilling-aging treatments of turkey carcasses in commercial practice. Some processing installations now freeze carcasses within 1 hr post-mortem. This represents a distinct change from the conventional method of aging the warm, freshly eviscerated carcass in ice-water for 6–12 hr followed by freezing. The latter method is still used in numerous processing plants, however.

Several reports have been published which suggest that either of the above processes may have detrimental effects on muscle properties. Luyet (1966) illustrated the drastic effects that pre-rigor freezing of muscle has when it is subsequently thawed and allowed to shorten—a process described as "thaw rigor." Locker and Hagyard (1963) established that exposure of pre-rigor bovine muscle to near-freezing temperatures stimulates a greater than normal degree of shortening. Marsh and Leet (1966) demonstrated that this "cold shortening" effect was closely related to shear values of the muscle. More recently, Smith et al. (1969) demonstrated that gross shortening of chicken and turkey muscle was significantly greater at 0°C than in the 12–18° range. Sarcomere length changes corresponded closely to changes in gross length.

These findings prompted Welbourn et al. (1968) to investigate the effects of different post-mortem chilling procedures on sarcomere length and shear values of the turkey pectoralis major and biceps femoris muscles. They reported that 0°C increased shear significantly in the biceps femoris muscle when compared to milder temperature treatments. However, sarcomere length did not appear to be related to shear values or chill treatment—a finding that implies that cold shortening is not a cause of toughening in turkey meat. However, Welbourn et al. (1968) did not monitor the post-mortem changes in muscle. Instead, they determined sarcomere length and shear values at only one time (ca 3 hr) post-mortem.

The post-mortem decrease in sarcomere length and the development of rigor mortis have long been considered to be equivalent. Bendall and Davey (1957) described rigor mortis as the shortening of the sarcomere and the irreversible combination of actin and myosin. More recently, however, Takahashi et al. (1967) reported that post-mortem sarcomere shortening can be reversible in muscles which are intact in the carcass. If pre-rigor muscle is excised, however, sarcomere shortening is irreversible.

The present study was designed to identify the specific patterns of post-mortem changes in turkey pectoralis major and investigate the possible relationship of these changes to electrical stimulation of muscle. In addition, the effects of postexsanguination physical activity (struggling) on these parameters were determined.

EXPERIMENTAL

THE STUDY WAS conducted in two parts utilizing 20 hens (Experiment 1) and 24 toms (Experiment 2). Experiment 1 proceeded as follows: hens were restrained by hand (permitting a moderate and variable amount of struggling), sacrificed by exsanguination, and, with no further processing, transferred to a 2°C cold room at 1 hr post-mortem. Experiment 2 was conducted in the same manner except that half the birds were permitted to struggle freely after exsanguination while the other half were restrained in a cone which greatly reduced their physical activity. Any bird in the unrestrained group that failed to struggle continuously until death was not used in the experiment. It should be explicitly stated that electrical stunning, scalding, picking and evisceration were not performed.

Sampling

Muscle (pectoralis major) samples were removed at 5 min post-mortem (referred to as 0-hr sample) for the determination of sarcomere length, fragmentation, response to electrical stimulation and time course of rigor mortis. Additional samples were obtained from the opposite (undisturbed, intact) pectoralis major at 1, 2, 6 and 24 hr for determination of sarcomere length and fragmentation.

Sarcomere length and fragmentation

Preparation of muscle samples was accomplished in a Virtis homogenizer (16,000 rpm for 40 sec) utilizing 2g muscle in 15 ml in 0.1M KCl, 0.039M borate buffer (pH 7.1) and 5 mM

Table 1—Means for response of muscle to electrical stimulation and time course of rigor mortis of turkey hen pectoralis major muscle.

Muscle parameter	Time course of rigor mortis[a] Short n = 4	Intermediate n = 8	Long n = 8	Mean[d]	S.D.[d]
Rigor completion (min)[a]	37 (25–48)[e]	93(84–107)	221(129–391)	133	97
Excitability threshold (volts)[b]	123(100–130)	58(25–130)	9 (2–25)	52	49
Contractility duration (sec)[c]	8 (0–30)	50 (0–102)	106 (48–156)	63	52

[a]Time to complete the decrease in extensibility.
[b]Minimum voltage that stimulated contraction.
[c]Time that muscle maintained ability to contract upon stimulation.
[d]Value for composite 20 birds.
[e]Range for each subgroup in parenthesis.

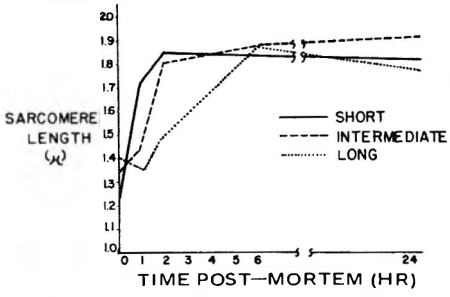

Fig. 1–Graph of sarcomere length as a function of time post-mortem for hen turkey pectoralis major muscle.

ethylenediaminetetraacetic acid (EDTA) (Takahashi et al., 1967). The myofibrils were observed with a phase-contrast microscope equipped with an eyepiece micrometer. Sarcomere length was determined as the average distance between z-lines for 15 myofibrils in each sample (including several sarcomeres per myofibril). Myofibrillar fragmentation was estimated by the method of Takahashi et al. (1967). The number of myofibrils fragmented to 1–4 sarcomere segments was determined and expressed as a percent of the total myofibrils and fragments present.

Rigor mortis

The time course of rigor mortis was determined in muscle strips (length, 3 cm; cross-sectional area, 0.5 cm^2) by the method of Briskey et al. (1962) as modified by Forrest et al. (1969). The muscle strip was suspended from the sample lever into the buffer chamber and was counter-balanced by attaching a weight to the opposite end of the lever. A 4 min loading cycle (2 min loaded, 2 min unloaded) was used. A 60g weight was placed on and removed from the lever on the side of the fulcrum opposite the sample. The temperature of the buffer (Krebs-Ringer Phosphate) was maintained at 27.5 ± 0.5°C for the entire experiment. The time course of rigor mortis was determined as the total time elapsed between exsanguination and the time at which the muscle reaches minimum extensibility.

Response to electrical stimulation

Excitability threshold voltage and duration of contractility were determined on muscle strips (length, 5 cm; cross-sectional area 0.5 cm^2) by the method of Forrest et al. (1966). Duration was measured at 130v using a stimulation frequency of 2/sec and stimulus duration of 0.1 millisec. Turkey pectoralis major rapidly loses its excitability and contractility properties postexsanguination. Therefore, although duplicate samples were determined, only the first sample was included in the results; the second sample was used to confirm the correctness of the first determination.

Statistical analysis

Data were subjected to analysis of variance and correlation analysis as outlined by Steel and Torrie (1960).

RESULTS

Experiment 1

A very high degree of variation was noted for threshold and duration of response to stimulation and time course of rigor mortis for turkey hens (Table 1). The mean value and range for rigor time course are similar to those reported by Briskey et al. (1962) for porcine longissimus dorsi. Also in agreement are the rigor pattern results of de Fremery and Pool (1960) and Pool (1963), although their data were obtained on chicken pectoral muscle by somewhat different techniques. Mean values for threshold voltage exceeded that reported (Forrest et al., 1966) for porcine muscle and the contractility duration was less. This indicates that turkey pectoral muscle is less responsive to electrical stimulation than porcine muscle.

The hens were arbitrarily divided into three groups based on time course of rigor mortis (Table 1). The results indicate that long rigor patterns occur in muscles which respond to electrical stimulation by exhibiting low excitability threshold voltage and prolonged contractility duration. Conversely, muscles which completed rigor quickly displayed high threshold and short contractility duration. These findings parallel the results of Forrest et al. (1966).

Changes in sarcomere length with time post-mortem (Fig. 1) demonstrated a sarcomere-lengthening that occurred within 1 hr in the fast-rigor group, 2 hr in the intermediate-rigor birds and 6 hr in slow-rigor muscle. The increases in sarcomere length in slow rigor birds may have occurred before 6 hr post-mortem but this was not determined. The increase in sarcomere length with post-mortem time agrees with the findings of Takahashi et al. (1967).

The degree of myofibrillar fragmentation increased with time post-mortem with 7.5%, 12.1% and 15.5% fragmented at 2, 6, and 24 hr respectively in homogenized samples. Perhaps more significant than segmental fragmentation was the finding of progressive parallel disarray of the myofibrils. This type of fragmentation occurred at an earlier time post-mortem and more generally throughout the post-mortem period than did segmental–yet, quantitation was not possible by visual observation. Nevertheless, such disarrangement suggests changes in the endomysial and/or perimysial connective tissue with time post-mortem. The fragmentation degree recorded in the present study is lower than that reported by Takahashi et al. (1967); however, they used a more prolonged homogenization which results in greater fragmentation (Fukazawa et al., 1969).

Correlations among excitability threshold, duration of contractility and time course of rigor mortis (Table 2) were highly significant ($P < .01$) and in agreement with results on porcine muscle (Forrest et al., 1966). Other significant correlation coefficients suggested that muscles with high threshold experience short ($P < .01$) sarcomeres at 0 hr but long ($P < .05$) sarcomeres at 1 and 2 hr post-mortem. This finding reflects the observation (Fig. 1) that muscle with the shortest sarcomeres at death experience the most rapid lengthening post-mortem. However, at 6 and 24 hr post-mortem these correlations were not significant ($P < .05$). Rigor completion time was negatively related ($P < .05$) to sarcomere length at 2 hr post-mortem, but not at 6 and 24 hr post-mortem. Sink et al. (1965) reported that 24 hr post-mortem sarcomere length in porcine muscle tended to be long in muscle with a prolonged rigor delay phase.

Table 2–Simple correlation coefficients among measurements of response to electrical stimulation, time course of rigor mortis, and sarcomere length of turkey hen pectoralis major muscle.[a]

	Excitability threshold	Contractility duration	Rigor completion	Sarcomere length			
				0[b]	1	2	6
Contractility duration	-.81**						
Rigor completion	-.69**	.69**					
Sarcomere length							
0[b]	-.60**	.60**	.29				
1	.49*	-.32	-.20	-.33			
2	.49*	-.60**	-.48*	-.17	.51*		
6	-.09	-.05	-.06	.14	.13	.34	
24	-.02	-.24	-.10	.12	-.29	.02	.21

[a]n = 20. *P < .05.

[b]Time (hr) post-mortem. **P < .01.

Experiment 2

The variation in muscle properties of hens may have been caused, in part, by the variation in struggling experienced during and after exsanguination. Since these birds were restrained by hand, physical activity was encountered which is known to decrease energy levels in muscle and, therefore, affect these properties (Forrest et al., 1966). Good evidence for this was obtained in Experiment 2 where postexsanguination struggling was accounted for in the experimental design (Table 3). Distinct differences ($P < .01$) were observed that

**P <.01

Fig. 2–Graph of sarcomere length as a function of time post-mortem for struggling and restrained tom turkey pectoralis major muscle. Treatment means were significantly (P <.01) different at 2 hr post-mortem.

Table 3–Effects of struggling on parameters of response to electrical stimulation and time course of rigor mortis of tom turkey pectoralis major muscle.

	Struggled (n = 12)			Restrained (n = 12)		
Muscle parameter	Mean	Range	S.D.	Mean	Range	S.D.
Rigor completion (min)	93	35–148	33	250**	87–461	107
Excitability threshold (volts)	29**	3–50	21	5	2–12	3
Contractility duration (sec)	93	36–167	33	202**	114–282	55

**P < .01.

demonstrated three effects of muscle activity: increase in excitability threshold, decrease in contractility duration, and reduction in time to rigor completion. Again, very wide ranges and some very long individual rigor patterns were noted.

Consistent with the results of Experiment 1 was the distinct increase in sarcomere length with time post-mortem (Fig. 2). This increase occurred more rapidly (P < .01) (as measured at 2 hr post-mortem) in the struggling group than in the restrained. Differences were not significant (P < .05) at 0, 1, 6 and 24 hr.

An apparent effect of struggling was observed on the correlation coefficients calculated among electrical stimulation parameters, rigor completion time and sarcomere length (Table 4). Within the struggler group, excitability threshold was related (P < .01) to duration of contraction. However, coefficients calculated between threshold and duration and rigor completion time in the struggling toms, and among these three measurements in the restrained toms, were not significant (P < .05). Nevertheless, these statistics do not reflect the degree to which these three parameters are interrelated. Visual inspection of the data from each group revealed that the lack of significance for the correlations resulted from a lack of variation in the data. Within the struggling group, nearly all birds displayed low threshold voltage, short duration and short rigor completion times; in the restrained birds the opposite was true. This observation is supported by the data (Table 3) which disclose relatively small data variation within each group. Correlation coefficients for the composite group (24 toms) were highly significant (P < .01, Table 4). These findings are consistent with those of Forrest et al. (1966).

Significant correlations were observed between threshold voltage and sarcomere length at 2 (P < .01), 6 (P < .01) and 24 (P < .05) hr for strugglers (Table 4). A similar correlation was noted in the restrained group at 0 hr (P < .05) but not at subsequent times.

Correlations from the combined strugglers and non-strugglers (Table 4) may be the most important since they reflect the commercial situation. Significant relationships (P < .01 and P < .05) were observed, indicating that electrical properties and extensibility characteristics of muscle are associated with the sarcomere-lengthening process.

Fragmentation data for tom turkey muscle demonstrated a similar pattern as was observed in Experiment 1–a progressive increase with time post-mortem and the finding that parallel disarray appeared to be more prominent than segmental fragmentation. No significant differences were found between restrained and struggling birds. No significant correlations were calculated that included fragmentation as a variable. Sayre (1969) noticed that fragmentation increased with aging time and that the persistence of a side by side (parallel) aggregation occurred in muscle which, possibly due to rapid glycolysis, resisted segmental fragmentation during aging.

DISCUSSION

ELECTRICAL STIMULATION data demonstrated that some turkeys exhibit muscle with electrical properties and rigor patterns similar to pale, soft and exudative porcine muscle (Forrest et al., 1966) which is characterized by rapid post-mortem glycolysis. It is, therefore, perhaps significant that de Fremery and Pool (1963) suggested that post-mortem lactic acid accumulation in avian muscle was related to toughness. Yet, it was precisely the same type of muscle (rapid rigor completion and high threshold voltage) that exhibited the most rapid increase in sarcomere length post-mortem.

The finding of some lengthy rigor times in the present study was perhaps the most significant discovery. These results suggest that thaw rigor may occur in a fraction of the turkeys processed in some commercial plants. Some processing installations freeze birds within approximately 1 hr post-mortem. de Fremery and Pool (1960) recorded the massive contraction that thaw rigor stimulated in excised chicken muscle. Presumably thaw rigor will occur in intact muscle but the severity of the condition may not be the same as in excised muscle. Nevertheless, Marsh and Leet (1966) noted that cold shortening does occur in isolated areas of isometrically restrained muscle.

The finding that some muscles retained a degree of extensibility after several hr post-mortem may provide insight into the unexplained variation in sarcomere length as reported by Welbourn et al. (1968). Their treatment means for sarcomere length (by weeks of experiment) ranged from 0.92 – 2.21 μ. The shortest single mean sarcomere in the present study (average of 15 determinations) was ascertained to be 1.06 μ. A sarcomere length of less than 1 μ would appear, from our microscopic observations, to indicate reverse-banding–an indication that the muscle is in a supercontracted state. Welbourn et al. (1968) determined sarcomere length at 3 hr post-mortem by homogenizing muscle in 0.08M KCl. In the absence of a calcium chelator, homogenization of pre-rigor muscle might stimulate contraction. The authors also reported that sarcomere length is not related to muscle shear values.

Further study will be required to explain the rapid contraction and lengthening of sarcomeres post-mortem. It was not possible in the present study to isolate any single myofibril which was completely relaxed at death. This was true in spite of the fact that EDTA was utilized in the homogenization buffer and otensibly prevented contraction induced by homogenization. Therefore, it is possible that a stimulus was provided for contraction at death. It is possible that a physiological process associated with exsanguination may have contributed such a stimulus. Or, perhaps the act of muscle excision (or homogenization) stimulated contraction. Koch et al. (1969) demon-

Table 4—Simple correlation coefficients among parameters of response to electrical stimulation, time course of rigor mortis and sarcomere length of tom pectoralis major muscle.

	Strugglers (n = 12)			Restrained (n = 12)			Combined (n = 24)		
	Excitability threshold	Contractility duration	Rigor completion	Excitability threshold	Contractility duration	Rigor completion	Excitability threshold	Contractility duration	Rigor completion
Contractility duration	-.63**			-.43			-.72**		
Rigor completion	-.39	-.18		-.05	.13		-.53**	.58**	
Sarcomere length									
0[a]	.31	-.28	.27	-.63*	.31	-.09	-.15	.33	.25
1	.24	-.10	-.03	-.28	-.17	-.28	.37*	-.34*	-.34*
2	-.76**	.44	.39	-.23	-.19	.12	.15	-.45**	-.34*
6	-.80**	.62*	.52*	.03	.30	-.25	-.75**	.56**	.30
24	-.59**	.37	.35	.02	.05	.53*	-.47**	.26	.38*

[a]Time (hr) post-mortem.
*P < .05.
**P < .01.

strated that excision at death stimulated glycolysis and decreased solubility of sarcoplasmic proteins in porcine muscle. Smith et al. (1969) reported that pre-rigor excision resulted in a 10% reduction in length of chicken muscle. Furthermore, it may be reasoned that excision at 1 hr post-mortem stimulated less contraction than at death because adenosine triphosphate levels were lower. Thus, excision may have stimulated contraction at death and less contraction at subsequent times post-mortem, thereby explaining the observed "increase" in sarcomere length.

Nevertheless, studies may be cited which support the notion that sarcomere-lengthening occurs as a normal post-mortem event and not as an artifact of pre-rigor excision. Goll (1968) presented good evidence that post-mortem contraction and changes in extensibility are independent processes. Jungk et al. (1967) demonstrated that excised muscle held isometrically begins to develop tension immediately after death which passes through a maximum, then declines. Goll et al. (1964) noted that bovine muscle incurs a decline in shear values post-mortem if left in the carcass whereas if excised, the muscle first increases in shear value followed by a decrease. Goll (1968) suggested that these changes which lead to decreased shear values are the "resolution of rigor mortis." Moreover, results of studies on the sarcoplasmic reticulum provide a possible mechanism for the rapid post-mortem sarcomere shortening reported in the present study. Greaser et al. (1967) discovered that the heavy sarcoplasmic reticulum fraction lost 40% of its calcium chelating capacity within 3 hr post-mortem in porcine muscle. It was subsequently determined (Greaser et al., 1969) that the inhibition of calcium-chelation was the result of pH and temperature conditions in muscle. Such an alteration of the sarcoplasmic reticulum may have caused the rapid sarcomere shortening noted in the present study. The turkey pectoralis major experiences post-mortem glycolysis at rates comparable to, or even exceeding, pale porcine muscle. In a group of 23 toms, the mean pH value for the pectoralis major at 15 min post-mortem was 5.89—five birds were below pH 5.70 (Wesala, W.D. 1970. Private communication).

Takahashi et al. (1967) noted that sarcomere lengthening was accelerated by increasing aging temperature. The rates of chilling of birds that they used were much higher than in this study. They recorded sarcomere length increases within 10 hr post-mortem whereas in the present study the increases occurred between 0 and 6 hr post-mortem, mostly with 2 hr. This temperature difference may explain the differences in rates of sarcomere lengthening between the two studies.

It may be significant that in Experiment 2 ultimate sarcomere length did not vary between treatments; rather, the rate of sarcomere lengthening varied as a function of rigor mortis completion time. Muscles which experienced rapid loss of extensibility demonstrated expeditious increase in sarcomere length. Literature citations have noted treatments which delay rigor also slow the rate of aging-tenderization. Sayre (1969) discovered that stimulation of glycolysis in chicken pectoralis major muscle had the effects of hastening the onset of rigor mortis, the development of maximum toughness and the tenderization process. Conversely, when glycolysis was slowed, he noted that rigor onset was delayed as was the development of maximum toughness and maximum tenderness.

The amount of struggling before slaughter has been implicated as a factor in the rate and extent of post-mortem tenderization of poultry muscle. Dodge and Stadelman (1960) noted that struggling increased tenderness at 2 hr aging time, but not at 5 hr. It is possible that the observed acceleration of sarcomere-lengthening by struggling in the present study may account for these findings. Stadelman and Wise (1961) studied effects of nembutal tranquilization of chickens on tenderization rate. They reported that nembutal treated birds, which did not struggle, first toughened more slowly, then tenderized more slowly than untreated birds. Thus, the well-established patterns of variation in shear values of post-mortem poultry muscle, as well as the effects of struggling on these changes, very closely resemble interrelationships among antemortem struggling, response to electrical stimulation, and post-mortem sarcomere length increases noted in this study.

The rate of sarcomere lengthening that has occurred in rapid vs. slow rigor completion muscles and struggler vs. restrained birds would appear to have special significance to the commercial processing of turkeys. These data suggest that some birds would be quite safely frozen within 2 hr post-mortem while in other (slow-rigor) birds this practice would most likely result in less tender meat. This is due to a shorter sarcomere length at freezing coupled with the possibility of additional shortening due to thaw rigor because the pectoralis major muscle in many of the short sarcomere birds is not in rigor at time of freezing. This informa-

tion may also be pertinent to turkey boning operations.

Research is in progress to develop a rapid, non-destructive electrical or physical measurement to identify unusual variations in muscle extensibility and tenderness of turkeys on the evisceration line (Stadelman, W. J. 1969. Private communication). Thus the significant correlation coefficients between electrical properties of muscle and extensibility data and sarcomere-length measurements may provide information to aid this research.

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L. D. SATTERLEE
Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska 68503

EFFECT OF A PORCINE PANCREATIC COLLAGENASE ON MUSCLE CONNECTIVE TISSUE

SUMMARY—Porcine and bovine pancreas as well as porcine spleen, liver and kidney were analyzed for possible collagenolytic activity. Both the porcine and bovine pancreas extracts possessed collagenolytic activity at pH 5.5 and 37°C. The ability of the pancreas extract to break down connective tissue was not due to tryptic or chymotryptic activity, but was due to a combination of collagenolytic and elastolytic activity. The collagenase was partially purified using Sephadex chromatography and was shown to degrade collagen into small soluble peptide fragments.

INTRODUCTION

COLLAGENOLYTIC ACTIVITY of the pancreas was first described by Ziffren and Hosie (1955) when they demonstrated that dog pancreatic juice would digest bovine Achilles tendon. Houck and Patel (1959) used a whole, desiccated, defatted porcine pancreas extract (Bridase) to reduce the viscosity and fiber-forming ability of acid soluble collagen solutions. Houck and Patel (1962) also demonstrated that the activity of the pancreas enzyme differed from the bacterial collagenase in that it was active at pH 5.5 and its activity was not calcium dependent. The ability of a porcine pancreas extract to hydrolyze the peptide backbone of collagen was shown by Houck and Patel (1964) not to derive from: (1) collagen mucoprotease activity; (2) nonspecific cathepsin activity; (3) general proteolysis of denatured collagen; or (4) contamination by bacterial collagenase.

This study was initiated in order to examine the mode of action of porcine pancreatic collagenase on connective tissue. The collagenolytic activity of other organs was investigated, since Woods and Nichols (1965) and Wynn (1967) found rat liver and kidney lysosomes to possess a collagenase.

The presence of a collagenolytic enzyme in the internal organs of the pig could yield an inexpensive source for the specific protease which may have possible use as a meat tenderizer. Secondly, determining the presence of a collagenolytic enzyme in the internal organs could yield information on how collagen is turned-over in the living animal.

EXPERIMENTAL

Source of commercial enzymes and substrates

The purified enzymes, papain and trypsin as well as purified elastin and denatured hemoglobin, were purchased from Sigma Chemical Company. Bovine protease was obtained from Nutritional Biochemicals Corporation. Each enzyme was dissolved in appropriate buffer at a concentration of 5 mg/ml. The elastin was suspended in buffer at a concentration of 1.7 mg/ml.

The buffers used throughout the experiment were: (1) 0.5M acetate, pH 4.5; (2) 0.5M acetate, pH 5.5; (3) 0.1M tris, pH 7.8; and (4) 0.1M tris, pH 9.0.

Preparation of internal organ extracts

The porcine and bovine pancreas as well as porcine liver, spleen and kidney extracts were prepared by a modification of the procedure of Houck and Patel (1964). All excess fat and connective tissue were removed from the organ, after which 250g of the trimmed, homogenized tissue was extracted with 450 ml of cold acetone. The slurry was suction filtered and resuspended in distilled water. The water slurry was then lyophilyzed to dryness. The defatted, desiccated powder from the various organs, as well as commercial Viokase powder, were extracted according to the procedure of Houck and Patel (1964). (Note: Viokase is a defatted, desiccated porcine pancreas powder obtained from Viobin Corporation, Monticello, Illinois.)

Partial purification of the collagenase

Partially pure collagenase was obtained by passing a crude Viokase solution through a 2.5 by 55 cm Sephadex G-100 column. Both the Viokase and column were equilibrated with 0.5 M acetate buffer, pH 5.5.

Preparation of the collagen substrates

Bovine muscle connective tissue was isolated from the fore-shank muscles by first scraping all muscle tissue from the connective tissue surrounding the muscles. The salt soluble collagen (SSC), acid soluble collagen (ASC) and acid insoluble collagen (AIC) components were isolated from the connective tissue by the procedure described by Heikkinen and Kulonen (1966). Each collagen suspension was diluted with the desired buffer to give a final concentration of 1.7 mg/ml. Collagen concentration was determined by the biuret technique, using purified gelatin (Nutritional Biochemicals Corporation) as the standard.

Assay procedures

Each assay was performed in duplicate by placing 3 ml of a collagen suspension in a small test tube along with 0.5 ml of an appropriate enzyme solution. The tubes were stoppered and placed in a 37°C rotating incubator for 24 hr. After 24 hr the digests were cooled and centrifuged to remove all insoluble collagen. The supernatant was then analyzed for hydroxyproline using the procedure of Woessner (1961). The samples were hydrolyzed for 6 hr, instead of 3 hr as described by Woessner. The amount of hydroxyproline found in the supernatant divided by the total amount of hydroxyproline in the initial 3 ml of collagen suspension, gave the percent hydroxyproline released.

A second qualitative technique was also used to determine collagenase activity. This technique consisted of placing 1 ml of the supernatant on a 0.9 by 55 cm Sephadex G-100 column and eluting the components. Each peak was identified by a column monitor set at 254 nm. The area under the hydroxyproline containing peak, eluted with 40 to 45 ml of buffer, was used to qualitatively determine the presence of collagenolytic activity.

Proteolytic activity was determined using denatured hemoglobin as the substrate according to a modification of the procedure of Ting et al. (1968). The 0.4M acetate buffer, pH 3.2 was replaced with 0.5M acetate buffer, pH 5.5.

Molecular weight of soluble collagen peptides

The appropriate molecular weight of peptides released from collagen by the collagenolytic enzyme was determined by placing the concentrated hydroxyproline containing peak, obtained from the Sephadex G-100 column, on a 1.5 by 85 cm Sephadex G-15 column. The peaks were identified using the ninhydrin procedure of Spies (1957) and the hydroxyproline procedure of Woessner (1961). The Sephadex column was standardized for molecular weight determination with vitamin B_{12}, riboflavin and tryptophan.

RESULTS & DISCUSSION

Collagenolytic activity of tissue extracts and commercial enzymes

Table 1 illustrates the effect of each internal organ extract on ASC at pH 5.5 and 7.8. Porcine pancreas extracts obtained from fresh pancreas and from commercial Viokase powder have the ability to hydrolyze a collagen suspension at pH 5.5. Bovine pancreas extract also has this ability, but to a smaller extent.

Table 1—Effect of organ extracts and enzymes on ASC at 37°C for 24 hr.[a]

Extract	pH	% OH-proline containing material solubilized
Porcine pancreas	5.5	22.0
(Viokase)	7.8	4.7
Porcine pancreas	5.5	25.7
Bovine pancreas	5.5	18.1
Porcine spleen	5.5	0.0
	7.8	7.3
Porcine kidney	5.5	0.0
	7.8	5.1
Porcine liver	5.5	0.0
	7.8	11.2
Papain	5.5	0.0
Trypsin	5.5	3.9
Bovine protease	5.5	4.3

[a]Each enzyme and extract was incubated with 5.10 mg of ASC at 37°C for 24 hr.

Porcine spleen, kidney and liver extracts were unable to hydrolyze collagen at pH 5.5. All extracts were able to hydrolyze a small amount of collagen at pH 7.8. Table 1 also illustrates the effect of papain, trypsin and bovine protease on ASC at pH 5.5. Papain, at pH 5.5 was unable to hydrolyze collagen, whereas, trypsin and bovine protease hydrolyzed only a very small amount of the collagen.

The presence of collagenolytic activity in porcine pancreas confirms the results of Houck and Patel (1964). The absence of collagenolytic activity in the liver confirms the observations of Woods and Nichols (1965). Woods and Nichols found only a trace amount of collagenolytic activity in rat kidney.

Action of pancreas extract on collagen

The activity of a Viokase extract at pH 4.5, 5.5, 7.8 and 9.0 is shown in Figure 1. The greatest activity was at pH 5.5.

The Viokase extract was incubated with each collagen component at timed intervals up to and including 24 hr. Figure 2A illustrates that the extract will hydrolyze 46% of the SSC in 8 hr and only an additional 3% in the next 16 hr. The action of Viokase on ASC is slow, when compared to the action on SSC. After 24 hr incubation, only 22% of the ASC was hydrolyzed. Incubation of Viokase and AIC for 24 hr resulted in hydrolysis of 34% of the collagen. Figure 2B illustrates that the extract also has the ability to hydrolyze purified elastin at a rate that is similar to the hydrolysis of the AIC.

The rapid enzymatic hydrolysis of SSC, when compared to hydrolysis of ASC, can be explained by the highly cross-linked nature of ASC and the subsequent difficulty in cleaving such a highly crosslinked collagen molecule. The crosslinkage of the AIC molecule, exceeds that of the ASC molecule and subsequently the molecule should be more difficult to cleave. The rapid cleavage of AIC could be the result of the elastase activity of Viokase, as illustrated in Figure 2B, on elastin in the AIC preparation. Elastin constitutes approximately one-fourth of the protein in muscle connective tissue, (Vognarova et al., 1968) and the purification procedure used in this paper is unable to remove the elastin from the AIC fraction.

When the supernatant from the ASC-Viokase digest was placed on a Sephadex G-100 column, a large hydroxyproline containing peak was eluted at 40 to 45 ml. Concentration of this peak and further separation of it on a Sephadex G-15 column yielded three major components. The two components retarded by the gel have approximate molecular weights of 2,600 and 1,200. Hydroxyproline was present in the component with molecular weight of 2,600. Figures 3A and 3B show the elution patterns for the Sephadex G-100 and the Sephadex G-15 columns.

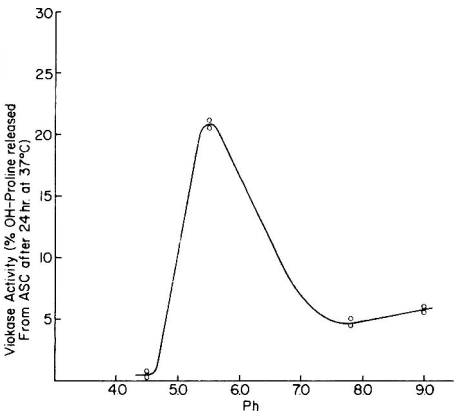

Fig. 1—Viokase activity on ASC at various pHs after 24 hr at 37°C.

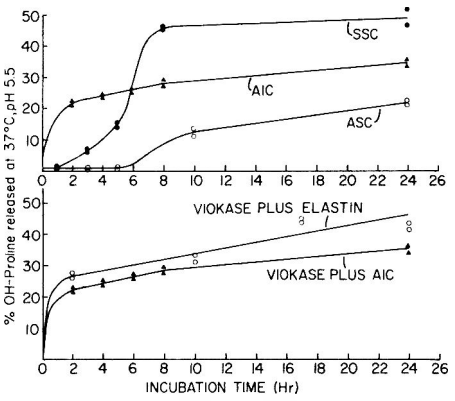

Fig. 2A—Activity of Viokase on each collagen component at pH 5.5, 37°C, for various times up to 24 hr.

Fig. 2B—Activity of Viokase on AIC and elastin at pH 5.5, 37°C, for various times up to 24 hr.

Partial purification of the collagenase

Partial purification was obtained by placing a Viokase extract on a 2.5 by 55 cm Sephadex G-100 column and eluting the fractions. Figure 4 shows the elution pattern for the Viokase extract. Figure 5 illustrates that fraction B is able to clear a suspension of ASC. When fractions A, B, C, and D were analyzed by ultraviolet spectrophotometry, A, B, and C had maximum absorption at 280 nm and D had its maximum absorption at 260 nm. Collagenolytic activity was found primarily in fraction B, with small amounts in A and C. Proteolytic activity was found in

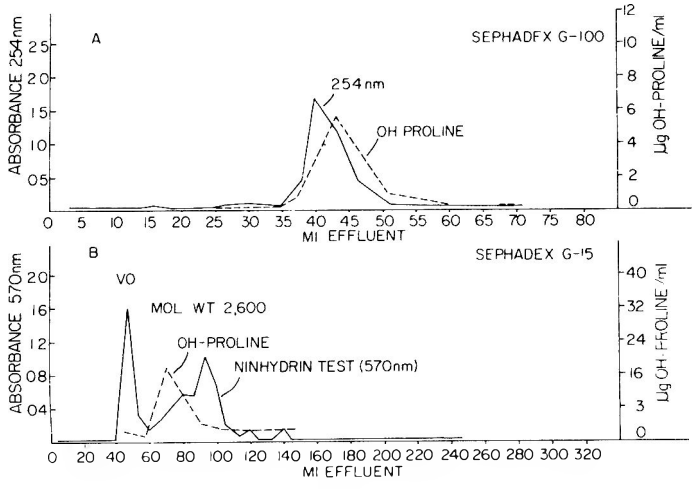

Fig. 3A—Elution pattern of a Viokase plus ASC hydrolyzate on a Sephadex G-100 column, 0.9 by 55 cm.

Fig. 3B—Elution pattern of the concentrated hydroproline containing peak from the Sephadex G-100 column on a Sephadex G-15 column, 1.5 by 85 cm.

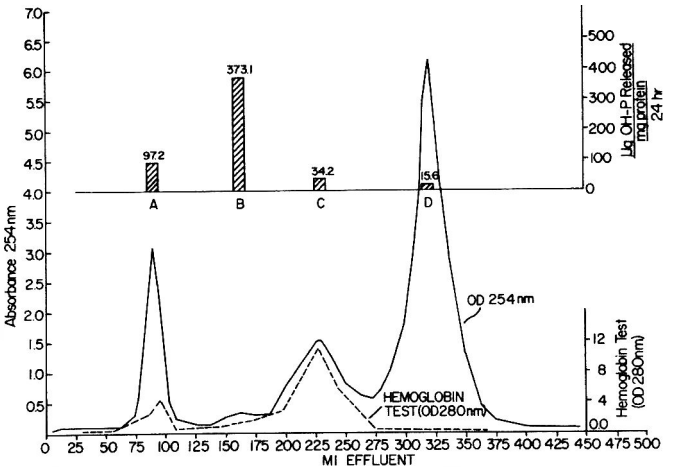

Fig. 4—Elution pattern for a Viokase extract on a Sephadex G-100 column, 2.5 by 55 cm.

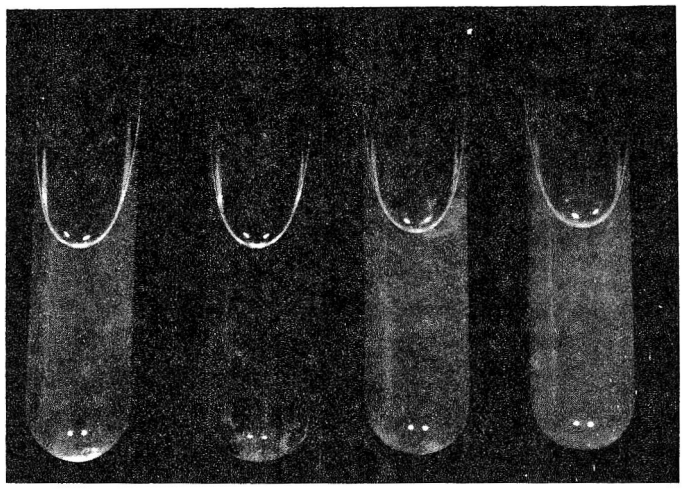

Fig. 5—Hydrolysis of ASC at pH 5.5 by fractions A, B, C and D.

A and C. Polyacrylamide disc gel electrophoresis of fractions A, B, C, and D indicated the presence of several protein components in peaks A and B, four protein components in peak C and no protein in peak D. The presence of 260 nm absorbing material in peak D plus the absence of protein from the gel electrophoresis of peak D, indicates that it consists of nonproteinaceous material, possibly nucleic acid. Gel electrophoresis demonstrated that peak B is not homogeneous, but that sephadex chromatography was able to separate the collagenolytic activity from the general proteolytic activity of the Viokase extract.

The results obtained indicate that a porcine pancreas extract does contain a collagenolytic enzyme capable of hydrolyzing bovine muscle collagen at a pH found commonly in post-mortem muscle. This extract also has elastolytic activity. Partial purification of the pancreas extract by sephadex chromatography was able to separate all proteolytic activity from the collagenase.

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C. -S. C. WU[1] and R. N. SAYRE
USDA Western Regional Research Lab, ARS, Albany, California 94710

MYOSIN STABILITY IN INTACT CHICKEN MUSCLE AND A PROTEIN COMPONENT RELEASED AFTER AGING

SUMMARY—Myosin from both red (myosin-R) and white (myosin-W) chicken muscle was extracted with Hasselbach-Schneider solution after the muscle had aged for 30 min and 24 hr post-mortem. Myosin-W from aged muscle contained a fast moving boundary in sedimentation velocity studies. When this fraction (component T) was separated on a DEAE-Sephadex column, the ATPase activity and sedimentation pattern of the resulting myosin were similar to those of chromatographed myosin from unaged muscle. The numbers of sulfhydryl groups in both myosin-R and myosin-W were not affected by aging. The extinction coefficient and absorption spectrum of component T were different from those of myosin, and component T had neither ATPase nor 5'-adenylic acid deaminase activity. Lowering the pH of myosin extracted from unaged muscle to the pH values found in muscle aged for 24 hr caused aggregation and loss of ATPase activity. These aggregates of myosin differed from component T in both sedimentation velocity and chromatographic pattern.

INTRODUCTION

TENDERIZATION and the associated post-mortem changes in muscle have been a subject of intensive study in the past decade, yet no general agreement has been reached on its exact nature. Most of the investigations in this area have been on extractability (Davey and Gilbert, 1968; Khan and van den Berg, 1964; Penny, 1968; Sayre, 1968; Weinburg and Rose, 1960) and proteolysis (Davey and Gilbert, 1966; Locker, 1960; Wierbicki et al., 1956) of the myofibrillar proteins, or on microscopic studies of the muscle (Davey and Gilbert, 1967; Sayre, 1970; Takahashi et al., 1967). Although it was thought that the changes took place within the myofibrils, no single myofibrillar protein has been purified from aged muscle and characterized intensively.

As an extension of a previous study (Wu, 1969), the effect of post-mortem aging on chicken myosin was investigated in red and white muscles to (1) disclose any changes in myosin taking place during storage of intact muscle and (2) gain information on the process of meat tenderization.

The results indicated that an additional protein fraction was extracted together with myosin from aged breast muscle, and that the amount of this fraction increased with time of aging. However, the myosin prepared from aged muscle had enzymic activity and other properties comparable to those of myosin from the fresh muscle.

EXPERIMENTAL

Materials

The type of chicken, the muscles used for extraction of myosin-R and myosin-W, and the reagents were the same as those described previously (Wu, 1969).

Extraction procedures

A modified Hasselbach-Schneider solution (0.5M KCl, 0.1M phosphate 0.01M pyrophosphate, 1 mM $MgCl_2$, pH 6.4) was used for the routine preparation of myosin in order to extract myosin from muscle in rigor mortis and to maintain similar extraction conditions for both fresh and aged muscles. For a better comparison, the 30 min and 24 hr preparations were made from the same chicken. After the chicken was sacrificed, the whole leg and the breast muscle from one side were placed in separate plastic bags and held at 3°C for extraction after 24 hr. The breast muscle was removed from the remaining side and the red muscles were dissected from the leg for extraction at 30 min post-mortem.

Both red and white muscle were passed through a meat grinder using a plate with 3 mm holes and extracted separately with 3 volumes of the above buffer for 30 min. All extractions and manipulations were conducted at 3°C. The supernatant obtained after centrifugation was diluted to approximately 0.04 ionic strength to precipitate the myosin. The precipitate was collected by centrifugation and dissolved in an equal volume of 2M KCl, 0.04M phosphate, pH 7.0. The solution was then diluted slowly to 0.3M KCl and centrifuged 1 hr at 105,000 x G in a Beckman Model L-2 preparative ultracentrifuge to remove actomyosin. The precipitation, redissolving, and centrifuging steps were repeated three more times except that the KCl concentration was made to 0.28M for the next two cycles and brought up to 0.5M for the last centrifugation. The myosin solutions in 0.5M KCl, 0.01M phosphate, pH 7.0 were stored at 3°C and used within 2 wk without chromatography unless otherwise specified.

Quantitative extraction of myosin was attempted using two modified Hasselbach-Schneider solutions containing pyrophosphate after the following treatments:

(A) The muscle was minced with scissors and 2g portions were extracted 5 times with 10 volumes of 0.47M KCl, 0.01M KH_2PO_4, 0.01M $Na_4P_2O_7$, pH 6.2 for a total extraction time of 6 hr.

(B) The muscle was minced as above, and extracted twice, each for 1 hr, with 10 volumes of 0.6M KCl, 0.1M KH_2PO_4, 0.01M $Na_4P_2O_7$, 1 mM $MgCl_2$, pH 6.4.

(C) The muscle was homogenized at a medium speed for 30 sec in a Sorvall Omni-Mixer equipped with a non-vortex forming cutter. The myofibrils were washed 5 times with 0.25M sucrose, 0.04M imidazole, 1 mM EDTA, pH 7.2. The washed myofibrils were then extracted as in B.

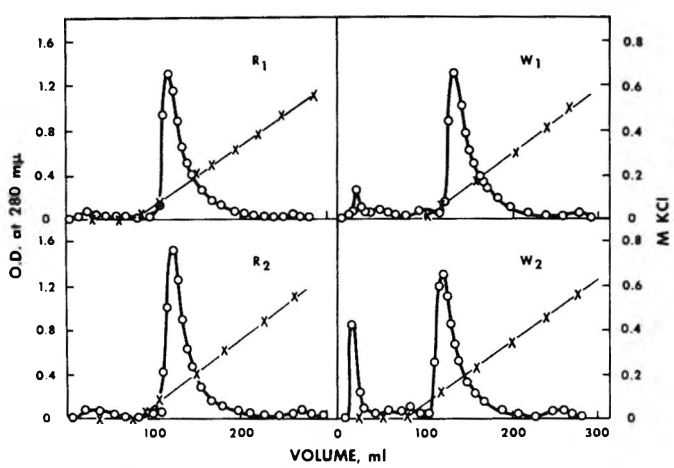

Fig. 1—DEAE-Sephadex (A-50) column chromatography of myosin-R and myosin-W extracted at 30 min (R_1, W_1) and 24 hr post-mortem (R_2, W_2). Column size: 1.5 x 30 cm; buffer: 0.04M pyrophosphate, pH 7.5 with linear gradient of KCl. o-o-o OD at 280 mμ. x-x-x M KCl.

[1] National Research Council Postdoctoral Resident Research Associate.

Table 1—Extraction of myosin with Hasselbach-Schneider solution

Method of extraction[a]	State of muscle before extraction	Yield of myosin mg/g fresh muscle[b]			
		R_1	R_2	W_1	W_2
A	Minced	21.4	28.9	31.9	46.8
B	Minced		24.2		40.1
C	Homogenized		22.0		40.1
D	Homogenized		19.2		34.8

[a]See "Experimental."

[b]R_1 = Myosin-R extracted at 30 min post-mortem.
R_2 = Myosin-R extracted at 24 hr post-mortem.
W_1 = Myosin-W extracted at 30 min post-mortem.
W_2 = Myosin-W extracted at 24 hr post-mortem.

Table 2—ATPase activity of myosin prepared from fresh and aged muscles.

Chromatography	Myosin	ATPase activity, μmole Pi/min/mg	
		Ca^{++}-activated	EDTA-activated
Before	R_1	0.32 ± 0.02[a]	0.66 ± 0.03[b]
	R_2	0.31 ± 0.03	0.64 ± 0.03
	W_1	0.37 ± 0.03	0.67 ± 0.04
	W_2	0.33 ± 0.04	0.49 ± 0.05
After	R_1	0.47[c]	0.79[c]
	R_2	0.48	0.78
	W_1	0.51	0.66
	W_2	0.51	0.75
	W_3[d]	0.51	0.73

[a]9 to 11 preparations.
[b]4 to 5 preparations.
[c]Figures represent average values of triplicate determinations of single preparation.
[d]W_3 = Myosin-W extracted at 48 hr post-mortem.

Table 3—Sulfhydryl groups of unchromatographed myosins prepared from fresh and aged muscles.[a]

(moles SH groups/10^5g myosin)			
R_1	R_2	W_1	W_2
8.59 ± 0.04	8.52 ± 0.16	8.64 ± 0.15	8.70 ± 0.11

[a]Average of 5 determinations.

(D)Same as C except that the homogenized myofibrils were washed 3 times with 0.1M KCl, 0.04M imidazole, pH 7.2.

In all cases, the ionic strength of the extract was lowered to 0.04 by dialysis against water. The precipitated myosin was collected by centrifugation and dissolved in 0.5M KCl, 0.05M phosphate, pH 7.0. The protein concentration was determined by the biuret method.

Protein characterization

ATPase activities were determined in 0.5M KCl, 0.05M tris-HCl, 2 mM ATP, containing either 10 mM $CaCl_2$, 2 mM EDTA, or 5 mM $MgCl_2$, at 25°C. The phosphate liberated was estimated either by the method of Fiske and SubbaRow (LeLoir and Cardini, 1957) or that of Taussky and Shorr (1953). The 5′-adenylic acid deaminase activity was assayed as described previously (Richards et al., 1967).

The sulfhydryl groups of myosin were determined according to Boyer (1954). In a series of test tubes approximately 0.7 mg of myosin, the buffer, and increasing amounts of PCMB were added to a final volume of 3 ml. The solutions were incubated at room temperature overnight and the optical density measured at 250 mμ.

For a direct titration of SH groups, 3 ml of 0.5M KCl, 0.05M phosphate, pH 7.0 was placed in cuvette #1 and the same amount of buffer containing 1.6×10^{-5}M PCMB was placed in cuvette #2. 50 μl of myosin solution (~5 mg/ml) was added to each cuvette before each reading. The optical density of the solution in cuvette #2 was read against cuvette #1. The optical density at 250 mμ was then plotted against the amount of myosin added, and the SH groups titrated were calculated from the end point.

Chromatography of myosin was performed on a DEAE-Sephandex A-50 column according to the procedure of Richards et al. (1967). Protein concentration was measured by the biuret method standardized by micro-Kjeldahl nitrogen.

Sedimentation velocity studies were carried out in a Spinco Model E analytical ultracentrifuge. Recording of the sedimentation patterns was performed with the photoelectric scanning system generally at 280 mμ. Experiments with dilute solutions containing 30–50 μg/ml protein were scanned at 235 mμ.

Viscosity was measured in a Cannon-Fenske Routine Viscometer #100 at 25°C. The efflux time for water was approximately 60 sec. This size was chosen in order to avoid possible aggregation of myosin during prolonged time of measurement. The buffer contained 0.5M KCl, 0.02M phosphate, pH 7.5. The protein concentration of each sample was determined by the biuret method after measurement of the efflux time. The viscometer was washed with cleaning solution and dried after each measurement, otherwise the protein film left on the wall of the viscometer introduced considerable error to the next determination.

RESULTS

Extractability of myosin

In order to make a fair comparison of all the myosin in the muscle, Hasselbach-Schneider solution was employed for the extraction of this protein. The results indicated, however, that the yield of myosin was rather low (Table 1) as compared to those reported for rabbit muscle by Hanson and Huxley (1957) and Bárány et al. (1965). Homogenization of the muscle or washing of the myofibrils did not improve extractability, nor did it alter the relative quantities of myosin-R and myosin-W. Difference in the yield of myosin-R and myosin-W was also noticed previously (Wu, 1969). More protein was extracted at 24 hr than at 30 min post-mortem from both types of muscles, with the greater increase from white muscle. Davey and Gilbert (1968) and Penny (1968) also reported increased protein extractability from aged bovine and rabbit muscles, respectively, with Hasselbach-Schneider solution.

ATPase activities

The Ca^{++}-activated ATPase of unchromatographed myosin-W appeared to decrease somewhat after 24 hr of aging in vivo (W_2), whereas that of unchromatographed myosin-R remained the same (Table 2). When EDTA was used as the activator, the loss of activity for myosin-W ATPase was more pronounced, decreasing to about 70% of the original value by 24 hr post-mortem. The Mg^{++}-activated ATPase activity was negligible in all cases, indicating there was no measurable actomyosin in the myosin solutions. The test for myosin-B by the superprecipitation method of Rice et al. (1963) also indicated the absence of this protein.

Sulfhydryl groups

In spite of the decreased enzymic activity of myosin-W from aged muscle, the sulfhydryl content of this protein did not differ from that of myosin-W from fresh muscle (Table 3). All four preparations contained 8.5–8.7 moles SH groups per 10^5g of myosin. This is in good agreement with the reported value for rabbit myosin (Bárány et al., 1964). When the SH groups were titrated directly (see Experimental), only about 6.5 moles SH groups per 10^5g of myosin reacted. The measurement of SH content by the first procedure, but incubated at 3°C instead of at room temperature, also showed low values, approximately 6–7 moles SH groups per 10^5g of myosin. This indicates that there are at least two slowly reacting SH groups per 10^5g of myosin. Slowly reacting SH groups in

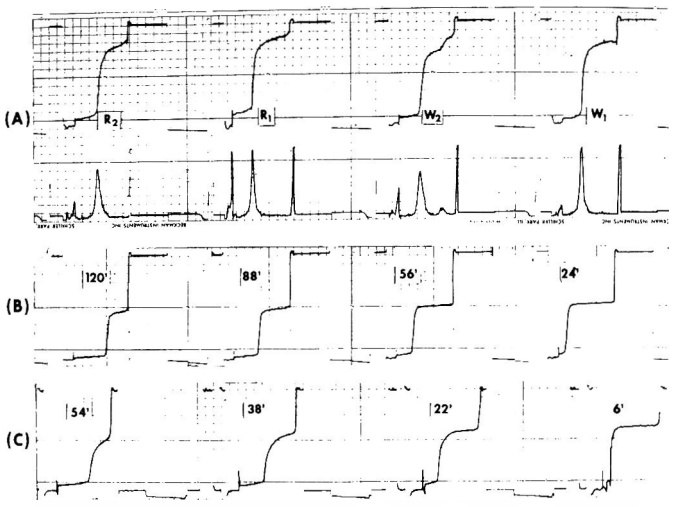

Fig. 2–Sedimentation velocity patterns. Rotor speed: 60,000 rpm; temperature: 4–8°C; wavelength: 280 mμ; buffer: 0.6M KCl, 0.025M histidine, pH 6.8. (A) Unchromatographed myosins 78 min after reaching speed. OD at 280 mμ in 1 cm cell is ~0.3. Both the functional and derivative patterns are shown. (B) Myosin-W extracted at 24 hr post-mortem after purification by a DEAE-Sephadex A-50 column, OD~ 0.3/cm. Only the functional patterns are shown. Minutes after reaching speed is indicated on each picture. (C) Component T after separation from myosin-W, OD ~ 0.6/cm.

Table 4–Sedimentation coefficients of purified myosins.[a]

Myosin	$S_{20,w}$
R_1	5.95 (Average of 3 determinations, 5.76, 5.73 and 6.35)
R_2	6.03 (Single determination)
W_1	5.94 (Single determination)
W_2	5.85 (Average of 2 determinations, 5.79 and 5.91)

[a]Determined at a concentration of 50 μg/ml using photoelectric scanner at 235 mμ and buffer containing 0.5M KCl 0.05M phosphate, pH 6.8.

Table 5–Properties of component T.

$S_{20,w}$	14.5 S
$E^{1\%}_{280\ m\mu}$	11.1
A_{280}/A_{260}	1.89
Tyrosine/tryptophan	2
ATPase activity	0
5'-Adenylic acid deaminase activity	3 units/mg

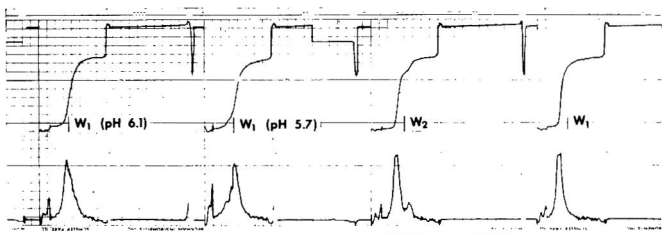

Fig. 3–Sedimentation velocity patterns of myosin-W treated at pH 5.7 and 6.1 and of unchromatographed myosin-W extracted from fresh and aged muscle. Rotor speed: 60,000 rpm; temperature: 4.6°C; 47 min after reaching speed, OD ~0.7/cm. The buffer contained 0.6M KCl, 0.025M histidine, pH 6.8.

rabbit and tuna myosins were also observed previously (Chung, 1967).

Chromatography and sedimentation velocity studies

The chromatography of myosin-W from aged muscle (W_2) on a DEAE-Sephandex A-50 column revealed a sharp peak eluted at the void volume of the column (Fig. 1). However, the myosin peak was normal and as sharp as that of myosin-W from fresh muscle (W_1). Also, the sedimentation velocity study of unchromatographed myosin-W from aged muscle showed a fast moving boundary, comprising approximately 10–15% of the total optical density (Fig. 2–A). The material comprising this fast moving boundary, component T, increased in muscle aged for 48 hr. No such component was found in myosin-R extracted at 24 hr post-mortem (R_2). However, preparations of myosin-R were not made from muscle aged longer than 24 hr.

The ATPase activity and the sedimentation coefficient of chromatographed myosin-W from aged muscle were similar to those of chromatographed myosin-W prepared from fresh muscle (Tables 2 and 4, respectively). In fact, the sedimentation boundary for chromatographed myosin-W_2 was sharper than that of unchromatographed myosin-W_1 (Fig. 2-B and 2-A, respectively. It is obvious, therefore, that the low ATPase activity of unchromatographed protein was due to the presence of component T in the preparation.

Effect of pH on myosin-R and myosin-W from fresh muscles

From the observations (1) that the 24 hr pH of white muscle (5.7) was lower than that of red muscle (6.1); (2) that there was a negligible amount of component T in myosin-R; and (3) that the positions occupied by component T in the chromatographic and ultracentrifugal patterns were very similar to those of highly aggregated myosin (Chung, 1967; Chung et al., 1967); it was thought that this extra protein in myosin-W might be composed of myosin aggregates. Therefore, myosin-R and myosin-W extracted from fresh muscle were dialyzed against 0.6M KCl, 0.025M histidine at pH 5.7 or 6.1 for 24 hr. The pH was then returned to 6.8 by dialysis for another 24 hr against the same buffer adjusted to pH 6.8. The sedimentation velocity patterns of myosin-W treated this way are shown in Figure 3. Myosin-W treated at either pH 5.7 or 6.1 showed continuous broad boundaries, presumably indicating various degrees of aggregation, whereas myosin-W extracted from aged muscle (W_2) exhibited two distinct boundaries in the ultracentrifuge. The chromatographic patterns of the pH adjusted myosin also differed from that of the myosin from aged muscle. Instead of a peak at the void volume of the column (Fig. 1), the aggregates appeared just before the myosin monomer peak (Fig. 4). Furthermore, the pH adjusted samples had very low Ca^{++}-activated ATPase activities, about 0.20–0.27 units for those treated at pH 6.1 and 0.01–0.02 units for those treated at pH 5.7 compared to 0.37 units for untreated myosin-W. Myosin-R treated at pH 5.7 and 6.1 behaved in a similar manner.

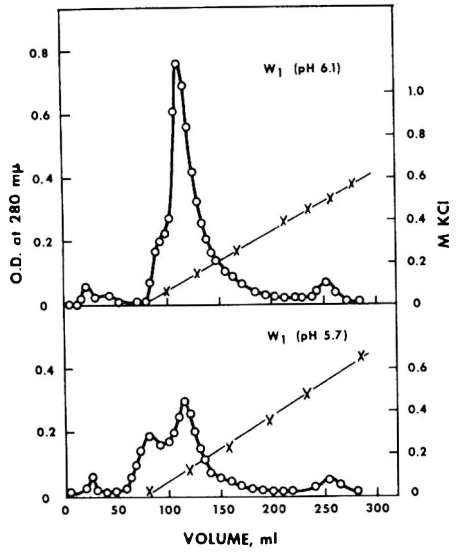

Fig. 4—Chromatography of myosin-W treated at low pH values on a DEAE-Sephadex A-50 column. All the experimental conditions are the same as those in Figure 1.

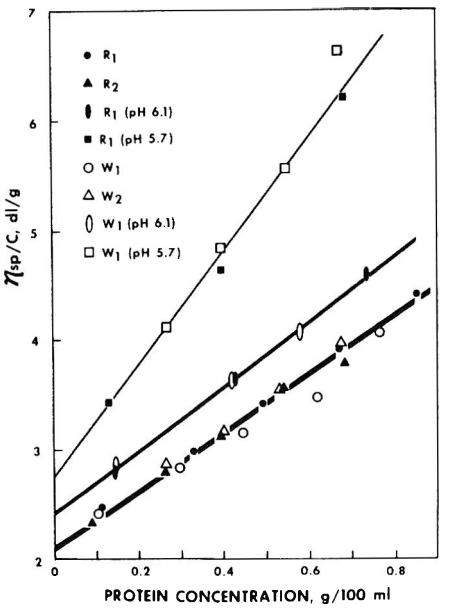

Fig. 5—Reduced viscosity of unchromatographed myosins vs. protein concentration.

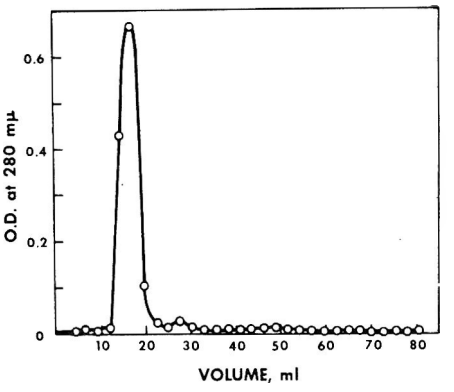

Fig. 6—Chromatography of component T on a Sephadex G-200 column. Component T was separated from myosin on a DEAE-Sephadex A-50 column using myosin-W extracted at 48 hr post-mortem. 3 ml of the solution was loaded on a column of 1.5 × 30 cm previously equilibrated with 0.04M pyrophosphate, pH 7.5 and eluted with the same buffer.

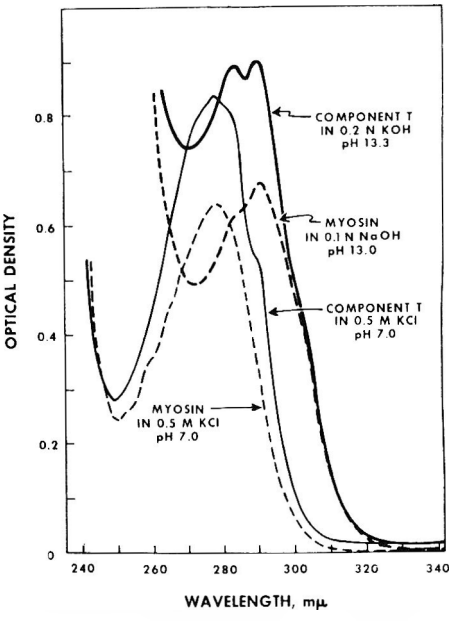

Fig. 7—Spectra of component T and myosin-W at neutral and alkaline pH values. Protein concentration of component T = 0.75 mg/ml, of myosin-W = 1.25 mg/ml.

The plot of reduced viscosity against protein concentration is shown in Figure 5. Myosin-R and myosin-W incubated at pH 6.1 and 5.7 showed higher intrinsic viscosity and steeper slopes than the "native" myosins. All the myosins not subjected to pH adjustment had the same intrinsic viscosity of 2.10 dl/g, and myosin-R and myosin-W treated at the same pH exhibited the same viscosity parameters. These experiments indicated that component T was not an aggregate of myosin.

Isolation and partial characterization of component T

Component T was separated from myosin on a DEAE-Sephadex A-50 column.

The sedimentation velocity pattern of component T is shown in Figure 2—C and can be compared with that of chromatographed myosin-W_2 in Figure 2—B. The shape of the boundary indicates that this preparation of component T is not homogeneous and may contain some faster moving material. Gel filtration of component T on a Sephadex G-200 column resulted in a slightly asymmetric peak near the void volume (determined by Blue Dextran 2,000) of the column and a trace of small molecular weight impurities (Fig. 6).

The spectra of component T at neutral and alkaline pH are shown in Figure 7 together with those of myosin as a comparison. Although the absorption maximum of component T at a neutral pH was the same as that of myosin (279 mμ), there was a shoulder at 290 mμ and the absorption minimum (249 mμ) was slightly lower than that of myosin (250 mμ). The alkaline spectra of these two proteins were also dissimilar. Myosin absorbed more strongly at 290 mμ than at 283 mμ whereas absorption at these two wavelengths were about the same for component T. The tyrosine to tryptophan ratios as calculated from these spectra were 5.0 for myosin and 2.0 for component T. The extinction coefficient of component T at 280 mμ was determined in 0.5M KCl, pH 7.0. Because of the limited quantity and dilute condition of this protein after chromatography, its concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. The results show that the extinction coefficient of component T ($E^{1\%}_{280}$ 11.1) was about twice that of myosin-W ($E^{1\%}_{280}$ 5.0).

The elution pattern of component T from the DEAE-Sephadex column was very similar to that of higher aggregates of myosin (Chung, 1967; Chung et al., 1967), or that of 5'-adenylic acid deaminase (Richards et al., 1967). However, the ATPase activity of this fraction was essentially negative, and the deaminase assay indicated a negligible activity.

The sedimentation coefficient was determined in a Model E ultracentrifuge at a concentration of 30 μg/ml using a photoelectric scanner at a wavelength of 235 mμ. The results together with some other properties of component T are summarized in Table 5.

DISCUSSION

HASSELBACH-SCHNEIDER solution has been known to extract selectively most, if not all, myosin from rabbit (Hanson and Huxley, 1957; Rárány et al., 1965; Penny, 1968) and bovine muscles (Davey and Gilbert, 1968). In the present study with chicken muscle, however, this buffer was found to be only about 50% effective if it is assumed that myosin constitutes 50—60% of the myofibrillar proteins. Thus, precautions have to be made when this buffer is used for the quantitative extraction of myosin. It is assumed in this investigation that the extracted myosin and that retained in the muscle have the same properties.

Davey and Gilbert (1968) and Penny (1968) attributed the increased protein extractability with Hasselbach-Schneider solution from aged muscle to the increase in actin and tropomyosin fractions. Although the yield of myosin was increased, no such fractions were obtained from aged chicken muscle as judged from the chromatographic study. The repeated precipitation and dissolving of the myosin before chromatography may have removed these fractions. Component T, which is not removed by this procedure, apparently contributed to the greater increase of myosin yield from aged white muscle (Table 1). The reason that component T was presented in extract from aged white muscle but not in that from aged red muscle cannot be explained at the present.

The decreased specific activity of Ca^{++}- and EDTA-activated ATPase of unchromatographed myosin extracted from aged muscle was apparently due to the presence of component T. However, since the amount of component T in myosin solution was less than 10%, the more than 25% decrease of EDTA-activated ATPase indicates that there was some type of inhibition in addition to the presence of inactive protein. Preliminary experiments on superprecipitation of reconstituted actomyosin also indicated that unchromatographed myosin extracted from aged white muscle behaved differently from that extracted from fresh muscle (Wu, unpublished data).

The ATPase activity of chromatographed myosin from aged muscles was unexpectedly high. The rather low pH values of the post-mortem muscle did not cause the myosin to aggregate. In view of the deleterious effect of similar pH values on purified myosin, the intact muscle must have some mechanism to protect myosin from denaturation.

The nature and source of component T is not known. From the fact that the quantity increased with time in the white muscle, it is likely a product of post-mortem aging. It has solubility properties very similar to that of myosin, hence it was extracted together with myosin and was not removed by repeated precipitation at low ionic strength. This component differed from the "extra protein" of Davey and Gilbert (1968) in terms of solubility. It is neither an aggregate nor a denaturation product of myosin because (1) there was no ATPase activity; (2) the ultraviolet absorption spectra, the extinction coefficient at 280 mμ, and the tyrosine to tryptophan ratio were different from those of myosin; and (3) the sedimentation behavior differed from that of myosin. None of the other known myofibrillar proteins resembled component T in all of the above criteria. However, the possibility of being a denatured or aggregated product of one of these proteins cannot be ruled out. Herring et al. (1969) have reported finding a protein component in their preparations of actomyosin from aged bovine muscle. This component was characterized only by its sedimentation coefficient of 12S to 13S and by its isolation with actomyosin. More understanding of this protein has to await further investigation.

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D. A. LEDWARD
CSIRO Div. of Food Preservation, Meat Research Lab., P.O. Box 12, Cannon Hill, Queensland, Australia

METMYOGLOBIN FORMATION IN BEEF MUSCLES AS INFLUENCED BY WATER CONTENT AND ANATOMICAL LOCATION

SUMMARY—The influence of relative humidity on the formation of metmyoglobin at 0–2°C, was studied. Decreased water content of semitendinosus muscle led to increased metmyoglobin formation, but only at degrees of dehydration that led to marked discoloration due to other causes e.g., heme concentration. Metmyoglobin formation was very dependent on the anatomical location of the muscle ($P < 0.001$). The susceptibility to metmyoglobin formation for the four muscles studied was biceps femoris > semimembranosus > longissimus dorsi = semitendinosus.

INTRODUCTION

DETRIMENTAL COLOR changes occur in fresh beef during storage. The changes are similar for both carcass beef during aging and retail cut meat during marketing.

If evaporation from the meat surface is prevented the most important color change is the oxidation of the heme pigment myoglobin, to the undesirable brown metmyoglobin. The accumulation of metmyoglobin at the surface of fresh beef depends on two opposing mechanisms: (a) the autoxidation of the reduced pigment in the presence of oxygen (Brooks, 1931; George and Stratmann, 1952) and (b) the enzymatic reduction of metmyoglobin to the reduced form (Stewart et al., 1965a). Ledward (1970) studied the effect of reduced oxygen and elevated carbon dioxide levels on the formation of metmyoglobin at the surface of moist refrigerated beef. Butler et al. (1953) and Robach and Costilow (1961) evaluated the influence of bacterial levels and specific bacteria upon metmyoglobin formation in beef. All these investigators interpreted their data in terms of metmyoglobin formed as a result of oxidation and enzymatic or microbial reduction.

In certain aging processes bacterial growth is inhibited by allowing evaporation from the meat surface to occur. Under these conditions meat takes on a dark, dull appearance due presumably to increased heme concentration and structural change (Brooks, 1937). Whether any of this darkening is due to increased metmyoglobin is not known and so experiments were performed to elucidate the contribution of metmyoglobin to the darkening in meat of reduced water content.

It has also been observed (Ledward, 1970) that semitendinosus muscles of normal water content, from different animals, tend to develop different levels of metmyoglobin during storage, the level developed being independent of the muscle pH. This observation was extended in the present study and attempts were made to compare metmyoglobin formation between muscles from animals of known age.

EXPERIMENTAL

Preparation and storage of the samples

Semitendinosus muscles, used in the relative humidity experiments, were sterilized by extensively flaming the outside surface. Slices (2.0 ± 0.5 mm thick) were prepared using the apparatus described by Kaess (1961) and dried to the appropriate water content (Kaess and Weidemann, 1968). The water content was maintained by storing the slices in the dark at 0°C in stainless steel frames (Kaess and Weidemann, 1962) over sulphuric acid solutions of the appropriate equilibrium relative humidity (E.R.H.).

To elucidate the effect of Cryovac L film on metmyoglobin formation, a sterile semitendinosus muscle was used to obtain 16 samples (15 ± 1 mm thick), 8 of which were randomized to storage in air at an E.R.H. of 99.3% (Ledward, 1970) and 8 to storage in Cryovac L film. The samples were stored for 10 days at 0°C.

After chilling for 24 hr at a commercial abattoir, 4 muscles (biceps femoris, semimembranosus, semitendinosus and longissimus dorsi) were removed from the carcasses of 16 steers of known age. The whole or halved muscles were wrapped in Cryovac L film and stored at 1 ± 1°C for 21 days. Bacterial growth was inhibited by storage in 25% carbon dioxide/air in a gas tight room (Bate, 1968). A layer 15 ± 1 mm thick was removed from the surface for the measurement of metmyoglobin concentration (Ledward, 1970). Several readings were taken on each muscle and averaged.

Determination of metmyoglobin concentration

Extraction procedure. The 2.0 mm thick slices were blended in a Waring Blendor with an equal volume of distilled water (4°C) and filtered through 1.2μ millipore filters. The metmyoglobin concentration of the clear solution was determined from the extinction coefficients at 572 and 525 nm, (Broumand et al., 1958; Stewart et al., 1965b). A Hitachi Perkin-Elmer spectrophotometer (Model 139) was used.

Reflectance spectrophotometry. Surface metmyoglobin concentrations were determined as described by Ledward (1970), except that a Beckman DB-G spectroreflectometer was used. At 572 nm and shorter wavelengths the 2 mm thick slices used in the E.R.H. experiments were "infinitely" thick while still yielding samples of relatively uniform water content.

Metmyoglobin concentrations are quoted as percentages of the total surface heme pigments.

Determination of color

C.I.E. color coordinates of the 2 mm slices were determined and corrected to infinite thickness, using white and black backgrounds of known reflectance (Judd, 1963). In these determinations care was exercised to ensure that a similar shape of meat was presented to the integrating sphere, against both backgrounds. The actual calculations, using the weighted ordinate method, were performed by a CDC 3200 computer. All measurements were performed on duplicate samples from the same slice.

RESULTS & DISCUSSION

Storage at different E.R.H.

In the 2.0 mm thick slices the metmyoglobin concentration determined by the extraction procedure was always less than the amount determined by spectroreflectometry, ($P < 0.001$). At 99.3% E.R.H. the difference was 8.9% with a variance of 3.24 on 12 samples.

Possible causes of the differences at this E.R.H. are that in the extraction procedure subsurface pigments, which may not be "seen" in the reflectance technique are analyzed, differences in solubility between the oxidized and reduced pigments may exist and also pigment changes may occur during the aerated extraction.

At the lower water contents the differences between the techniques was greater and more variable. At an E.R.H. of 98.0% the difference was 11.0% with a variance of 38.44 on 13 samples. This is to be expected since, at these decreased water contents, meat becomes very dark due to changes in structure and heme concentration (Brooks, 1937) and the reflectance technique cannot adequately correct for these gross changes. For this reason all the metmyoglobin concentrations quoted in the relative humidity experiments were determined by the extraction procedure. The influence of water content on the formation of metmyoglobin in 5 semitendinosus muscles is shown in Figure 1.

Figure 1 indicates that the formation of metmyoglobin in fresh, sterile beef is aided by dehydration at the surface. This is contrary to the observation of Brooks (1937) who stated that hemoglobin is apparently oxidized more slowly in partly dried tissue, but agrees with Lawrie's (1966) argument that desiccation causes

Table 1—Effect of age on the metmyoglobin (Mb^+) concentration[a] in 4 muscles, from 16 animals. Storage was for 21 days at 1°C in 25% CO_2/air at an E.R.H. of 99.3%.

No. of animals	(Age yr)	Muscle: Biceps femoris	Semi-membra-nosus	Longis-simus dorsi	Semi-tendi-nosus	Mean
4	1–1½	80.9	41.4	40.1	41.4	50.9
4	2–2½	60.3	47.0	39.2	43.2	47.4
8	3–3½	57.4	56.2	39.6	40.3	48.4
16	Mean	64.0	50.2	39.6	41.3	48.0

[a]Expressed as a percentage of the total heme pigments.

increased salt concentrations which by analogy with pure solutions (Brooks, 1931) should facilitate metmyoglobin formation.

It has previously been found (Ledward, 1970) for 15 mm slices at 0°C and an E.R.H. of 99.3%, that the surface concentration of metmyoglobin was virtually constant from about 5 to 14 days' storage. This is not the case for 2 mm slices (Fig. 1). This failure to maintain the "equilibrium" in thin slices may be due to the complete oxygen penetration of the samples so that the "ferrimyoglobin reductase activity" (metmyoglobin reducing activity, MRA) is depleted by oxygen relatively quickly. In thick slices, where anaerobic conditions exist under the surface, reserves of the reducing enzymes or essential substrates may exist. Such a system could maintain the "equilibrium" over a longer period.

The reflectivity or "brightness" of the slices from the 5 semitendinosus muscles was recorded in terms of the C.I.E. Y coordinate (Y = 0 for a matt black surface and Y = 100 for powdered magnesium oxide). At an E.R.H. of 99.3% the Y values, after 8 and 15 days storage were 12.0 and 11.7 with standard error 0.5 on 8 degrees of freedom. At an E.R.H. of 98.0% the respective Y values were 6.0 and 6.1 with standard error 0.3 on 8 degrees of freedom. Since significant increases in metmyoglobin concentration from the 8th to 15th day (Fig. 1) caused no change in the whiteness/blackness index it would appear that the C.I.E. Y coordinate is not responsive to metmyoglobin formation. The effect of heme concentration upon darkness is evident due to the reduction of the Y coordinate from ~12 to ~6 as a result of E.R.H. reduction from 99.3 to 98.0%. Thus, increased metmyoglobin formation does not contribute, to any marked extent, to the darkening observed in meat of decreased water content.

Storage in polythene

Polythene (Cryovac L film) had no effect on the formation of metmyoglobin at 1 ± 1°C. The mean metmyoglobin concentrations in the sterile semitendinosus samples stored in Cryovac L film and air were 22.9% and 21.6% with standard error 1.9% on 14 degrees of freedom.

Effect of anatomical location

The values found for the surface concentration of metmyoglobin in muscles from 16 animals of known age wrapped in the moisture impermeable Cryovac L film, after storage for 21 days in an atmosphere of 25% carbon-dioxide/air at 1 ± 1°C are summarized in Table 1.

A gaseous environment of 25% carbon dioxide/air was chosen as this atmosphere is known to inhibit bacterial growth (Shaw and Nicol, 1969) without significantly affecting the formation of metmyoglobin (Ledward, 1970). Storage for 21 days was selected as this is normally considered the maximum aging time necessary at this temperature. However, time was not critical as an independent experiment using halved longissimus dorsi muscles from 8 animals showed that the surface metmyoglobin concentration after 2 wk storage was not significantly different from that after 4 wk storage. The values obtained were 31.2% and 32.2% with standard error 2.9% on 14 degrees of freedom. This extends the previous observation that in several intact semitendinosus muscles the concentration of metmyoglobin is virtually constant from 5 to 14 days storage (Ledward, 1970). Thus, over a considerable period, a pseudo-equilibrium concentration of metmyoglobin exists at the surface of beef, presumably due to the opposing effects of the autoxidation and enzymatic reduction.

Analysis of variance of the results summarized in Table 1 showed that animal and age effects were not significant in determining the mean concentration of metmyoglobin developed during storage, where the mean is the average of that found in the biceps femoris, semimembranosus, longissimus dorsi and semitendinosus. Highly significant ($P < 0.001$) differences in metmyoglobin concentrations were found between muscles from different anatomical locatiions.

The final level of *Pseudomonas* in these experiments performed in 25% carbon dioxide/air, which inhibits the growth of these organisms, was always less than $10^4/cm^2$ in a total bacterial population of $10^6/cm^2$ or less (Nicol et al., unpublished). Independent experiments showed that this level of *Pseudomonas* contamination caused no increased metmyoglobin formation during air storage at 0°C (Ledward, unpublished).

When stored in this atmosphere the bacterial flora becomes predominantly *Microbacterium* and *Lactobacillus* (Shaw and Nicol, 1969). Robach et al. (1961) found that *Lactobacillus plantarum* had

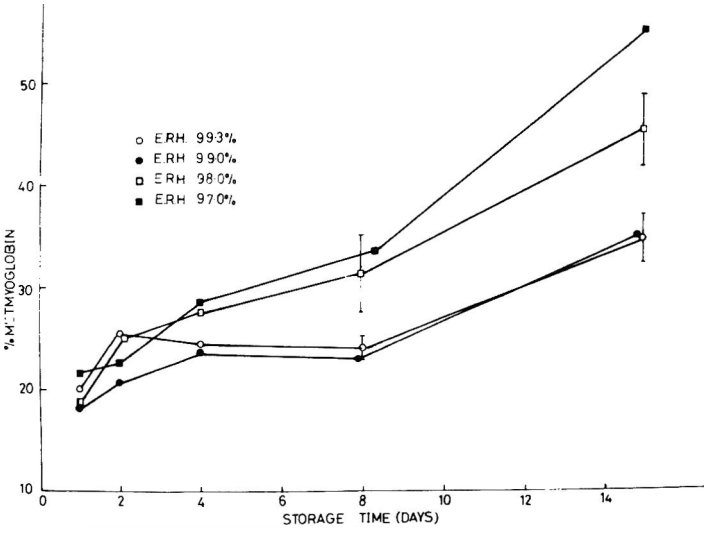

Fig. 1—The effect of equilibrium relative humidity (E.R.H.) on the formation of metmyoglobin in semitendinosus muscles, at 0°C, as a function of storage time. Samples (2.0 ± 0.5 mm thick) were dried for 0–9 hr at 0°C. Points drawn with their standard deviations are averages from 5 muscles; other points were determined on 1 muscle only.

no effect on the formation of metmyoglobin and this observation was confirmed in the present study with *Lactobacillus* (CSIRO Meat Res. Lab. strain 58) (Ledward, unpublished). *Microbacterium* (CSIRO Meat Res. Lab. strain 22), at levels greater than about $10^5/cm^2$, was found to cause a slight, but significant, increase in the concentration of metmyoglobin (Ledward, unpublished). The maximal increase observed, at *Microbacterium* (22) levels of up to $10^8/cm^2$, was only about 10% of the total surface heme pigments. Thus, this oxidation is not of sufficient magnitude to explain the different metmyoglobin concentrations observed between the muscles, especially as the level of contamination ($10^5-10^6/cm^2$) was similar in all cases, (Nicol et al., unpublished).

The *Microbacterium* contamination may explain the higher "equilibrium" concentrations of metmyoglobin found in the semitendinosus muscles used in the present study compared with the concentrations found in the sterile slices used previously (Fig. 1; Ledward, 1970).

The pH of the muscles studied were all within the range 5.5–6.0 and thus, although both the rate of autoxidation (Brooks, 1931) and enzymatic reduction (Stewart et al., 1965a) are pH dependent, over this small increment pH would not be expected to be a significant factor in determining the concentration of metmyoglobin developed (Ledward, 1970). An analysis of covariance using initial muscle pH as the covariate confirmed this conclusion.

What the important factors are that cause these marked differences in metmyoglobin developed between muscles from the same carcass will have to await further study, but the fact that differences do occur may limit the use of oxygen-containing atmospheres for the storage (aging) of certain retail cuts at chiller temperatures.

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C. E. DAVIS,[1] E. E. FINNEY and D. R. MASSIE
USDA, Market Quality Research Div., ARS, Beltsville, Maryland 20705

USE OF SONIC AND ULTRASONIC MEASUREMENTS ON BOVINE BONE TO ESTIMATE CHRONOLOGICAL AGE

SUMMARY—Sonic and ultrasonic measurements were made on bovine bone to explore their possible value in objectively determining chronological age of a beef animal carcass. The sonic resonant frequency of the bone and the transmission rate of an ultrasonic pulse through the bone were measured on the right large metacarpal bone of slaughtered beef animals of different ages of the same breed. Thirty-four bones from animals approximately 6, 12, 18, 24, and 30 months of age were measured. The simple correlation coefficient between animal age and the sonic resonance parameter, $(FL)^2$ was 0.875 and between age and the ultrasonic velocity parameter, $(cm/\mu sec)^2$ was 0.866.

INTRODUCTION

MATURITY is one of the factors evaluated and used in the final determination of a USDA grade for carcass beef (USDA, 1965). Presently, maturity is determined subjectively by visual evaluation of the size, shape, and degree of ossification of the bones and cartilages, particularly the split chine bones, and the color and texture of the lean tissue. Numerous workers, (including Alsmeyer et al., 1959; Goll et al., 1965; Romans et al., 1965a; Romans et al., 1965b; Suess et al., 1966; Tuma et al., 1962; Walter et al., 1965), have reported on meat quality characteristics associated with known carcass age or apparent carcass maturity.

Audio-frequency instrumentation and resonant-frequency techniques have been used (Finney and Norris, 1968; Abbott et al., 1968) to measure textural quality of fruits and vegetables. These applications have shown that sonic energy can be used to estimate internal characteristics of fruits and vegetables by measuring their sonic resonance. Similar resonant-frequency measurements have been made on bones by (Selle and Jurist, 1966) to detect senile osteoporosis. In their work the resonant frequency, F, and the length, L, of the human ulna were measured and a parameter, FL, was calculated. Average FL values (Hz-cm) ranged from 2729 for osteoporotic females to 6173 for normal males. Because of the large differences, the resonant-frequency measurements were reported to be more sensitive than current radio-graphic methods of detecting changes in bone density and bone degeneration associated with senile osteoporosis.

Lang (1969) calculated and reported the elastic stiffness coefficients of dried bovine phalanx and femur and fresh bovine phalanx from measurements of the velocity of an ultrasonic pulse through small prepared sections of these bones.

The object of this work was to explore the use of resonant frequency and ultrasonic pulse velocity in determining the age of an animal from measurements made on the right metacarpus bone from slaughtered beef animals.

METHODS

THE RIGHT METACARPUS, consisting of a large metacarpal and a small lateral metacarpal bone, from a group of 34 Black Angus steers was removed after slaughter as animals attained the approximate ages of 6, 12, 18, 24, and 30 months. These bones with surrounding hide and other tissues were wrapped in aluminum foil and held at about 0°F until all bones had been collected. This resulted in the bones being held in frozen storage for varying lengths of time as follows: 19–24 months, 15–18 months, 9–12 months, 8 months–1 week for the animal age groups 6, 12, 18, 24 and 30 months respectively. Prior to measurement, the bones were placed at 34–36°F for 24 hr. At the time of measurement, the bones were unwrapped and measured for length from the condyle ridges to the medial facet of the proximal end of the large metacarpal bone. After this, sonic resonance and ultrasonic velocity measurements were made on the large metacarpal bone with the accompanying tissue and surrounding hide intact.

The ultrasonic velocity parameter was determined by measuring the rate at which an ultrasonic wave is transmitted through the bone. In this method (Fig. 1), a low-frequency oscillator was used to trigger a high-voltage pulse generator. The high voltage pulse was applied to a barium-titanate crystal which produced an ultrasonic pulse at the condyles on the distal end of the bone. The voltage pulse also opened a gate in an electronic counter which began scaling the pulses from a 1 MHz oscillator. After passing through the bone, the ultrasonic pulse was received by an accelerometer on the medial facet of the proximal end of the bone. This signal was amplified and used to close the gate in the electronic counter. The counter then printed the number of microseconds for the ultrasonic pulse to pass through the bone. The bone in position for measurement is shown in Figure 2.

A series of times was recorded on each bone. Each series was averaged and divided into the corresponding bone-length measurement. This gave a pulse-velocity value in centimeters per microsecond. This value was squared giving the quantity $(cm/\mu sec)^2$, which is related to the elasticity of the bone (Lang, 1969).

Sonic resonance of the bone was measured using an electromagnetic vibrator and the resonant frequency test instrumentation described by Finney and Norris (1968). This instrumentation consisted of an audio signal generator, an output power amplifier, a vibrator exciter, a vibration detector, an a-c voltmeter with a pick-up amplifier, and an electronic counter. The specimen was placed vertically on the vibration exciter (MB Electronics Model EA 1250) with the condyles resting on the moving element of the exciter. A small hole was drilled in the medial facet of the proximal end of the bone and an accelerometer (Endevco Model 2221 D) attached using a screw. The frequency range

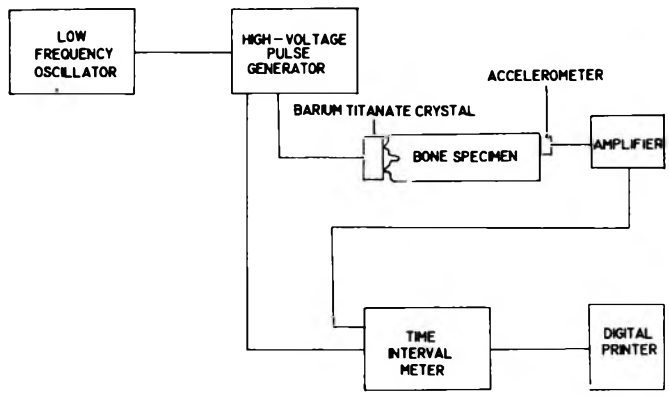

Fig. 1—Instrumentation for generating ultrasonic impulse and measuring time for impulse passage through bone specimen.

[1]Present address: USDA, Richard B. Russell Agricultural Research Center, ARS, P.O. Box 5677, Athens, Ga. 30604.

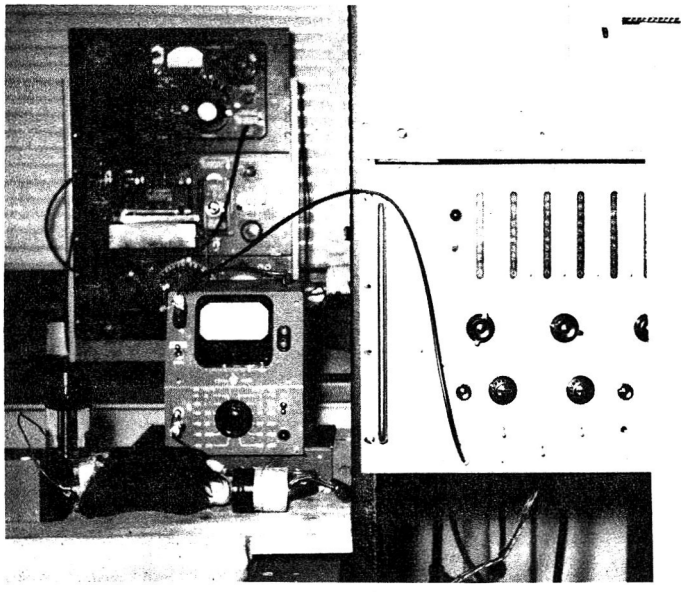

Fig. 2—Experimental arrangement for measuring the through-transmission rate of an ultrasonic pulse on an intact bone.

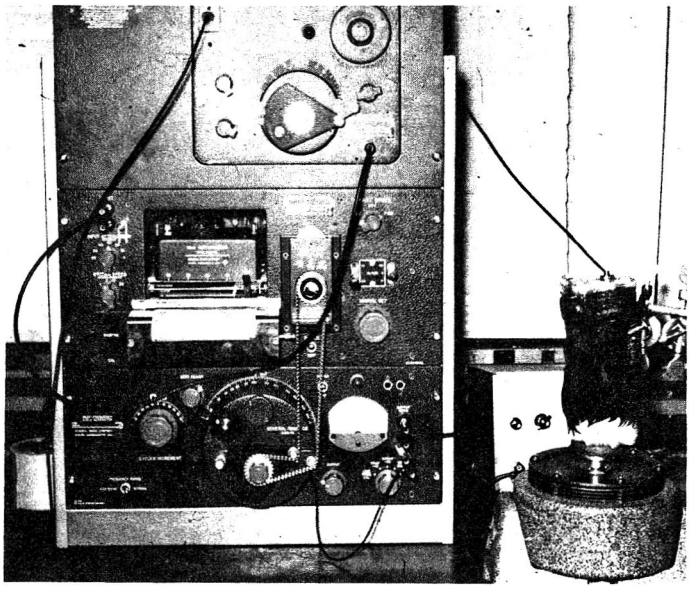

Fig. 3—Experimental arrangement for exciting and measuring resonance within an intact bone.

from 100–10,000 Hz was scanned and that frequency where the signal from the accelerometer reached a maximum was observed and recorded as the resonant frequency, F, of the bone specimen. This and the bone length were used to derive the quantity $(FL)^2$; F is the resonance frequency, in Hertz units, at maximum amplitude; L is the length of the bone in centimeters. In general, $(FL)^2$ is related to Young's modulus of elasticity (Finney and Norris, 1968). Thus, it is a general measure of the elastic stiffness of the bone. The bone in position for measurement is shown in Figure 3.

RESULTS

THE RELATIONSHIP between the ultrasonic velocity measurement, expressed as $(cm/\mu sec)^2$, and chronological age of the animal was tested for linear regression (Snedecor and Cochran, 1967). This relationship was highly significant with a sample standard deviation from the regression line of 0.0082 $(cm/\mu sec)^2$ units (Fig. 4). The simple correlation between days of age and the ultrasonic velocity parameter was r = 0.866 accounting for 74.9% of the variability. This measurement over the range of age from about 200 to about 900 days indicates the sound traveled through the bone at a faster rate in the more mature samples.

The relationship between the quantity $(FL)^2$ and chronological age of the animal was tested for linear regression (Snedecor and Cochran, 1967). This relationship was highly significant with a sample standard deviation from the regression line of 1667×10^6 $(FL)^2$ units (Fig. 5). The simple correlation between days of age and $(FL)^2$ was r = 0.875 accounting for 76.6% of the variability. This measurement over the age range from about 200 days to about 900 days indicates that the frequency of resonance increased in the more mature samples. In addition to the sonic resonance and ultrasonic velocity measurements, the length of the bone and animal age were correlated. The length-age relationship was linear from about 200 to 600 days of age, after which the length remained relatively constant (data not shown). The simple

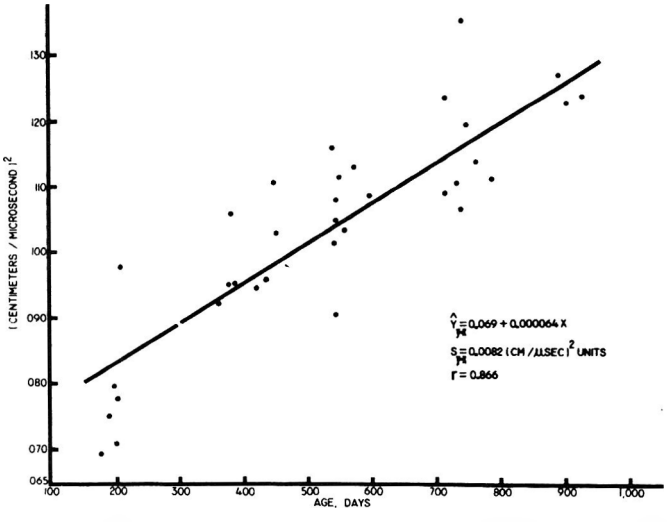

Fig. 4—Relation between animal age (days) and the ultrasonic velocity parameter (cm/microsec).[2]

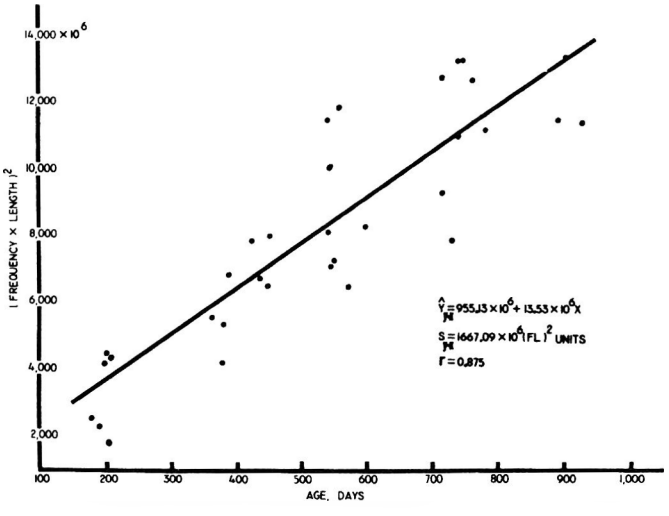

Fig. 5—Relation between animal age (days) and the resonance parameter (frequency × length).[2]

correlation between days of age and bone length was r = 0.765 accounting for 58.6% of the variability.

This limited study indicates that measurements of resonance could be used to determine dynamic mechanical properties of bone. These properties, which seem to be related to the aging process of bone, might be used to determine objectively bone and carcass maturity. In addition, measurement of bone with ultrasonic techniques could provide another approach to the study of bone maturation.

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Mention of specific instruments or trade names is made for identification purposes only and does not imply any endorsement by the U.S. Government.

GRETCHEN A. PELROY and JOHN SPINELLI
National Marine Fisheries Service, Seattle, Washington 98102

AVAILABILITY OF AMINO ACIDS IN SARCOPLASMIC FISH PROTEINS COMPLEXED WITH SODIUM HEXAMETAPHOSPHATE

SUMMARY—Comparisons were made on the availability (enzyme digestion) of 4 amino acids (lysine, methionine, tryptophan, and threonine) in sarcoplasmic fish proteins and sarcoplasmic fish proteins complexed with sodium hexametaphosphate. The protein complexes were formed by reacting 1% solutions of sarcoplasmic fish proteins at pH values ranging from 2–4 and at phosphate concentrations ranging from 0.005–0.05 M. Uncomplexed sarcoplasmic protein was obtained by precipitation from 70% isopropanol. The samples were digested with the enzyme pronase and the available amino acids were measured with a bio-assay system. No significant differences in the availability (enzyme digestion) of amino acids were found between complexed and noncomplexed samples. Assays of samples dried at 75°C showed that nearly 100% of the methionine, threonine and tryptophan and 85% of the lysine were available. When the samples were subjected to moist heat (50% moisture, 100°C) for up to 24 hr, availability of lysine, methionine, tryptophan and threonine was reduced by 35, 22, 39 and 34%, respectively. Results of this study indicate that the nutritional characteristics of fish proteins are unimpaired when they are complexed with sodium hexametaphosphate.

INTRODUCTION

REACTION between condensed phosphates and proteins is well known, and the modifying effect that these phosphates induce in proteinaceous food has been used to advantage by the food industry in a variety of products. Examples range from increasing the water-holding capacity of frozen and thawed fish (Mahon, 1962) to improving the foaming power and foam stability of egg albumen (Chang et al., 1970). Recent work at this laboratory suggests that the reaction between condensed phosphates and proteins to form insoluble protein-phosphate complexes might also be utilized to recover proteins economically from dilute aqueous concentration, the reaction being particularly useful in the recovery of proteins from the effluent of plants processing proteinaceous products.

Although the utility and the chemistry of the reaction has been investigated (Briggs, 1940; Van Wazer, 1958; McKee and Tucker, 1966), the nutritional characteristics of the protein-phosphate complexes that are formed have generally been neglected. Spinelli and Koury (1970) determined chemical available lysine values (Carpenter, 1960) and protein efficiency ratios (PER) on complexes formed with sodium hexametaphosphate (HP) and sarcoplasmic fish proteins isolated under one particular set of conditions with respect to pH, protein and phosphate concentration. Their data showed that neither the PER nor the available lysine values differed significantly from sarcoplasmic fish protein prepared by precipitation from 70% isopropanol.

These investigators and others (Lyons and Siebenthal, 1966), however, have shown that protein and condensed phosphates form complexes of varying composition, the composition of the complex being dependent on the concentration of phosphate and protein in solution and the pH at which the complex is formed. It is possible, therefore, that proteins complexed under different conditions could have different nutritional characteristics.

In the present study, sarcoplasmic fish proteins were prepared under various conditions with respect to phosphate concentration and pH. Some of the samples were abused by subjecting them to moist heat for varying periods. A measure of nutritional value of the isolated complexes was then assessed by determining the availability (enzyme digestion) of the essential amino acids lysine, methionine, threonine and tryptophan in the complexes. These particular amino acids were chosen for evaluation because the over-all nutritional value of protein foods is often limited by them. Knowledge of their availability would be useful for predicting the value of hexametaphosphate complexes of fish sarcoplasmic protein in mixed diets or as a supplement with other proteins. A microbiological assay system that offered a rapid means for this assessment was adopted from the work of Ford and Salter (1966).

MATERIALS & METHODS

Preparation of complexes

Sarcoplasmic proteins were obtained from rockfish (*Sebastodes melanops*) fillets and precipitated with hexametaphosphate (HP) according to the procedure described by Spinelli and Koury (1970). A noncomplexed control was prepared by precipitating the sarcoplasmic protein from 70% isopropanol (IPA). The samples were dried in a vacuum (water aspirator; 25 in. vacuum) at 80°C for 16 hr, then ground in a mortar and redried for 2 hr to remove traces of trapped IPA and stored under refrigeration.

Heat abuse of complexes

Samples containing 100 mg N were sealed in glass ampules in the presence of an equal weight of water. After sealing, the samples were equilibrated with the water by mechanical vibration. The ampules were heated to 100°C for 6, 12 and 24 hr.

Nitrogen

Total nitrogen was determined by the Kjeldahl method, Official Methods of Analysis (AOAC, 1960).

Amino acid determinations

Microbiological assay system. *Streptococcus zymogenes* (NCDO 592) was used for the methionine assay, *Streptococcus faecalis* (ATCC 9790) for the lysine and threonine assays and *Lactobacillus arabinosus* (ATCC 8014) for tryptophan. Media for the maintenance of stock cultures, growth of inoculi and bio-assay were all obtained from Difco. Their general procedures were followed (Difco Supplementary Literature, 1962) except that the assay media were sterilized by filtration rather than autoclaving. Growth was measured turbidimetrically at 580 mμ. Duplicate tubes were run in each determination and each amino acid value represents the mean of at least 2 and usually more determinations.

The inherent error of the bio-assay technique has been reported to be ± 15% (Stokes and Gunness, 1945), although this can be reduced by replicate determinations and careful attention to details.

S. faecalis (ATCC 9790) is subject to rapid autolysis when l-lysine is exhausted from the growth medium (Shockman et al., 1961). Timing of the turbidity readings was critical, therefore, since small differences in concentration of lysine in the standards vs. the concentration of lysine in the material being assayed could result in differences in the time of lysis onset.

Total amino acids. Total contents of lysine, methionine and threonine were determined by bio-assay on acid hydrolysates of the samples. Acid hydrolysis was accomplished by refluxing samples containing 100 mg N for 24 hr in 30 ml of 6–8 N HCl. The hydrolysate was adjusted to pH 7, diluted to 500 ml and filter-sterilized. Aliquots of this solution were diluted further to the analytical range for the bio-assay.

Available amino acids. Values for available amino acids represent the level of amino acid found available to the test microorganisms after the samples were digested with the enzyme Pronase (Calbiochem). Samples containing 100 mg N were suspended in 20 ml of 0.2 M beta-glycero-phosphate and digested with Pronase according to the procedure described by Ford and Salter (1966). Suspension of the sample in the buffer was accomplished by wet-grinding in a mortar. After digestion, the hydrolysates were heated to 100°C for 5 min, neutralized, diluted and filter-sterilized.

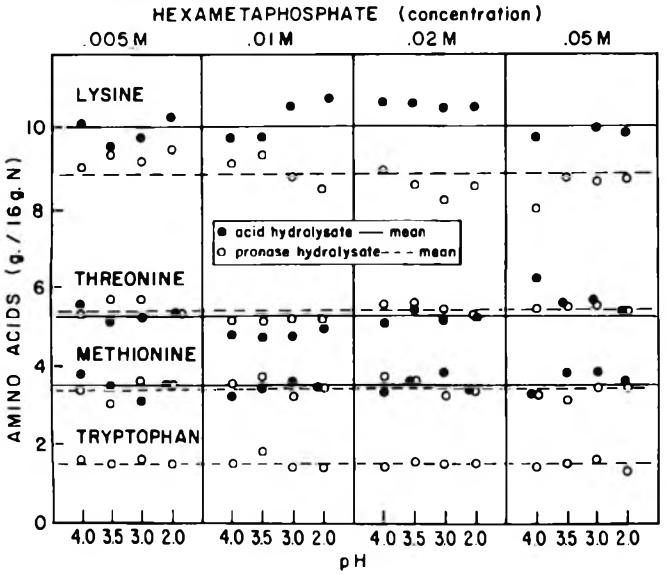

Fig. 1—Total and available amino acid content of sarcoplasmic fish protein complexed from solution in the presence of 0.005–0.05 M sodium hexametaphosphate at pH values ranging from 2.0–4.0.

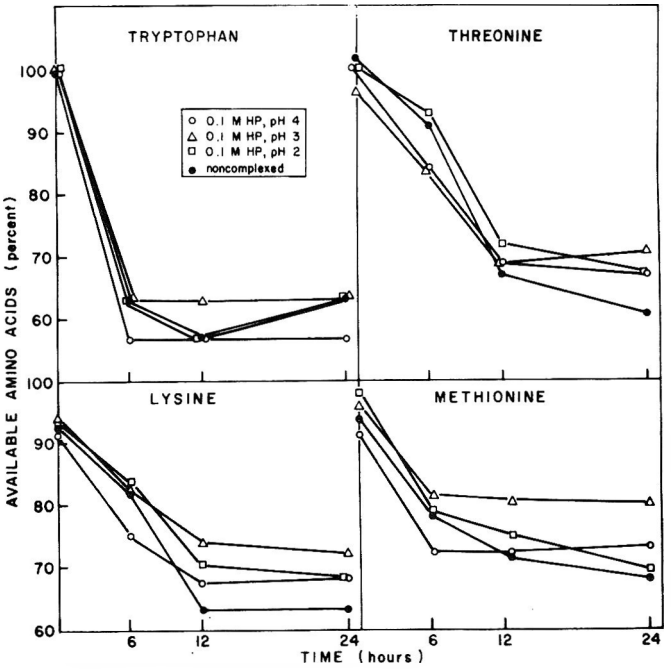

Fig. 2—Available amino acids in phosphate-complexed and noncomplexed fish sarcoplasmic protein heated to 100°C in the presence of an equal weight of water.

RESULTS

Total amino acid content

Total contents of lysine, methionine and threonine were determined by bioassay on acid hydrolysates of complexes prepared at phosphate concentrations ranging from 0.005–0.05 M and at pH values from 4.0–2.0 and on the IPA-precipitated control protein. Total tryptophan was not determined because of destruction during acid hydrolysis. Threonine is partially destroyed by the acid and the values as directly determined represent about 94% (Rees, 1946) of the actual threonine concentration.

Differences in the total content of the 4 amino acids among the various complexes (Fig. 1) were within ± 10% from the general mean. The mean content of lysine in the complexes was 10.11 g/16 g N, of threonine 5.25 g/16 g N, and of methionine 3.50 g/16 g N. For the noncomplexed control protein, these values were 9.85, 5.84 and 3.47 g/16 g N, respectively. The mean value for the complexes taken as a group was compared with the mean value for the noncomplexed control protein for the significance of the difference between them by the "t" test. The difference was not significant at the 1% level.

Available amino acid content

Among the complexes prepared under various conditions of molarity of phosphate and pH, there were no significant individual differences in the levels of available threonine, methionine and tryptophan (Fig. 1). The mean value for available threonine was 5.40 g/16 g N, for methionine 3.42 g/16 g N and for tryptophan 1.50 g/16 g N. Nor were these values significantly different from those obtained for the noncomplexed control protein, which were 5.99, 3.46 and 1.40 g/16 g N, respectively.

When the available amino acid content is compared with the total amino acid content, few differences are found. Mean values for available and total methionine are very nearly identical. The value for available threonine is slightly higher than the total threonine, but if a correction is made for loss of threonine in acid hydrolysis, then these values are also very nearly identical.

Lysine is the only amino acid in this study that showed a loss in availability when compared to the total values. The mean loss for the complexed samples was 12.47% and for the noncomplexed control 11.58%.

Differences in availability of lysine among the various HP-complexes were grouped both according to pH and molarity of HP and were compared statistically using the "t" test. The pH at which the complexes were formed did not appear to affect the availability of lysine in the various complexes when they were compared to each other nor when they were compared to the noncomplexed control protein.

When the lysine values were grouped according to the phosphate molarity of preparation, it appeared that the availability of lysine decreased as the concentration of phosphate was increased. Complexes prepared in the presence of 0.005 M and 0.01 M phosphate had mean values of 9.30 and 8.94 g available lysine/16 g N, respectively, compared to 9.29 g/16 g N for the noncomplexed samples. These differences were not significant. The mean value for available lysine in the complexes prepared from 0.02 M phosphate was 8.85 g/16 g N and this was significantly lower than the lysine available in the noncomplexed protein. The mean value for the 0.05 M phosphate preparations was 8.63 and this was significantly lower at the 5% level.

To test the validity of this trend, an additional complex was prepared using 0.1 M phosphate at pH 3.5. The available lysine content for this preparation, however, was not significantly different from the noncomplexed protein, indicating that phosphate concentration did not influence availability of lysine.

Effect of moist heat on availability of amino acids

Sarcoplasmic proteins precipitated at pH 2, 3 and 4 in 0.1 M phosphate and the IPA-precipitated sarcoplasmic proteins were sealed in ampules with an equal weight of water and heated to 100°C for up to 24 hr. Values for lysine and methionine (Fig. 2) were calculated as the ratio of the mean values from the enzymatic hydrolysate divided by the mean value from the acid hydrolysates (Fig. 1). Since tryptophan was destroyed by acid hydrolysis and threonine partially destroyed, the mean values from the enzymatic

hydrolysates were arbitrarily assumed to represent 100% availability prior to heat abuse.

Under the conditions of this experiment (Fig. 2), availability of lysine, methionine, tryptophan and threonine was reduced by 35, 22, 39 and 34%, respectively.

The greatest loss of tryptophan and methionine occurred in the first 6 hr of heating; the greatest loss of lysine and threonine in the first 12 hr. From 12–24 hr, there was relatively little further loss of any of the amino acids. There was no significant difference in rate or magnitude of destruction of availability between the complexes prepared at the various pH's nor between them and the noncomplexed sample.

DISCUSSION

FORD and Salter (1966) have pointed out that the term "availability" is meaningful only when the particular system used for measuring it is carefully defined. The method employed here combines microbiological assay with in vitro enzymic digestion. An acid and enzyme hydrolysis of each sample gives total and available amino acids, respectively. Any type of processing damage that reduces digestibility will be revealed. Products of the Maillard reaction, for example, that are not resistant to acid hydrolysis can be present in such enzymic digests and these bound amino acids may not be available to microorganisms for growth. Indeed, the quantity of amino acids in enzymatically digested proteins that had been damaged by heat, interaction with sugars or peroxy fats has been found to be reduced (Carpenter and March, 1961; Carpenter et al., 1963; Miller et al., 1965; Tannenbaum et al., 1969; Horn et al., 1952; 1968).

Although the damage caused by heat abuse was measured by reduced enzymatic digestibility in this study, the results have only relative, not absolute, values for predicting the in vivo nutritional value of the protein. This is because in severely damaged protein, factors other than digestibility may affect amino acid metabolism in the intact animal. This was demonstrated by Ford and Salter (1966) and Buraczeuski et al. (1967) in collaborative studies on preparations of cod fillets subjected to various heat treatments. They found that the enzymic release of several amino acids from the less severely heated preparations was consistent with the results of feeding tests with rats and with microbiological and chemical assays. In the severely heated preparations, however, more amino acids were released in vitro than were biologically available to the rat.

Results of the present study indicate that the digestibility of sarcoplasmic fish proteins is unimpaired by complexing in the presence of 0.005–0.05 M HP in the pH range of 2–4 when compared to a noncomplexed sarcoplasmic protein. Although the mechanism of the complexing reaction is different, dependent upon pH and protein-phosphate ratio, the variation in these conditions in the present study did not cause formation of complexes resistant to enzymic digestion.

Lysine was the only amino acid showing a lower value for available as compared to the total lysine content. The decrease in lysine availability may have been a result of the drying step in the preparation of the samples, since available lysine is readily destroyed by heat (Carpenter, 1960; Carpenter and March, 1961). Lyons and Siebenthal (1966) suggested that the site of binding of condensed phosphates to protein is probably with lysine and arginine. Since the loss of available lysine was no greater in the complexes prepared under the higher molarities of phosphate than in the noncomplexed proteins, it must be assumed that HP was not bound irreversibly under any of the conditions made in this study.

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G. DUBE,[1] V. D. BRAMBLETT, R. D. HOWARD, B. E. HOMLER,
H. R. JOHNSON, R. B. HARRINGTON and M. D. JUDGE
Departments of Home Economics and Animal Sciences, Purdue University, Lafayette, Indiana 47907

DIETARY EFFECTS ON BEEF COMPOSITION. 4. Processing and Palatability Attributes

SUMMARY—The influence of dietary regimen of 104 half-sib Angus steers allotted to 1 of 13 combinations of hay, corn silage and corn concentrate, and of slaughter weights of 284 vs. 340 kg and 409 vs. 454 kg (live weight) on processing qualities and palatability of beef was studied. Measurements included postmortem glycolytic activity, water- and fat-binding capacity, textural properties, cooking losses, flavor and juiciness of selected muscles. Results showed that feeding regimens had a greater effect than did slaughter weight on qualities studied. Corn silage in the early dietary regimen of cattle weighing 284 and 340 kg resulted in significantly lower pH values (5 and 45 min post-mortem), higher amounts of glycogen at slaughter and greater water- and fat-binding capacity as compared to hay. Similar effects of early diet were noted for 5- and 45-min pH and water-binding capacity in cattle fed to heavier weights (409 and 454 kg); however, in these animals the early-hay diet resulted in greater muscle glycogen deposition than the early-silage diet. Steaks from animals fed corn silage in the early period were determined to be more tender by both shear values and panel scores and were more juicy and flavorful than those from hay-fed animals. These differences in palatability due to early diet were still evident after the cattle had been fed to heavier (409 and 454 kg) weights. When steers weighing 409 and 454 kg were grouped together, the effects of feeding regimen during the intermediate period were considerably less pronounced than those of the early feeding period.

INTRODUCTION

OUR RESEARCH on beef composition and quality has shown that the dietary regimen of the animals is important in the control of variability in many economically important characteristics. The carcass traits used im marketing (Garrigus et al., 1969), patterns of fat deposition (Johnson et al., 1969) and the composition of muscle (Judge et al., 1969) were found to be controlled to a large extent by the level of dietary energy available to the animals. These studies demonstrated that the traditional systems of feeding (large quantities of concentrates) do not necessarily produce the most palatable cuts of beef.

The purpose of this study was to identify the influences, if any, of the dietary regimen of bovine animals on the processing qualities and palatability of beef. This report includes measures of postmortem glycolytic activity, water- and fat-binding capacity, textural properties, cooking losses, flavor and juiciness.

EXPERIMENTAL

ANIMALS used in this research were 104 half-sib Angus steers born within a 3-week period. They were allotted to 1 of 13 treatment groups. 1 group was slaughtered without further dietary treatment and designated as "control" animals. Details of the experimental design, including rations fed and weight gain, were described by Garrigus et al. (1969). The specific diets fed are shown in Tables 1–10.

Postmortem muscle properties

5 min after exsanguination of the animals, samples of the rectus abdominus muscle were secured (near the insertion on the pubis) for pH and glycogen analyses. Subsequent pH values were observed on samples secured at 45 min and 48 hr post-mortem. pH determinations were made with a combination probe electrode on the freshly cut muscle surface. The glycogen analyses were performed using the method of Seifter et al. (1950).

At 48 hr post-mortem, samples of the adductor, biceps femoris, longissimus dorsi and semitendinosus muscles were secured for determination of water-holding capacity. The method of Wierbicki et al. (1957) was used on ground meat samples heated for 30 min at 60°C and centrifuged at 230 g. The results were expressed as juice loss as a percent of total moisture (determined by oven drying at 94°C under vacuum).

At 48 hr post-mortem, samples of biceps femoris muscle were used to determine fat-emulsifying capacity. The model system described by Swift et al. (1961) was used with corn oil to determine maximum binding capacity. Results were expressed as ml of oil emulsified per g of fat-free tissue (determined by ether extraction).

Cooking and palatability evaluations

At 7 days post-mortem, boneless steaks 3.8 cm thick were cut from the longissimus dorsi and the biceps femoris muscles. Steaks were marked for identification, wrapped individually, frozen at −30°C and stored for subsequent cooking and taste panel evaluation.

When steaks were to be tested they were thawed in the original wrapping in a refrigerator at approximately 2°C for 18–24 hr just prior to cooking. Steaks were oven broiled at 177°C to an internal temperature of 65°C for the longissimus dorsi and to 71°C for the biceps femoris using the method described by Bramblett et al. (1963). A record at 15-sec intervals of oven temperature and internal temperature of the meat was made possible by using thermocouples and a multipoint temperature recorder. Total cooking time was noted and percentage total, volatile and dripping losses calculated.

Objective measurements

Measurements of both raw and cooked meat included firmness readings of steaks at 22°C using a Precision Penetrometer equipped with a multipoint spike pressure head. The depth of penetration by the spikes in 15 sec was recorded at 3 locations on each side of the steak and the 6 readings then averaged. A Beckman Expanding Scale pH Meter obtained the pH (both raw and cooked meats) of a slurry prepared from 20 g of finely chopped muscle and 40 ml of distilled water.

Raw and cooked samples were removed from adjacent locations on the same test steak. The weighed samples (0.5–1.0 g) were dried in a vacuum oven at 65°C under vacuum equivalent to 28 in. of mercury for 18 hr to determine moisture content.

Measurements on the cooked meat included percentage of press fluid using the 1-min method of Sanderson and Vail (1963), percentage of bound water and Warner-Bratzler shear values on 12, 1.3-cm-diameter cores cut parallel to the fibers.

Subjective evaluations

The organoleptic qualities of the steaks were rated by a panel of 8 experienced judges on a 1–9-point scale. Scores were given for broth flavor and intensity and steak flavor, flavor intensity, juiciness, tenderness (initial impression) and the amount of residue remaining in the mouth after a bite was masticated. Tenderness based on the number of chews was recorded.

Statistical analysis

Variance analyses were used to determine the effects of feeding regimen at various growth stages on the observed characteristics. Among cattle slaughtered at 284 and 340 kg live weight (groups 2, 3, 4 and 5), mean squares for early diet and slaughter weight effects were calculated. Mean squares for early diet, intermediate diet and slaughter weight were calculated for cattle slaughtered at 409 and 454 kg live weight (groups 6, 7, 8, 9, 10, 11, 12 and 13). The analysis was used to test for 2- and 3-way interactions between or among early diet, intermediate diet and slaughter weight.

RESULTS & DISCUSSION

Postmortem muscle properties

Corn silage vs. hay feeding in the early dietary regimen resulted in significant ($P < 0.05$) differences in 5- and 45-min postmortem pH of the rectus abdominus muscles with lower values attributed to the corn silage diet (Table 1, groups 2 and 4 vs. 3 and 5). Differences in pH were even more pronounced ($P < 0.01$) for the 5- and 45-min periods when the effects of the early diets were contrasted in cattle fed to heavier weights (Table 2, groups 6, 8, 10 and 12 vs. 7, 9, 11 and 13). Although pH values followed the same pat-

[1] Present address: Department of Home Economics, University of Ottawa, Ottawa, Canada.

Table 1–Means and analysis of variance for muscle properties of cattle slaughtered at 284 and 340 kg live weight.

Treatment	Group	Rectus abdominus pH 5 min	Rectus abdominus pH 45 min	Rectus abdominus pH 48 hr	Glycogen (mg %)	Juice loss as % total moisture[a,b] (%) A	Juice loss as % total moisture[a,b] (%) LD	Juice loss as % total moisture[a,b] (%) BF	Juice loss as % total moisture[a,b] (%) St	BF[a] fat-emulsifying capacity[c] (ml/g)
					Means					
Control[d]	1	6.42	6.18	5.59	326.32	32.00	28.51	25.68	29.96	53.43
Silage to 284 kg	2	6.43	6.20	5.60	992.87	33.50	30.95	28.89	32.16	51.09
Hay to 284 kg	3	6.46	6.25	5.73	731.24	36.28	30.29	32.22	34.26	45.65
Silage to 340 kg	4	6.43	6.22	5.64	1,021.43	35.29	29.38	30.08	31.18	49.65
Hay to 340 kg	5	6.57	6.35	5.73	655.42	36.92	31.91	31.58	32.00	47.70
Analysis of variance					Mean squares					
Source	d.f.									
Diet (D)[e]	1	0.06*	0.07*	0.01	787,867.38**	38.96	7.02	46.78*	17.13	109.15**
Slaughter wt (SW)[f]	1	0.02	0.03	0.003	4,466.47	11.80	0.005	0.63	20.99	0.75
D × SW	1	0.03	0.01	0.003	21,784.09	2.64	20.40	6.73	3.26	24.33*
Error	28	0.01	0.01	0.01	33,283.73	9.49	5.66	9.32	12.37	4.81

*P < .05. **P < .01.

[a]A = adductor, LD = longissimus dorsi, BF = biceps femoris, St = semitendinosus.

[b]After heating for 30 min at 60°C and centrifugation at 230 g.

[c]Ml corn oil emulsified per g of fat-free tissue.

[d]Control cattle (216 kg slaughter weight) were not included in analysis of variance.

[e]Hay vs. silage.

[f]284 vs. 340 kg.

Table 2–Means and analysis of variance for muscle properties of cattle slaughtered at 409 and 454 kg weight.

Treatment	Group	Rectus abdominus pH 5 min	Rectus abdominus pH 45 min	Rectus abdominus pH 48 hr	Glycogen (mg %)	Juice loss as % total moisture[a,b] (%) A	Juice loss as % total moisture[a,b] (%) LD	Juice loss as % total moisture[a,b] (%) BF	Juice loss as % total moisture[a,b] (%) St	BF[a] fat-emulsifying capacity (ml/g)
					Means					
Silage to 409 kg	6	6.50	6.24	5.70	577.09	39.35	29.38	31.98	33.22	48.40
Hay to 340 kg Silage to 409 kg	7	6.69	6.40	5.64	718.19	40.51	33.82	31.38	32.65	48.59
Silage to 340 kg Corn to 409 kg	8	6.52	6.19	5.69	521.62	35.82	29.67	30.21	32.33	48.63
Hay to 340 kg Corn to 409 kg	9	6.76	6.39	5.72	725.52	39.61	32.53	32.30	33.33	48.01
Silage to 340 kg Corn to 454 kg	10	6.56	6.29	5.68	533.09	37.00	30.65	30.38	33.20	49.11
Hay to 340 kg Corn to 454 kg	11	6.72	6.38	5.59	658.05	41.55	32.35	32.35	33.16	48.70
Silage to 409 kg Corn to 454 kg	12	6.57	6.28	5.69	631.93	37.21	30.78	31.91	33.70	49.06
Hay to 340 kg Silage to 409 kg Corn to 454 kg	13	6.74	6.36	5.63	874.37	40.07	31.99	32.13	32.81	47.18
Analysis of variance	d.f.				Mean squares					
Source										
Early diet (ED)[d]	1	0.58**	0.29**	0.04	507,542.59**	153.02**	95.25**	13.44	0.24	7.43
Intermediate diet (ID)[e]	1	0.003	0.001	0.0003	131,996.15	9.95	1.49	4.67	0.13	1.50
Slaughter wt (SW)[f]	1	0.01	0.01	0.03	24,031.90	0.29	0.01	0.82	1.77	0.18
ED × ID	1	0.001	0.001	0.01	2,990.75	18.72	0.40	19.70	5.91	0.46
ED × SW	1	0.01	0.04*	0.02	501.48	6.03	15.60	0.51	1.84	3.52
ID × SW	1	0.01	0.01	0.01	71,300.07	32.58	2.82	0.21	0.003	3.71
ED × ID × SW	1	0.003	0.001	0.01	32,500.95	0.91	2.63	0.88	0.49	5.18
Error	56	0.01	0.01	0.02	38,384.71	8.18	8.62	10.14	5.75	8.23

[a]A = adductor, LD = longissimus dorsi, BF = biceps femoris, St = semitendinosus.

[b]After heating for 30 min at 60°C and centrifugation at 230 g.

[c]Ml corn oil emulsified per g of fat-free tissue.

[d]Hay vs. silage to 340 kg.

[e]Silage vs. corn from 340–409 kg.

[f]409 vs. 454 kg.

*P < .05. **P < .01.

tern at 48 hr post-mortem, differences were not significant. Results also indicated that slaughter weight had little or no effect on pH.

The amount of glycogen in the rectus abdominus muscles was significantly ($P < 0.01$) higher in the corn silage- than the hay-fed steers (Table 1). However, when the 409- and 454-kg weight steers were considered, the effects of feeding regimen during the early feeding period were reversed and the hay-fed steers had higher ($P < 0.01$) muscle glycogen than silage-fed animals (Table 2). These results suggest that glycogen level was associated with rate of growth of the animals. The report of Garrigus et al. (1969) indicated that the hay-fed animals subsequently fed high-concentrate diets gained more rapidly in the late growth stages than those continuously on high-energy diets.

In general, muscles from corn silage-fed animals had superior water-holding capacity in the early postmortem period. This is shown by a greater loss of juice, as a percentage of total moisture, in muscles from steers fed hay than from those fed corn silage in all feeding regimens (Tables 1 and 2). In the lightweight animals, differences were significant ($P < 0.05$) for the biceps femoris only, but when the animals were fed to heavier weights the early diet resulted in significant differences in juice loss from the adductor ($P < 0.01$) and the longissimus dorsi ($P < 0.01$) muscles.

Even though the fat-emulsifying capacity was significantly higher ($P < 0.01$) in the biceps femoris from corn silage than from hay-fed steers (Table 1), the effect was not apparent when the cattle were continued to heavier weights (Table 2).

Results depicted in Tables 1 and 2 indicate that the feeding regimen can influence the properties of bovine muscle that are altered during its conversion to meat. The effects were evident in the early stages of growth and many of these early effects persisted after continued growth had taken place. In one instance (glycogen level), the early dietary effects were reversed in heavyweight animals. The interrelationships among pH, glycogen level and water-binding properties were not expected. However, it should be borne in mind that these dietary regimens resulted in large differences in animal age at slaughter. Further research is required to more fully explain the basis for these results.

Meat quality measurements

Although considerable information is available on the influence of diet and feeding on body composition of beef (cited by Garrigus et al., 1969), fewer studies report the influence of feeding on bovine muscle as food. In meat aged for 7 days, the effects of feeding regimen on longissimus dorsi firmness, pH, percentage of moisture in the raw meat, water-holding capacity (bound water) and cooking losses were not significant for the early diet period (Table 3). These data substantiate findings of Jacobson and Fenton (1956), who reported that level of nutrition seemed to have no influence on cooking losses.

Differences in over-all meat quality of the longissimus dorsi muscle due to the early dietary regimen became somewhat pronounced when the cattle were fed to heavy weights (Table 4). In these animals, the early corn silage diet resulted in lower ($P < 0.01$) pH of cooked meat, greater ($P < 0.05$) firmness of raw meat and lower moisture content of both raw ($P < 0.01$) and cooked ($P < 0.01$) meat as compared to the early hay diet. With an increase in slaughter weights from 409–454 kg, significant decreases in moisture content of both raw ($P < 0.01$) and cooked ($P < 0.05$) longissimus dorsi steaks were obtained. These differences were expected, since moisture content is known to be inversely related to the intramuscular fat content of raw meat (Doty and Pierce, 1961) and we observed in part II of this study (Johnson et al., 1969) that an increase in intramuscular fat in the longissimus dorsi occurred as weight increased from 409–454 kg.

The biceps femoris, unlike the longissimus dorsi, showed significant ($P < 0.05$) differences in percentages of total cooking losses, drip losses and evaporation losses (Table 5) attributed to the early diets (greater in silage-fed groups), but were not significant when the effects of the early diet were contrasted in cattle fed to heavier weights (Table 6). There were also highly significant ($P < 0.01$) differences in pH (Table 5) of the raw biceps femoris of cattle weighing 284 and 340 kg as influenced by the early diet period (high in silage-fed groups). Inconsistent treatment effects were noted when the 48 hr pH of the rectus abdominus was compared to the 7 day pH of the biceps femoris. Several possibilities exist to account for these differences including effects of muscle activity, effects of 7-day aging and effects of freezing. When cattle were fed to heavier weights, pH values of the raw meat were similar in all groups and pH for cooked meat (Table 6) was influenced ($P < 0.01$) by early diet (greater for hay-fed groups).

Firmness of cooked biceps femoris steaks was greater ($P < 0.05$) in hay-fed animals at the end of the early feeding period (Table 5), but was greater ($P < 0.01$) in raw steaks from early silage-fed animals fed to heavier weights (Table 6).

Meat flavor

The feeding regimens resulted in marked differences in palatability characteristics of the meat. Corn silage, as compared to hay during the early feeding period, resulted in more desirable broth flavor ($P < 0.01$) and steak flavor ($P < 0.01$) as well as greater intensity of broth flavor ($P < 0.05$) in the longissimus dorsi (Table 7). Differences due to early diet were highly significant ($P < 0.01$) for all measures of flavor in the biceps femoris (Table 9).

When the effects of the early diet of cattle fed to heavier weights were contrasted, silage-fed cattle were superior in flavor scores for longissimus dorsi ($P < 0.01$) and biceps femoris ($P < 0.01$) steaks and in scores for intensity of flavor of broth ($P < 0.05$) and steaks ($P < 0.01$) from the longissimus dorsi (Tables 8 and 10). Some significant but less pronounced effects of intermediate diet and slaughter weight were also noted in the heavy (409 and 454 kg) cattle.

Explanations for the observed influence of early diet on flavor desirability and intensity are not obvious. No direct association between flavor and intramuscular fat levels existed, as evidenced by the observations that the early corn silage diet improved flavor scores in both light (284 and 340 kg) and heavy (409 and 454 kg) weight cattle, and Johnson et al. (1969) reported that this feeding regimen reduced intramuscular fat in the lightweight cattle but increased it in the heavyweight cattle. Additionally, one might expect more pronounced flavor in meat from animals of advanced age, yet the silage-fed cattle were younger at slaughter than those fed hay.

Meat tenderness

Steers fed corn silage were determined to be more tender than those fed hay during the early feeding period. All criteria of tenderness (shear values, taste panel scores for tenderness and residue and the number of chews required) were significantly better in longissimus dorsi and biceps femoris steaks from corn silage-fed animals (Tables 3, 5, 7 and 9). Tenderness of steaks was also influenced by the early diet in cattle fed to heavier weights (Tables 4, 6 and 8).

It is likely that the observed dietary effects on tenderness were associated with the chronological age of the animals. The early silage diets resulted in more rapid weight gains than the hay diets; consequently, the animals were younger when they reached slaughter weight (Garrigus et al., 1969). This would explain the persistent influence of early diet after the cattle had been fed to live weights of 409 and 454 kg.

Meat juiciness

Steak juiciness scores were generally higher from corn silage- vs. hay-fed steers, although the differences in scores were not significant for the longissimus dorsi (Table 7) but were highly significant ($P \leqslant 0.01$) for the biceps femoris (Table

Table 3–Means and analysis of variance for longissimus dorsi properties from cattle slaughtered at 284 and 340 kg live weight.

Treatment	Group	Cooking losses (%) Total	Drip	Evaporation	Firmness Raw	Firmness Cooked	pH Raw	pH Cooked	Total moisture (%) Raw	Total moisture (%) Cooked	1-min press fluid (%)	Bound water (%)	Warner-Bratzler shear (lb)
						Means							
Control[a]	1	17.26	1.36	15.85	185.28	117.88	5.59	5.74	73.96	67.91	50.96	21.20	4.00
Silage to 284 kg	2	15.38	1.28	14.08	181.51	112.15	5.60	5.73	73.44	67.94	51.38	21.06	3.65
Hay to 284 kg	3	15.31	0.95	14.38	168.03	113.10	5.54	5.70	73.26	68.86	52.08	20.51	5.10
Silage to 340 kg	4	15.38	0.93	14.44	179.95	122.34	5.59	5.75	73.09	67.86	53.73	19.99	3.70
Hay to 340 kg	5	14.54	0.90	13.64	169.09	108.59	5.49	5.68	73.26	68.91	52.84	20.28	4.66
Analysis of variance						Mean squares							
Source	d.f.												
Diet (D)[b]	1	1.62	0.25	0.50	1,185.85	327.68	0.06	0.02	0.00	7.80*	0.07	0.14	11.64**
Slaughter wt (SW)[c]	1	1.20	0.32	0.28	0.50	64.41	0.01	0.0001	0.25	0.001	19.38*	3.45	0.03
D × SW	1	1.20	0.18	2.42	13.78	432.18	0.003	0.002	0.25	0.03	5.04	1.40	0.48
Error	28	2.84	0.18	3.20	594.90	359.20	0.02	0.006	0.89	1.54	5.83	2.41	1.14

*P < .05. **P < .01.
[a]Control cattle (216 kg slaughter weight) were not included in analysis of variance.
[b]Hay vs. silage.
[c]284 vs. 340 kg.

Table 4–Means and analysis of variance for longissimus dorsi properties from cattle slaughtered at 409 and 454 kg live weight.

Treatment	Group	Cooking losses (%) Total	Drip	Evaporation	Firmness Raw	Firmness Cooked	pH Raw	pH Cooked	Total moisture (%) Raw	Total moisture (%) Cooked	1-min press fluid (%)	Bound water (%)	Warner-Bratzler shear (lb)
						Means							
Silage to 409 kg	6	14.43	1.04	13.40	165.64	105.10	5.46	5.65	69.84	65.56	50.89	21.56	3.23
Hay to 340 kg Silage to 409 kg	7	14.91	1.31	13.60	185.40	118.85	5.47	5.71	72.39	66.95	51.49	20.99	4.10
Silage to 340 kg Corn to 409 kg	8	14.90	1.28	13.64	176.31	116.39	5.48	5.69	69.79	64.54	51.15	21.10	3.68
Hay to 340 kg Corn to 409 kg	9	13.76	1.14	12.66	190.40	112.09	5.47	5.70	70.96	68.18	52.31	20.60	3.73
Silage to 340 kg Corn to 454 kg	10	15.35	1.50	13.83	179.73	110.11	5.44	5.66	69.71	64.74	51.75	20.59	3.59
Hay to 340 kg Corn to 454 kg	11	14.08	1.43	12.64	183.08	113.68	5.48	5.70	70.13	65.08	52.46	20.31	3.85
Silage to 409 kg Corn to 454 kg	12	14.50	1.21	13.58	189.75	115.86	5.49	5.67	68.26	65.44	52.11	20.43	3.55
Hay to 340 kg Silage to 409 kg Corn to 454 kg	13	13.74	0.84	12.94	195.23	121.60	5.49	5.70	68.99	65.79	45.19	17.89	3.50
Analysis of variance						Mean squares							
Source	d.f.												
Early diet (ED)[a]	1	7.22	0.10	6.76	1,821.16*	351.56	0.001	0.02**	23.64**	32.63**	19.80	15.11	1.29*
Intermediate diet (ID)[b]	1	0.26	0.88	0.56	42.25	83.72	0.002	0.001	1.24	1.47	64.00	3.02	0.21
Slaughter wt (SW)[c]	1	0.11	0.05	0.11	901.50	77.88	0.0001	0.00004	34.66**	17.54*	18.71	25.38	0.06
ED × ID	1	4.57	0.13	2.98	60.84	409.05	0.00004	0.0001	2.85	5.01	67.24	5.46	0.26
ED × SW	1	1.93	0.35	1.10	626.25	0.02	0.001	0.0001	6.70	18.81*	63.60	3.02	0.51
ID × SW	1	3.47	0.66	0.42	1,432.62	331.24	0.004	0.002	15.40*	2.60	33.93	11.82	0.10
ED × ID × SW	1	1.24	0.51	0.39	12.60	252.02	0.003	0.004	1.13	5.12	50.06	4.79	1.29*
Error	56	2.52	0.32	1.86	395.41	231.94	0.002	0.002	2.83	4.36	44.85	7.77	0.32

[a]Hay vs. silage to 340 kg.
[b]Silage vs. corn from 340–409 kg.
[c]409 vs. 454 kg.
*P < .05. **P < .01.

Table 5–Means and analysis of variance for biceps femoris properties from cattle slaughtered at 284 and 340 kg live weight.

Treatment	Group	Cooking losses (%) Total	Drip	Evaporation	Firmness Raw	Firmness Cooked	pH Raw	pH Cooked	Total moisture (%) Raw	Total moisture (%) Cooked	1-min press fluid (%)	Bound water (%)	Warner-Bratzler shear (lb)
						Means							
Control[a]	1	19.27	3.20	16.55	169.40	108.62	5.68	5.82	74.62	70.40	52.68	21.22	4.32
Silage to 284 kg	2	18.48	2.65	16.54	174.89	106.63	5.63	5.78	73.91	68.84	51.41	21.80	5.13
Hay to 284 kg	3	15.55	1.60	13.95	156.71	84.39	5.49	5.74	74.83	68.81	53.03	21.11	7.14
Silage to 340 kg	4	20.48	2.40	18.08	164.81	98.81	5.59	5.75	73.73	68.58	52.35	21.35	5.58
Hay to 340 kg	5	17.50	2.35	15.15	159.03	87.35	5.51	5.74	74.23	69.80	52.54	21.14	7.61
Analysis of variance						Mean square							
Source	d.f.												
Diet (D)[b]	1	69.62*	2.42*	60.78*	1,148.40	2,271.38*	0.09**	0.003	3.99	2.88	6.48	1.62	32.81**
Slaughter wt (SW)[c]	1	31.21	0.50	14.99	120.51	47.05	0.001	0.001	1.24	1.05	0.41	0.36	1.71
D × SW	1	0.01	2.00	0.23	306.90	232.20	0.01	0.002	0.24	3.13	4.06	0.45	0.001
Error	28	11.09	0.48	8.25	400.00	485.62	0.01	0.003	1.26	4.44	9.60	3.91	0.78

[a]Control cattle (216 kg slaughter weight) were not included in analysis of variance.
[b]Hay vs. silage.
[c]284 vs. 340 kg.
*P < .05. **P < .01.

Table 6–Means and analysis of variance for biceps femoris properties from cattle slaughtered at 409 and 454 kg live weight.

Treatment	Group	Cooking losses (%) Total	Drip	Evaporation	Firmness Raw	Firmness Cooked	pH Raw	pH Cooked	Total moisture (%) Raw	Total moisture (%) Cooked	1-min press fluid (%)	Bound water (%)	Warner-Bratzler shear (lb)
						Means							
Silage to 409 kg	6	17.76	2.30	15.48	160.05	88.48	5.49	5.68	72.45	66.60	52.35	21.04	6.33
Hay to 340 kg Silage to 409 kg	7	19.73	2.88	16.88	187.74	100.76	5.52	5.74	72.05	66.80	51.44	21.29	7.10
Silage to 340 kg Corn to 409 kg	8	18.28	2.31	15.96	166.13	93.65	5.51	5.72	71.54	66.50	52.83	20.74	6.89
Hay to 340 kg Corn to 409 kg	9	17.13	2.58	14.54	184.00	98.19	5.53	5.76	72.18	66.45	51.36	21.30	7.48
Silage to 340 kg Corn to 409 kg	10	18.65	3.31	15.34	166.85	100.93	5.50	5.70	72.06	65.64	51.54	20.75	6.28
Hay to 340 kg Corn to 454 kg	11	17.13	2.61	14.51	178.00	98.24	5.51	5.71	72.15	66.74	52.68	20.79	7.60
Silage to 409 kg Corn to 454 kg	12	18.44	3.58	14.88	175.35	97.31	5.52	5.71	71.40	65.38	50.80	21.54	6.85
Hay to 340 kg Silage to 409 kg Corn to 454 kg	13	16.68	2.38	14.29	180.94	109.38	5.55	5.73	65.53	70.56	53.73	20.05	6.98
Analysis of variance						Mean squares							
Source	d.f.												
Early diet (ED)[a]	1	6.13	1.13	2.07	3,875.06**	686.44	0.01	0.02**	1.96	0.26	2.85	0.41	7.91**
Intermediate diet (ID)[b]	1	2.03	0.10	1.35	83.72	24.26	0.002	0.0001	1.05	2.14	0.01	0.11	0.98
Slaughter wt (SW)[c]	1	4.00	3.29*	14.73	10.40	613.80	0.001	0.002	9.46	4.15	0.58	1.53	0.01
ED × ID	1	8.27	0.04	9.38	18.49	506.25	0.002	0.001	0.49	3.85	5.46	3.38	1.03
ED × SW	1	16.81	7.49**	1.93	830.88	55.50	0.0001	0.0047	1.21	0.98	41.44	5.12	0.01
ID × SW	1	7.56	0.07	6.44	189.75	102.52	0.01	0.008	3.71	9.23*	0.51	0.06	0.79
ED × ID × SW	1	11.22	0.66	6.70	236.39	49.00	0.0001	0.0004	1.44	0.01	1.53	1.47	1.93
Error	56	10.45	0.58	8.47	307.82	290.50	0.004	0.003	3.85	2.10	10.79	2.80	1.00

[a]Hay vs. silage to 340 kg.
[b]Silage vs. corn from 340–409 kg.
[c]409 vs. 454 kg.
*P < .05. **P < .01.

Table 7–Means and analysis of variance for longissimus dorsi palatability from cattle slaughtered at 284 and 340 kg live weight.

Treatment	Group	Broth[a]		Steak[a]					Steak
		Flavor	Intensity	Flavor	Intensity	Juiciness	Tenderness	Residue	No. chews
					Means				
Control[b]	1	5.69	5.98	5.56	5.89	6.15	6.38	6.46	34.04
Silage to 284 kg	2	5.65	6.13	5.75	5.93	6.15	6.78	6.98	33.81
Hay to 284 kg	3	4.45	5.63	4.38	5.54	5.71	4.56	5.43	41.70
Silage to 340 kg	4	5.66	6.26	6.26	6.14	6.31	6.96	6.96	28.23
Hay to 340 kg	5	4.69	5.60	4.66	6.08	5.90	5.21	5.56	42.81
Analysis of variance					Mean squares				
Source	d.f.								
Diet (D)[c]	1	9.46**	2.70*	17.70**	0.41	1.45	31.40**	17.41**	1010.25**
Slaughter wt (SW)[d]	1	0.13	0.03	1.28	1.13	0.25	1.40	0.03	40.05
D × SW	1	0.10	0.05	0.10	0.21	0.001	0.43	0.05	89.78
Error	28	0.67	0.46	0.34	0.35	0.49	1.00	0.52	26.86

[a]Maximum score = 9.0.
[b]Control cattle (216 kg slaughter weight) were not included in analysis of variance.
[c]Hay vs. silage.
[d]284 vs. 340 kg.
*P < .05. **P < .01.

Table 8–Means and analysis of variance for longissimus dorsi palatability from cattle slaughtered at 409 and 454 kg live weight.

Treatment	Group	Broth[a]		Steak[a]					Steak
		Flavor	Intensity	Flavor	Intensity	Juiciness	Tenderness	Residue	No. chews
						Means			
Silage to 409 kg	6	5.63	6.36	6.44	6.44	6.86	7.30	7.54	31.75
Hay to 340 kg Silage to 409 kg	7	5.76	5.74	5.66	5.71	6.39	6.16	6.95	37.55
Silage to 340 kg Corn to 409 kg	8	5.56	6.19	6.18	6.46	6.66	7.09	7.26	30.60
Hay to 340 kg Corn to 409 kg	9	5.53	5.85	5.86	6.34	6.39	6.26	7.10	35.56
Silage to 340 kg Corn to 454 kg	10	5.76	6.09	6.01	6.50	6.21	6.84	7.36	33.19
Hay to 340 kg Corn to 454 kg	11	5.55	5.63	6.03	6.16	6.58	6.64	7.25	33.30
Silage to 409 kg Corn to 454 kg	12	5.84	6.03	6.48	6.58	6.93	7.21	7.36	30.53
Hay to 340 kg Silage to 409 kg Corn to 454 kg	13	5.83	6.10	6.34	6.49	6.95	6.90	7.33	31.71
Analysis of variance						Mean squares			
Source	d.f.								
Early diet (ED)[b]	1	0.03	1.82*	1.47**	1.63**	0.13	6.13**	0.81*	145.50**
Intermediate diet (ID)[c]	1	0.49	0.23	0.70*	0.06	1.66*	0.56	0.04	1.24
Slaughter wt (SW)[d]	1	0.20	0.09	0.51	0.60*	0.13	0.60	0.20	45.40
ED × ID	1	0.11	0.06	0.38	0.12	0.29	0.18	0.12	3.66
ED × SW	1	0.08	0.33	0.93*	0.18	1.29*	2.10*	0.36	89.54**
ID × SW	1	0.00	0.12	0.51	1.10**	0.79	0.28	0.003	54.58*
ED × ID × SW	1	0.01	0.68	0.10	0.72*	0.02	0.04	0.25	0.06
Error	56	0.39	0.32	0.15	0.15	0.28	0.38	0.14	11.90

[a]Maximum score = 9.0.
[b]Hay vs. silage to 340 kg.
[c]Silage vs. corn from 340–409 kg.
[d]409 vs. 454 kg.
*P < .05. **P < .01.

Table 9—Means and analysis of variance for biceps femoris palatability from cattle slaughtered at 284 and 340 kg live weight.

Treatment	Group	Broth[a]		Steak[a]					Steak
		Flavor	Intensity	Flavor	Intensity	Juiciness	Tenderness	Residue	No. chews
					Means				
Control[b]	1	5.58	5.97	5.67	5.82	5.10	6.25	5.87	32.53
Silage to 284 kg	2	6.03	6.46	6.23	6.13	5.91	5.83	6.05	34.08
Hay to 284 kg	3	4.90	5.86	4.34	5.50	3.44	4.56	3.33	47.79
Silage to 340 kg	4	5.90	6.59	5.96	6.36	5.46	5.95	5.10	35.84
Hay to 340 kg	5	4.80	5.56	4.68	5.68	3.54	5.09	3.61	47.90
Analysis of variance					Mean squares				
Source	d.f.								
Diet (D)[c]	1	9.90**	5.28**	20.16**	3.45**	38.72**	9.03**	35.49**	1328.70**
Slaughter wt (SW)[d]	1	0.10	0.06	0.01	0.34	0.25	0.85	0.88	7.03
D × SW	1	0.001	0.36	0.72	0.01	0.61	0.32	3.06*	5.44
Error	28	1.03	0.29	0.56	0.41	0.53	0.80	0.55	21.41

[a]Maximum score—9.0.
[b]Control cattle (216 kg slaughter weight) were not included in analysis of variance.
[c]Hay vs. silage.
[d]284 vs. 340 kg.
*P < .05. **P < .01.

Table 10—Means and analysis of variance for biceps femoris palatability from cattle slaughtered at 409 and 454 kg live weight.

Treatment	Group	Broth[a]		Steak[a]					Steak
		Flavor	Intensity	Flavor	Intensity	Juiciness	Tenderness	Residue	No. chews
						Means			
Silage to 409 kg	6	5.14	5.94	5.99	6.01	5.66	4.80	4.84	40.63
Hay to 340 kg Silage to 409 kg	7	5.40	5.80	5.16	6.30	5.28	4.11	4.49	45.51
Silage to 340 kg Corn to 409 kg	8	5.30	6.08	5.69	6.16	5.49	4.44	4.54	43.40
Hay to 340 kg Corn to 409 kg	9	5.28	5.73	5.59	6.36	5.90	4.26	4.44	45.94
Silage to 340 kg Corn to 454 kg	10	5.73	6.10	5.94	6.18	5.23	4.53	4.74	43.09
Hay to 340 kg Corn to 454 kg	11	6.03	5.80	5.23	5.96	5.60	4.09	4.06	44.51
Silage to 409 kg Corn to 454 kg	12	5.51	5.55	6.24	6.54	5.75	4.43	4.50	44.54
Hay to 340 kg Silage to 409 kg Corn to 454 kg	13	5.63	6.14	5.56	6.01	5.76	4.70	4.40	43.18
Analysis of variance						Mean squares			
Source	d.f.								
Early diet (ED)[b]	1	0.42	0.04	5.35**	0.06	0.17	1.05	1.50	56.06
Intermediate diet (ID)[c]	1	0.42	0.08	0.26	0.04	0.06	0.53	0.20	9.53
Slaughter wt (SW)[d]	1	3.15*	0.003	0.29	0.02	0.0002	0.02	0.36	0.03
ED × ID	1	0.01	1.21	0.47	0.05	1.35	0.04	0.11	0.19
ED × SW	1	0.03	0.60	0.21	1.50*	0.13	0.49	0.11	54.21
ID × SW	1	0.33	0.02	0.58	0.39	1.29	0.09	0.06	10.97
ED × ID × SW	1	0.23	0.46	0.58	0.16	0.19	1.50	0.68	26.39
Error	56	0.53	0.39	0.33	0.32	0.63	0.46	0.47	24.75

[a]Maximum score = 9.0.
[b]Hay vs. silage to 340 kg.
[c]Silage vs. corn from 340–409 kg.
[d]409 vs. 454 kg.
*P < .05. **P < .01.

9). It is likely that the higher juiciness scores in biceps femoris muscle from silage-fed animals resulted from greater fat content, since these same animals had greater cooking losses in this muscle.

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WILLIAM F. HENRY, MORRIS H. KATZ, FRANCIS J. PILGRIM and ALFRED T. MAY
The Pillsbury Company, 311 Second Street S. E., Minneapolis, Minn. 55414

TEXTURE OF SEMI-SOLID FOODS: SENSORY AND PHYSICAL CORRELATES

SUMMARY—Texture profile analyses (TPA) were conducted on a series of commercial desserts (puddings, custards, and gelatin) and on whipped toppings and marshmallow creme. Textural attributes of firmness, elasticity, cohesiveness, gumminess, and chewiness were determined on each product, (a) under compression as in conventional TPA, and (b) under tension to provide five new additional textural parameters. They are referred to as firmness under compression (F_C), firmness under tension (F_A), elasticity under compression (E_C), elasticity under tension (E_A), etc. Additional attributes of stringiness, maximum tensile force, and work of extension, were also measured. The products were also evaluated by a taste panel for fifteen sensory attributes related to texture. 18 samples were prepared from several products, each of which was altered mainly by using various liquid levels to change the physical and sensory properties. These changes were measured by the conventional TPA method and by the additional procedures described. Precision of the physical test values and precision and reliability of the sensory rating were evaluated. Precision and reliability were deemed sufficient to warrant including all of the physical responses and all of the sensory ratings in a further analysis of the data. Separate factor analyses were performed on the physical test values and on the sensory ratings. Four factors were identified for each set of data, and these accounted for more than 90% of the variation in each case. Multiple regression equations were established for predicting the four factors derived from the sensory ratings by means of the physical test values. Although 15 physical values were available, only 8 were needed for the prediction of the 4 sensory factors. It should be emphasized that some of the additional physical test values obtained under tension, were needed for these predictions as well as the standard physical test values obtained under compression.

INTRODUCTION

THE LACK OF adequate methods for finding mechanical (physical) substitutes for sensory evaluations of food texture has long been recognized. Szczesniak (1963) stressed the need for a rational and consistent system for describing and translating textural qualities into precisely defined, measurable rheological properties. She classified textural characteristics into mechanical, geometrical and other categories. Brandt et al. (1963) elaborated on this classification of sensory qualities. Szczesniak et al. (1963a) developed rating scales for correlations between objective and sensory evaluations of texture. Szczesniak (1966) discussed the principles and problems of instrumental texture description relative to characteristics manifested during food consumption. She classified physical textural measurements into three classes: fundamental, empirical, and imitative. Szczesniak et al. (1963b) used the approach of a work association test, which was applied to 100 people to determine their degrees of texture consciousness and terms used to describe texture. The results suggested that texture is an important influence on the consumer's image of a food. Friedman et al. (1963) described the food texturometer, at that time a new instrument for physical measurements on food texture. Szczesniak (1968) discussed relationships between sensory and physical textural measurements by the use of correlation coefficients with appropriate conditions and transformations of variables to arrive at better correlations.

Bourne et al. (1966) adopted the Instron for use in textural studies (texture profile analysis) and applied it to such foods as cherries, potato chips, apples, and peas. Bourne (1968) subsequently pointed out the desirability of considering textural measurements as multi-point, rather than single-point, values. His method allowed separate values for hardness, brittleness, elasticity, cohesiveness, gumminess, and chewiness; hence it constituted an amplification of the pioneering work done by Szczesniak and others.

Henry et al. (1968) introduced a method for measuring stringiness (or inversely, shortness) of certain fluid and semi-solid foods. This method involved contacting a plastic disc with a flat surface of the material to be tested and pulling the material away from the disc at a fixed rate. The distance the surface of the material had to be moved downward to obtain rupture of the strand of material adhering to the disc was a measure of the stringiness or shortness of the material.

Morrow et al. (1968) approached texture characterization by a different technique. They used dynamic methods including direct stress-strain methods, transducer methods, resonance methods and wave propagation, in which the results appear as storage and loss moduli.

Scott-Blair (1938) studied the elastic and plastic properties of cheese curd. Scott-Blair (1941) related the consistency of cheese curd at the pitching point to the firmness and quality of the finished cheese. Probably the first use of factor analysis in connection with foods was that of Harper and Baron (1948), in their work on Cheddar cheese. They reported further work on cheese at a later date (1951) and in 1952 they reported on factor analysis as a technique for handling complex data on foodstuffs. Harper (1947, 1949) discussed firmness of deformable materials. He also published a paper on psychological and psychophysical studies of craftsmanship in dairying (1952). Harper et al. (1964) studied the subjective hardness of compound materials.

The purpose of our present work is to measure the textural parameters of a certain class of semi-solid foods (dessert puddings, pie fillings, and whipped toppings). To do this, the physical parameters involved must first be measured, including the texture profile parameters under compression (conventional) and under tension (new values); and also including the new parameters of stringiness, maximum tensile force, and work of tension. Second, the sensory qualities must be evaluated by a taste panel. Finally, correlations must then be sought between the relevant physical and sensory attributes by application of standard statistical methods, such as multiple regression analysis and factor analysis.

EXPERIMENTAL

Preparation of foods for testing

A number of commercially available packaged desserts and toppings were selected for this study.[1] They were made up according to instructions on the packages, except the marshmallow creme which was used "as is." In addition, the desserts were prepared using (a) more liquid than called for in the recipe, and (b) less liquid, to vary the "consistency" of each one to cover a range of rheological and sensory qualities. An additional sample of marshmallow creme was prepared by heating it to 80°C while stirring until its volume decreased by about 50% due to the release of part of the incorporated air with a resultant increase in density. This treatment altered its rheological properties to a degree deemed sufficient for the purposes of this study. A sufficient amount of each sam-

[1] Sources of Products—Marshmallow Creme: Kraft Foods; Fluffy White Frosting: The Pillsbury Company; Whipped Topping: "Dream Whip," General Foods Corporation; Vanilla Cooked Pudding: General Foods Corporation, Jello Brand; Vanilla Instant Pudding: General Foods Corporation, Jello Brand; Lemon Gelatin: General Foods Corporation, Jello Brand; Golden Egg Custard: General Foods Corporation, Jello Brand.

ple was prepared for evaluation by a taste panel of 26 members. All samples were poured into custard dishes (about 3 × 2 in.) and allowed to remain in the refrigerator (40°F) for 2–4 hr after preparation before testing, except the marshmallow creme which was kept at room temperature (72°F). The sample which was treated by stirring and heating to 80°C was merely allowed to return to 72°F before testing.

Physical testing of food samples

For testing, a cylindrical core (about 1 in. high by 1 in. dia) of the food was cut from the supply in its dish and placed carefully on the table (c) of the Instron. (See Fig. 1) The top of the specimen was trimmed with a knife or razor blade with minimum disturbance until it was horizontal and flat. Table (c) rested on top of load cell CB, balanced to zero at the recorder scale mid-point and calibrated to an appropriate full scale load at both ends of the chart scale. The crosshead was fitted with a plastic anvil (A) made of plexiglass with the lower surface roughened by rubbing with emery cloth. The crosshead was lowered manually until the bottom anvil surface just contacted the test sample (B). The gage length was then set to zero and the pen balanced at the zero (chart mid-point) line. Then the crosshead was allowed to descend at the rate of 0.5 in. per min to a total deformation of 0.25 in. Then the direction was reversed and the crosshead was allowed to move upward at the same speed until the gage length scale was back to zero. Then a second down-and-up cycle was run on the same sample. Figure 2 represents a typical curve obtained on this type of semi-solid food, reversed so that one reads it from left to right in the normal manner.

The height of the first peak (end of first downstroke) is the force resisting compression at 0.25 in. deformation (Fig. 2). It is referred to as the firmness (F_c). The distance d, from the rise of the force curve above zero on the second downstroke, to the end of the second downstroke, is a measure of the elastic recovery of the material in inches (E_c). The area A_2 represents the work done during the second down (compression) stroke and area A_1 is the work of compression on the first downstroke. The ratio (A_2/A_1) represents the cohesiveness of the material under compression (C_c). In addition to these three primary parameters, two secondary quantities are derived from them:

G_c = Gumminess = $F_c \times C_c$
Ch_c = Chewiness = $F_c \times C_c \times E_c$

Note that on the upstroke, part of the curve lies below the zero line, where "negative" forces represent forces of cohesion while tension is applied to the previously compressed sample. We can define five new parameters of tension,

F_A = maximum depth of force curve during first upstroke (grams) (firmness under tension)
E_A = elasticity under tension = d′ (inches) (elastic recovery)
C_A = ratio of areas (A_4/A_3) (cohesiveness under tension)
G_A = gumminess under tension = $F_A \times C_A$
Ch_A = chewiness under tension = $F_A \times C_A \times E_A$

In addition, a stringiness test (S-test) was run on the same samples. This test was described by Henry et al. (1968). A small petri dish was filled from the supply in the custard cup. It was leveled by running a spatula across the top. Use was made of the "A" tension cell in the Instron. A plastic (plexiglass) disc, suspended from the tension cell, was contacted with the sample surface so that the material adhered to the disc by wetting it. When the platform carrying the dish was lowered at 0.5 in./min, the extension of the material at cleavage (S-value), the maximum tensile force (T), and the work of extension or area under the force-distance curve (W) were recorded.

Sensory testing of food samples

For taste panel evaluation, some of each product was poured into 4-oz. clear plastic cups. They were all kept under refrigeration (40°F) except the marshmallow creme (72°F) for 2–4 hr before evaluation. The cups were coded so that the panel members were not informed as to the specific nature of the materials being tasted. On the first day of testing, the panel members familiarized themselves with eight examples of the products and with the rating scales. On each of three successive days, the panel evaluated six of the 18 products. Each of the 26 panel members rated each sample according to each of 15 attributes (chewy, stringy, springy, gummy, oily, grainy, breakable, firm, frangible, fluffy, sticky, smooth, fat-like, lumpy, and elastic) on a 7-point rating scale. For example, in the case of the attribute "stringy,"

Degree of Stringiness	Numerical Rating
Extremely stringy	7
Very stringy	6
Quite stringy	5
Fairly stringy	4
Somewhat stringy	3
Slightly stringy	2
Not at all stringy	1

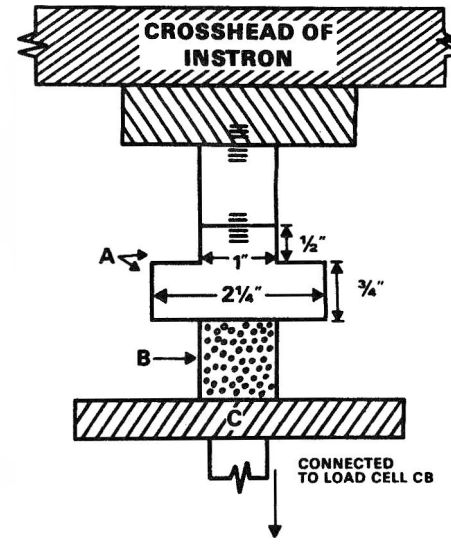

Fig. 1–Arrangement for texture profile analysis (TPA) of food products using the Instron.

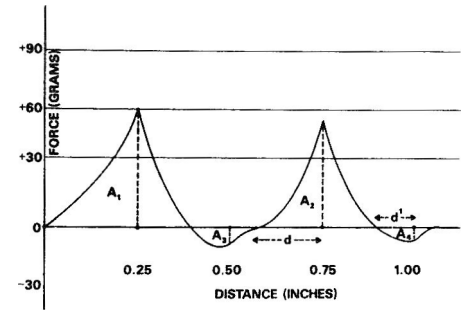

Fig. 2–Typical force-distance curve obtained by TPA of a dessert pudding.

RESULTS & DISCUSSION

Physical tests

The 15 test results (5 obtained from Instron curves while the sample was under compression; 7 from similar curves while sample was under tension; and 3 parameters obtained from conducting the S-test) were recorded as averages of two values. Duplicate tests were run on each sample, the second test following the first by approximately 2 hr. The precision of the various physical measurements is compared in Table 1. The statistics used to evaluate precision are the 95% confidence limit and the coefficient of variation. The coefficient of variation is the ratio of the standard deviation to the mean value. Since this statistic is a ratio, one can compare the relative precision of the various physical measurements. Some of the more precise measurements were elasticity under compression (E_c), firmness under compression (F_c), and stringiness (S). Some of the least precise were work of extension (W), gumminess under tension (G_A), chewiness under tension (Ch_A), and firmness under tension (F_A).

Table 1 is a summary of the physical test data. The lowest and highest mean values indicate the range of the physical test values for each product tested. The coefficient of variation is given for each product as well as the standard deviation, to show the amount of variation in each case.

The correlation coefficients among the physical measurements are given in Table 2. Chewiness under compression (Ch_c), chewiness under tension (Ch_A), gumminess under tension (G_A), and A_4 (a tensile work value as in Fig. 2) all had very high correlations with other measurements. (Correlation coefficients were 0.98 or higher.) This indicates that these four values were redundant and contributed little additional information with regard to the physical properties of the 18 products in the study.

Sensory tests

Table 3 is a summary of the sensory data. 26 ratings were averaged for each of the 15 attributes. Again, the lowest and

highest mean values are given to indicate the range of the sensory values for each product tested. The reliabilities of the mean ratings were obtained as described by Ebel (1951).

Some of the most reliable ratings were of fluffiness, stickiness, firmness, and chewiness. Some of the least reliable ratings were of oiliness, smoothness, lumpiness, and graininess.

The correlations between the sensory ratings are given in Table 4. Frangible and breakable, gummy and stringy, gummy and sticky, elastic and firm, all had correlations of at least 0.90, indicating that there was some degree of redundancy. For correlation of sensory and physical values see Table 5.

Table 1—Summary of physical test data.

Measurement	Overall mean	Lowest product mean		Highest product mean		Standard deviation of replicate measurements	Coefficient of variation
F_c	56.2	18.8	Whipped topping (high water)	122.5	Custard (medium water)	7.66	13.6%
E_c	0.184	.148	Cooked vanilla (high water)	.228	Marshmallow (as is)	0.0104	5.7%
C_c	0.676	.414	Cooked vanilla (high water)	1.18	Marshmallow (stirred)	0.0558	8.3%
Ch_c	7.03	2.25	Cooked vanilla (high water)	22.1	Marshmallow (stirred)	1.68	23.9%
G_c	37.3	12.05	Whipped topping (high water)	97.4	Marshmallow (stirred)	7.65	20.5%
F_A	13.3	3.50	Custard (high water)	47.2	Marshmallow (stirred)	4.16	31.3%
E_A	0.170	.0640	Gelatin (low water)	.264	Marshmallow (stirred)	.0121	7.1%
C_A	1.01	.774	Instant pudding (high water)	1.19	Custard (high water)	.0945	9.4%
Ch_A	2.58	.666	Custard (low water)	4.99	Cooked pudding (low water)	.858	33.3%
G_A	10.8	4.18	Custard (high water)	45.2	Marshmallow (stirred)	4.10	37.1%
A_3	.213	.0232	Custard (high water)	.912	Marshmallow (stirred)	.0454	21.3%
A_4	.207	.0275	Custard (high water)	.872	Marshmallow (stirred)	.0407	19.7%
S	.481	.091	Instant pudding (high water)	3.53	Marshmallow (stirred)	0.356	7.4%
T	12.5	5.9	Cooked vanilla (high water)	38.0	Gelatin (low water)	1.74	13.9%
W	.585	.0965	Cooked vanilla (medium water)	2.64	Marshmallow (as is)	.361	61.7%

Table 2—Correlation between physical measurements. (See text for definitions of F_c, E_c, etc.)

	F_c	E_c	C_c	Ch_c	G_c	F_A	E_A	C_A	Ch_A	G_A	A_3	A_4	S	T	W
F_c	1.00														
E_c	−0.15	1.00													
C_c	−0.12	.86	1.00												
Ch_c	0.65	.49	.60	1.00											
G_c	0.79	.34	.46	.98	1.00										
F_A	.09	.56	.57	.65	.53	1.00									
E_A	−.52	.33	.17	−.17	−.31	.54	1.00								
C_A	.31	−.24	−.26	.05	.12	−.02	−.31	1.00							
Ch_A	.05	.55	.55	.59	.47	.99	.62	.00	1.00						
G_A	.15	.49	.50	.63	.53	.98	.46	.17	.96	1.00					
A_3	−.12	.60	.58	.48	.33	.91	.75	−.25	.94	.82	1.00				
A_4	−.09	.60	.57	.50	.35	.94	.74	−.16	.97	.87	.99	1.00			
S	.01	.67	.79	.61	.49	.75	.46	−.12	.79	.68	.83	.83	1.00		
T	−.05	.20	.30	.15	.13	−.28	−.51	−.26	−.37	−.32	−.32	−.36	−.12	1.00	
W	−.14	.74	.84	.42	.30	.38	.22	−.20	.40	.32	.49	.48	.82	.34	1.00

Analysis of data

Factor analysis. Two separate factor analyses were performed, one on the 15 physical measurements and another on the 15 sensory rating scales. Factor analysis can be viewed as setting up a new set of reference axes so as to arrive at the simplest possible representation of the data. In terms of the original set of physical data it takes 15 numbers to characterize a given product, but through a rotation to a new set of axes, it is possible to adequately characterize a given product with only four numbers.

A factor analysis was made of the 15 physical measurements with the purpose of identifying basic underlying physical

Table 3–Summary of sensory evaluation data.

Characteristic	Overall mean	Lowest product mean		Highest product mean		Standard deviation of replicate judgments	Reliability of mean rating
Chewiness	2.45	1.00	Whipped topping (high water)	5.19	Marshmallow (as is)	1.04	.97
Gumminess	2.48	1.27	Whipped topping (medium & high water)	4.96	Marshmallow (stirred)	1.16	.97
Breakability	2.26	1.42	Marshmallow (stirred)	3.73	Gelatin (low water)	1.08	.94
Fluffiness	2.80	1.12	Gelatin (high water)	6.08	Whipped topping (medium water)	.97	.99
Frangibility	2.12	1.42	Fluffy frosting	3.42	Gelatin (low water)	1.09	.91
Elasticity	2.58	1.23	Whipped topping (medium water)	4.19	Gelatin (medium water)	1.33	.92
Stringiness	1.42	1.04	Custard (low water)	3.62	Marshmallow (as is)	.72	.96
Firmness	3.84	1.65	Whipped topping (high water)	5.69	Marshmallow (as is)	1.01	.98
Stickiness	2.58	1.42	Whipped topping (high water) Custard (medium water)	6.15	Marshmallow (as is)	1.04	.98
Springiness	2.96	1.65	Whipped topping (medium & high water)	5.58	Gelatin (medium water)	1.13	.96
Fat-like	2.46	1.38	Gelatin (medium water)	3.00	Custard (low water)	1.05	.83
Smoothness	4.82	3.92	Instant pudding (low water)	5.38	Whipped topping (medium water) Cooked pudding (medium water)	1.19	.74
Oiliness	2.12	1.35	Gelatin (low water)	3.04	Cooked pudding (low water)	.91	.75
Lumpiness	1.36	1.00	Whipped topping (medium water)	1.73	Custard (medium water)	.67	.68
Graininess	1.42	1.04	Gelatin (medium water)	1.81	Instant pudding (low & medium water)	.70	.66

Table 4–Correlations between sensory ratings.

	Chewy	Gummy	Breakable	Fluffy	Frangible	Elastic	Stringy	Firm	Sticky	Springy	Fat-like	Smooth	Oily	Lumpy	Grainy
Chewy	1.00														
Gummy	.76	1.00													
Breakable	.07	−.43	1.00												
Fluffy	−.59	−.22	−.58	1.00											
Frangible	.06	−.46	.98	−.54	1.00										
Elastic	.88	.53	.34	−.62	.34	1.00									
Stringy	.76	.91	−.37	−.11	-.39	.53	1.00								
Firm	.86	.47	.51	−.83	.49	.90	.44	1.00							
Sticky	.69	.93	−.60	−.01	-.62	.46	.88	.31	1.00						
Springy	.43	−0.12	.80	−.69	.81	.74	−.14	.75	−.21	1.00					
Fat-like	−.27	.03	−.49	.30	−.49	−.61	.02	−.45	.09	−.70	1.00				
Smooth	−.86	−.47	−.24	.57	−.23	−.77	−.49	−.81	−.46	−.54	.20	1.00			
Oily	−.26	.11	−.36	.11	−.39	−.54	−.05	−.29	.05	−.57	.78	.20	1.00		
Lumpy	.31	−.17	.70	−.74	.66	.38	−.23	.62	−.25	.69	−.19	-.60	-.02	1.00	
Grainy	.24	.51	-.76	.30	-.78	-.01	.46	−.16	.72	−.48	.54	-.23	.29	−.28	1.00

characteristics of the eighteen samples. For example, firmness on compression (F_c) is correlated with chewiness and gumminess on compression (Ch_c and G_c respectively). The correlations indicate that there is some common attribute or factor which these values are measuring. The factor loadings obtained for each of the four factors are given in Table 6 (Cattell, 1965).

Since a factor is a hypothetical construction, judgment is required to name it. We named the factors derived from the physical data and computed the factor scores (Table 7) for each product by applying the corresponding loadings of Table 6. Examples of products that exhibit the properties of the several factors are given under each factor.

Factor I– "Resistance to tension after compression"
High score–stirred marshmallow
Low score–egg custard with high water addition

Factor II– "Resistance to compression"
High score–stirred marshmallow creme
Low score–whipped topping with high water addition

Factor III– "Resistance to tension without compression and recovery from compression"
High score–stirred marshmallow
Low score–cooked pudding with high water addition

Factor IV– "Cohesiveness under tension after compression"
High score–cooked pudding with low water addition
Low score–instant pudding with high water addition

A similar factor analysis of the sensory data was made. It is possible to adequately represent the 15 responses to a given product with only four numbers. The loadings obtained for each of the four factors are given in Table 8.

Table 5—Correlation of sensory and physical measurements. (See text for definitions of F_C, E_C, etc.)

	F_c	E_c	C_c	Ch_c	G_c	F_A	E_A	C_A	Ch_A	G_A	A_3	A_4	S	T	W
Chewy			.65	.52	.50								.65		.73
Gummy						.65			.67	.72	.56	.62	.67		.47
Breakable						−.57	−.86		−.62	−.57	−.64	−.66		.60	
Fluffy							.67								
Fat-like			−.53											−.68	
Frangible						−.59	-.86		−.65	-.59	−.67	−.69		.64	
Smooth	−.48			-.51	.55			-.51							
Elastic			.72	.54	.51								.52		.63
Stringy		.61	.68			.58	.47		.63	.55	.67	.69	.90		.85
Oily			−.50											−.67	
Firm			.51	.51	.55		−.55								.53
Sticky		.51	.57			.73	.55		.77	.74	.75	.79	.81		.62
Lumpy	.58						-.83		−.47		−.62	−.59			
Springy							-.80							.60	
Grainy						.60	.60		.66	.66	.57	.63		−.65	

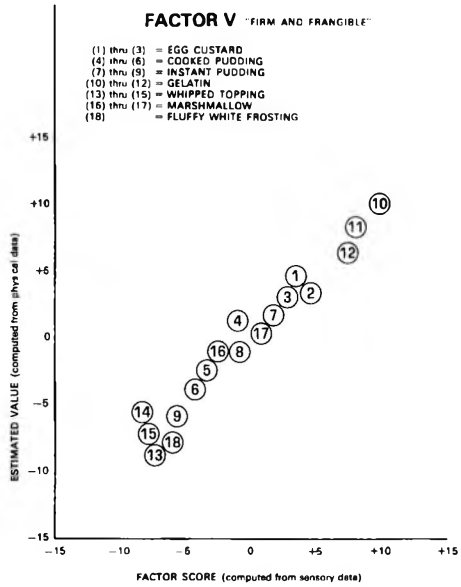

Fig. 3—A plot of the scores for Factor V estimated from the physical measurements, against the actual scores for Factor V.

Table 6—Rotated factor matrix of physical variables.

	Variable	Factor Loadings I	II	III	IV
F_c	Firmness on compression	−.04	.91	−.23	.13
E_c	Elasticity on compression	.36	.00	.80	−.11
C_c	Cohesiveness on compression	.31	.16	.89	−.12
Ch_c	"Chewiness" on compression	.34	.84	.37	−.02
G_c	"Gumminess" on compression	.16	.90	.27	.09
F_A	Firmness under tension	.90	.25	.28	−.01
E_A	Elasticity under tension	.78	−.56	.03	−.18
C_A	Cohesiveness under tension	−.04	.19	−.17	.96
Ch_A	"Chewiness" under tension	.94	.18	.26	.04
G_A	"Gumminess" under tension	.86	.28	.23	.18
A_3	Area	.92	.02	.32	−.19
A_4	Area	.94	.04	.32	−.09
S	Stringiness	.66	.18	.64	−.01
T	Tension maximum	−.62	.17	.54	−.29
W	Work of extension	.19	.03	.92	−.04
Variance accounted for by factor, %		39.9	19.6	24.5	7.8
Total variance accounted for		39.9	59.5	84.0	91.8

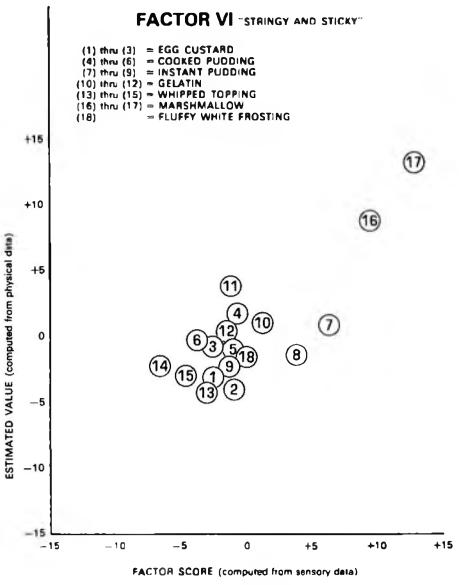

Fig. 4—A plot of the scores for Factor VI estimated from the physical measurements, against the actual scores for Factor VI.

We named the factors derived from the sensory data and computed the factor scores in Table 7 by applying the weights in Table 8. Examples of products that exhibit the properties of the several factors are given under each factor.

Factor V– "Firm and frangible" (leading to pieces of lumps in the mouth; geletin-like)
High score–gelatin
Low score–whipped topping

Factor VI– "Stringy and sticky" (chewy, gummy aspects; marshmallow-like)
High score–marshmallow
Low score–whipped topping with medium water addition

Factor VII– "Fat-like"
High score–cooked pudding with low water addition
Low score–gelatin

Factor VIII– "Grainy"
High score–instant pudding with low water addition
Low score–gelatin

Multiple regression equations. Multiple regression equations were established for predicting the four factors derived from the sensory ratings by means of the physical test values. A summary of this analysis is given in Table 9. Note that only 8 of the available 15 physical values

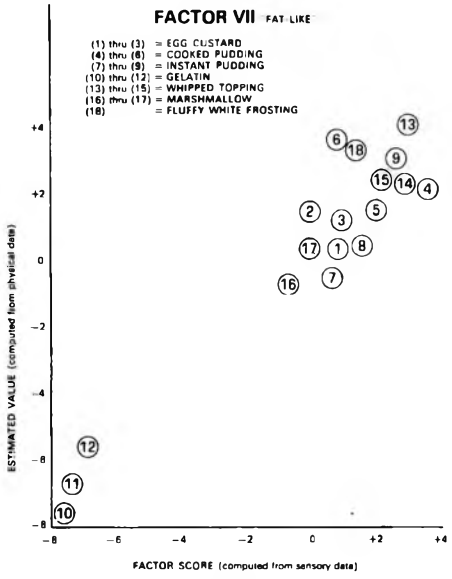

Fig. 5–A plot of the scores for Factor VII estimated from the physical measurements, against the actual scores for Factor VII.

Table 7–Factor scores of products on physical and sensory factors.

Product	Liquid level (or other treatment)	Physical factor scores I	II	III	IV	Sensory factor scores V	VI	VII	VIII
Egg custard	low	−4.6	2.5	−3.0	−0.6	3.5	−2.2	0.6	−1.6
Egg custard	med	−4.6	3.2	−3.8	2.2	4.4	−2.5	0.0	−1.6
Egg custard	hi	−5.9	0.2	−4.5	1.9	3.2	−2.7	1.0	−1.2
Cooked pudding	low	3.1	3.5	−1.1	3.2	−0.4	−0.8	3.1	−0.1
Cooked pudding	med	−0.3	−1.7	−2.7	0.8	−2.3	−2.1	2.0	−0.2
Cooked pudding	hi	−1.8	−2.6	−4.5	1.4	−3.4	−3.3	1.1	−0.9
Instant pudding	low	−1.4	0.1	−1.7	0.6	2.5	6.8	0.6	3.4
Instant pudding	med	−3.4	−2.4	−2.5	0.1	−0.2	3.9	1.6	2.9
Instant pudding	hi	−0.9	−3.1	−2.0	−2.1	−5.1	−2.3	2.5	0.8
Gelatin	low	−5.0	4.5	4.2	−0.4	9.8	0.2	−7.8	−3.4
Gelatin	med	−5.2	−0.3	0.5	−0.1	8.1	−0.8	−7.6	−3.9
Gelatin	hi	−3.7	−0.4	0.3	−1.0	7.6	−1.6	−7.0	−3.7
Whipped topping	low	4.4	−0.4	1.5	−1.1	−6.7	−4.1	3.0	0.9
Whipped topping	med	0.0	−3.0	−0.3	−0.7	−7.6	−5.7	2.8	−0.1
Whipped topping	hi	−1.0	−3.8	−0.7	−1.1	−7.0	−5.2	2.7	0.6
Marshmallow	as is	6.4	−1.5	7.8	−1.4	−1.4	10.0	−0.3	3.1
Marshmallow	stirred	22.0	− 8.1	13.0	−1.0	0.8	13.0	0.0	2.8
Fluffy white frosting		2.0	- 2.9	−0.8	−0.6	−5.7	−0.7	1.9	2.0

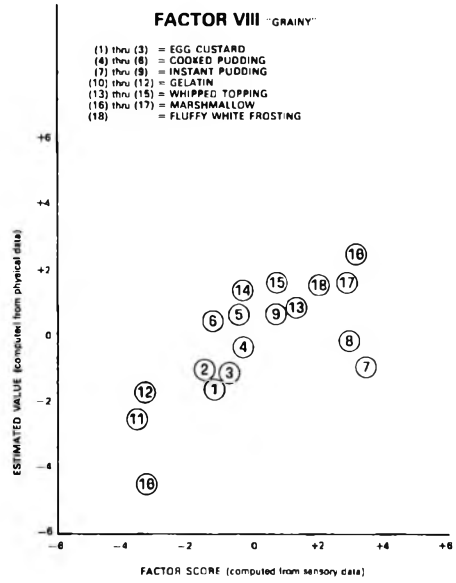

Fig. 6–A plot of the scores for Factor VIII estimated from the physical measurements, against the actual scores for Factor VIII.

Table 8–Rotated factor matrix of sensory variables.

Variables	Factor Loadings V	VI	VII	VIII
Chewy	.44	.86	−.22	.05
Gummy	.06	.89	.14	.38
Breakable	.73	−.36	−.33	−.41
Fluffy	−.83	−.28	.03	.27
Fat-like	−.19	−.03	.88	.27
Stringy	−.13	.95	.00	−.05
Oily	−.05	−.02	.96	.00
Firm	.75	.56	−.27	−.19
Sticky	−.19	.92	.04	.29
Lumpy	.96	−.12	.02	.08
Springy	.77	−.02	−.60	−.11
Grainy	−.28	.48	.31	.75
Frangible	.71	−.38	−.36	−.40
Smooth	−.68	−.57	.16	−.31
Elastic	.51	.63	−.56	−.01
Variance accounted for by factor, %	32.6	32.7	19.1	9.5
Total variance accounted for	32.6	65.3	84.4	93.9

Table 9—Regression coefficients for the sensory factors.[a]

Coefficients for regression equations	Factor V	Factor VI	Factor VII	Factor VIII
Constant	+19.5	+11.2	−7.08	−3.67
F_c	+ 0.0289	—	+ 6.0202	—
E_c	−87.3	—	+54.8	—
C_c	+17.7	—	−16.7	—
Ch_c	−0.279	−0.815	—	—
G_c	—	—	—	—
F_A	—	+ 0.315	+ 0.0678	—
E_A	−93.7	−76.0	+40.8	+26.0
C_A	—	—	—	—
Ch_A	—	—	—	—
G_A	—	—	—	—
A_3	—	—	—	—
A_4	—	—	—	—
S	+ 1.97	+ 6.70	—	—
T	—	—	−0.0573	−0.0605
W	—	—	—	—
No. of regressor variables	6	4	6	2
Multiple R	.991	.887	.963	.736
% variation accounted for	98.3	76.9	92.7	54.2
F ratio for regression model	103.9	10.84	23.41	8.86
Level of significance	.1%	.1%	.1%	1%

[a]—signifies that the relevant variable is not included in the regression model.

were needed in obtaining these prediction equations. They were F_c, E_c, C_c, Ch_c, F_A, E_A, S, and T. It is of importance here to note that the inclusion of the new values F_A and E_A, obtained under tension, is necessary, along with the customary compressional values (F_c, E_c, C_c, Ch_c), in arriving at the prediction equations for the sensory ratings. The values S and T (obtained from the S-test), are also needed.

The regressions were significant at the 0.1% or the 1.0% level.

Figures 3 through 6 illustrate graphically the effectiveness of the regressions. These figures also show the relative positions of the various products with respect to the corresponding factor. For example, on Factor V (Fig. 3) all gelatins cluster at one end of the scale and the whipped toppings at the other end.

CONCLUSIONS

ALTHOUGH THE application of TPA and the S-test has yielded 15 physical values for each of the products tested in this study, some of these values are interrelated. Application of factor analysis reduced them to four dimensions in a physical "space." The four dimensions represent the basic underlying physical characteristics of these products. Similarly, the application of factor analysis reduced the 15 sensory ratings of these products to four dimensions in sensory "space."

Multiple regression analysis showed that physical values can predict the essential sensory attributes. The accuracy of these predictions was improved by the inclusion of "tension-derived" TPA values and S-test values along with the "compression-derived" or conventional TPA values.

The specific methods employed here and the results obtained from factor analysis and regression equations apply only to the particular types of desserts and toppings used in this study. The S-test values can be obtained only with certain liquid and semi-solid foods. On such solid foods as cake and bread, the TPA method will normally yield only those physical values obtained by compression, since there is little or no adhesion of the anvil to the material during the upstroke portion of the cycle.

Mention should also be made of the fact that the values arising from the S-test are not measures of the adhesive force. Instead, these values arise from the internal body forces and the surface forces involved in placing the liquid or semi-solid material under tension.

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J. M. WILSON[1], I. BEN-GERA[2] and A. KRAMER
Department of Horticulture, University of Maryland, College Park, Maryland 20742.

QUANTITATIVE DETERMINATION OF COMPOSITION OF MODELS OF FOOD SYSTEMS BY THE INFRARED ATTENUATED TOTAL REFLECTANCE TECHNIQUE

SUMMARY—Attenuated total reflectance (ATR), offering a possible rapid infrared spectrophotometric method for quantitative measurements of major nutritional components in foods materials, was employed in this study for evaluating composition of model systems representing solid foods: system #1—starch and protein; system #2—starch, protein and fat. Using a band ratio technique, correlations were determined between the ratio of intensities for carbohydrate and protein peaks and their proportion in the systems studied. When the log of the ratio of these two peaks was plotted against the composition, correlation coefficients of .9699 and .9358 for systems #1 and #2 respectively were obtained. Errors assignable to the systems' composition and those inherent to the ATR technique are discussed.

INTRODUCTION

IN SPITE OF its wide application in organic chemistry, the use of infrared spectrometry in food science, and particularly in the direct study and evaluation of food systems, is rather limited. This may, at least in part, be explained by inherent disadvantages of the methods used for the preparation and presentation of the sample. Present methods require thin films for liquid sample and very small quantities of solid sample pressed into a halide pellet or mixed in a mull. When the infrared radiation is passed through the sample to produce so-called transmission spectra, it comes in limited contact with the sample. Thus, the amount of material exposed to the energy beam may not represent accurately the sample from which it was drawn.

Nevertheless, the fact that valuable information can be obtained by infrared spectrometry of food systems under the above conditions has been demonstrated. Ben-Gera and Norris (1968) have developed a technique for the quantitative determination of moisture and fat content in meat products based on direct spectrophotometry in the near infrared, on thick, light scattering samples. Goulden (1964) developed an infrared spectrophotometer and a procedure for direct quantitative determination of fat, protein and lactose in milk. Lin and Pomeranz (1965) studied the infrared spectra of different wheat flours and related the information obtained from these spectra to the bread making properties of the flours. Strum et al. (1966) were relatively successful in determining the quantitative presence of vulgare wheat flour in durum wheat flour and pasta, based on information obtained from the IR spectra of wheats in the wavelength range of 8–10 microns. Johnston and Watts (1965) studied the infrared spectra of glandless cotton seed meals which were shown to have different nutritive values as a result of different methods of extraction and heat treatments. They found that changes in nutritive value can be detected by studying the IR spectra in the range of 2.5–6.5 microns.

The ability to characterize the major food components, protein, carbohydrates and lipids as containing or lacking certain functional groups and comparison with knowns has also been demonstrated. Bellamy (1958), Jones and Sanderfy (1956) and Randal et al. (1949), offer general information concerning infrared qualitative and quantitative analysis and specific literature reviews concerning functional groups and spectra of knowns. Numerous papers have been published containing valuable collections of spectra as well as information depicting the functional groups of the major food components. Kuhn (1950), Whistler and House (1953), Neely (1957) and White et al. (1958) reported on the infrared spectrum of carbohydrates; Sutherland (1952) and May and Callahan (1965) on proteins and polypeptides; and Wheeler (1954) on lipids.

The opportunity to produce spectra similar to transmission spectra in a direct and rapid manner using samples which are relatively unrestricted in size and therefore in the case of food more accurately representing the average properties of the material was made possible by the work of Harrick (1960) and Fahrenfort (1961). This system is called "Attenuated Total Reflection" (ATR) or multiple internal reflection technique, and is discussed in detail by Harrick (1967).

In this technique a beam of electromagnetic radiation is directed to the surface of a transparent crystal in the form of a prism. The energy beam is propagated through the prism by total internal reflection. As long as the reflection remains total no spectrum is produced. If the infrared radiation is incident on a transparent prism of high refractive index, which is in contact with the sample having a lower refractive index, the radiation will be attenuated as it is reflected internally if at an angle above the critical angle. The critical angle is that angle of incidence for which the angle of refraction is 90°. Under such circumstances a recording of the attenuation of the energy beam as a function of the wavelength will result in a spectrum similar to that of a transmission spectrum (Anon., 1965).

Gore (1965) demonstrated the applicability of the attenuated total reflectance technique by showing and interpreting spectra of food and drink that might be found in a multiple course meal. Cameron (1967) assessed the potential application of ATR for qualitative analysis of food systems. Kliman and Pallansch (1967) studied the attenuated total reflectance of infrared energy by dairy products. They showed a quantitative relationship between the concentration of butteroil in benzene and attenuation of reflectance at 1735, 2820, and 2880 wavenumber.

Ben-Gera and Kramer (1968) reported on the problems involved in the quantitative application of the attenuated total reflectance technique for studies and evaluation of composition of foods. The study presented in this paper reports on the quantitative relationship between composition of food materials as represented by multicomponent model systems and their infrared spectra, produced by the ATR technique.

EXPERIMENTAL PROCEDURE

SPECTRA WERE obtained by using a Beckman IR-8 infrared spectrophotometer with a scale expansion unit and a Beckman 10-in. recorder. The ATR attachment used was the Wilks Scientific Model 12 double beam internal reflection unit. This unit is equipped with two KRS-5 (thallous-bromide-iodide) reflector plate prisms (50 × 20 × 2mm) and sample holders.

In order to demonstrate that a quantitative relationship exists between multicomponent food analysis and attenuated total reflection over a wide range of sample composition, it was decided to use model systems. Two basic model systems were used for this experimentation having the following composition:

System #1 (protein-starch)

The following compositions are listed as egg albumin and soluble starch (analytical grade): 10%–90%, 25%–75%, 40%–60%, 50%–50%, 60%–40%, 75%–25%, 90%–10%.

[1] Address as of 4/1/71: Department of Food Science, University of Maine, Orono, Me. 04473.

[2] Present address: Division of Food Technology, Volcani Institute of Agricultural Research, P. O. Box 15, Rehovot, Israel.

Table 1–Means and standard deviations for system #1 (starch and albumin).[a]

% Composition		Ratio	Std	Coefficient of
Protein	Starch	Mean	Deviation	Variability[b]
90	10	.459	.054	11.8
75	25	1.231	.129	10.5
60	40	2.080	.203	9.8
50	50	2.211	.261	11.8
40	60	3.788	.626	16.5
25	75	5.437	.746	13.7
10	90	9.106	1.590	17.5

[a]Data based on one set of samples in which each sample was mounted 5 times and duplicated twice.
[b]In percent of the mean.

Table 2–Means and standard deviation for system #2 (starch, casein, and 10% cotton seed oil).[a]

% Composition		Ratio	Std	Coefficient of
Protein	Starch	Mean	Deviation	Variability[b]
80	10	1.701	.384	22.6
67.5	22.5	4.115	.873	21.2
55	35	7.969	1.541	19.3
45	45	9.517	2.055	21.6
35	55	11.164	1.629	14.6
22.5	67.5	19.597	9.135	46.6
10	80	37.775	10.333	27.4

[a]Data based on one set of samples in which each sample was mounted 5 times and duplicated twice.
[b]In percent of the mean.

System #2 (protein-starch-fat)

Two types were prepared. Composition X, with constant fat content (10% cottonseed oil) and variable casein and corn starch contents: 80%–10%, 67.5%–22.5%, 55%–35%, 45%–45%, 35%-55%, 22.5%–67.5% and 10%–80%; and composition Y, variable casein, corn starch and cottonseed oil contents: 8.33%–83.33%–8.33%, 16.66%–66%–16.66%, 33.33%–50%–16.66%, 50%–33.33%–16.66%, 83.33%–8.33%–8.33%.

Three complete sets of system #1, one set of system #2 (composition X) and two sets of system #2 (composition Y) were made up at different times with spectra being run on each set immediately following preparation.

A typical spectrum was obtained in the following manner: (1) approximately half of the sample was placed on the bottom plate of the sample holder covering an area approximately the size of the prism; (2) the KRS-5 prism was placed on the sample and the remaining sample was placed on top of it; (3) the top plate of the sample holder was bolted into place. Following the mounting of the sample, the sample holder was placed into the Wilks model 12 double beam internal reflection unit. The intensity of an ATR spectrum varies according to the angle of incidence with the attenuation increasing until its maximum at the critical angle. The optimum angle of incidence was found empirically for each sample by adjusting the mirrors of the reflection unit. In all cases the IR-8 was operated as a double beam instrument using a reference beam containing only the sample holder and KRS-5 crystal.

Every sample from each set was mounted three times and duplicate spectra were run from each mount giving a total of six spectra per sample. Two extensions of this procedure were carried out in order to obtain meaningful standard deviation data: (1) one sample from one set in each of the two model systems was replicated ten times; (2) two complete sets of samples, one from each model system, were mounted five times with duplicate spectra run for each mount.

RESULTS & DISCUSSION

TYPICAL SPECTRA of the two model systems and of the 100% protein, starch and fat used in this study, are given in Figures 1 and 2. In spite of similarity between the different spectra, which can be explained on the basis of similarities in the molecular structures of fats, proteins and carbohydrates, differences in the spectra are easily detectable. The two pronounced absorption bands in the transmission spectrum of the protein are the Amide I at 1660–1640 cm^{-1} (C = 0 stretching) and the Amide II at 1570–1530 cm^{-1} (N–H plane-bending and C–N stretching). Water has an absorption peak which interferes with the Amide I peak; therefore, Amide II was used in this study (Cameron, 1967). At 1740 cm^{-1} there is a strong absorption band in the spectrum of the fat, (C = 0 stretching of the carboxyl of esterified fatty acids) and a background absorption only for carbohydrates and proteins. In the 960–990 cm^{-1} the three components absorb, but the carbohydrate peaks very strongly. The possibility of establishing qualitatively the presence of the studied components in a mixture is based on the fact that for each there is a range in the infrared spectrum where its contribution to the intensity of the spectrum is easily detected and recognized. It should be noted that internal reflection absorptivity with respect to transmission absorptivities increases with the wave length so that new absorptivity values must be determined (Anon., 1965; Cameron, 1967).

In our attempts to analyze quantitatively the spectra produced, a band ratio and baseline technique was used (White, 1964). A ratio of the intensity of the Amide II peak at 1520 wavenumbers for protein and the intensity at 990 wavenumbers for carbohydrate as related with the sample composition was studied.

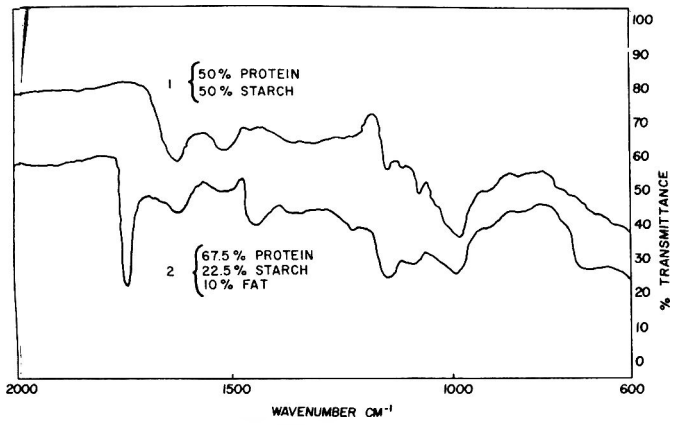

Fig. 1–ATR spectra of model systems #1 and #2.

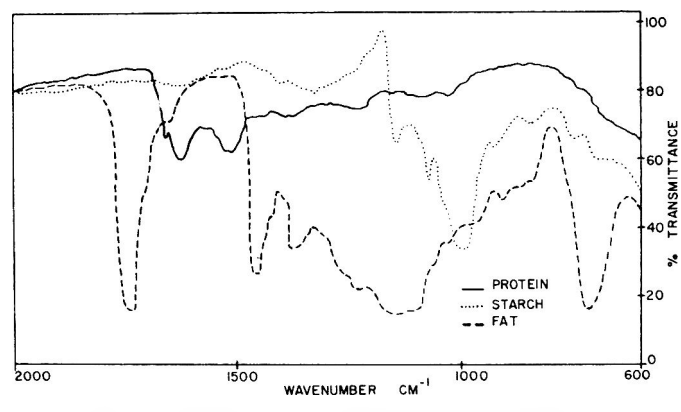

Fig. 2–ATR spectra of 100% protein, starch and oil.

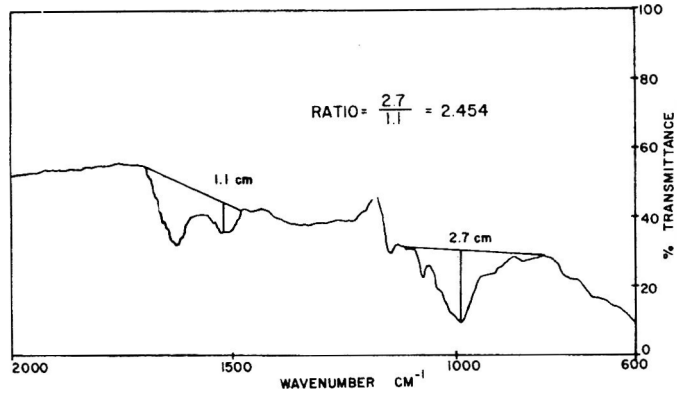

Fig. 3—Baseline drawing and peak intensity computation procedure (system #1).

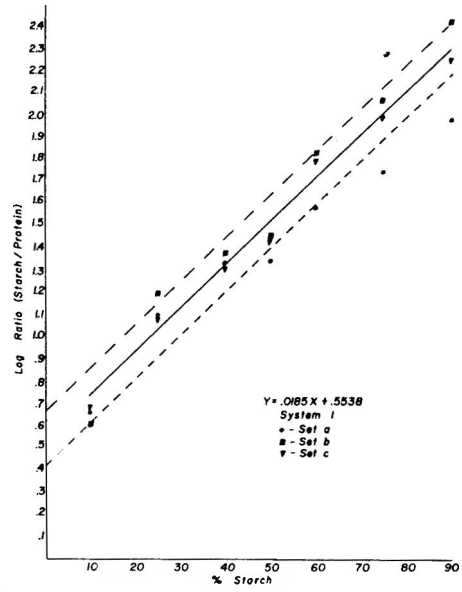

Fig. 4—The relationship between the starch content and the ratio of attenuation at 1,520 and 990 wavenumbers for system #1.

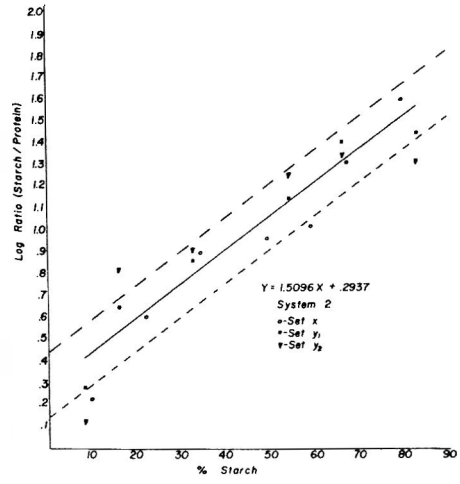

Fig. 5—The relationship between the starch content and the ratio of attenuation at 1,520 and 990 wavenumbers for system #2.

These intensities were selected as most suitable after preliminary experimentation of numerous possibilities. Peak intensity is a function of the percent transmittance and is obtained by the difference between the peak height and a baseline at the peak's wavenumber.

The baselines for these two intensity peaks were drawn in a straight line tangential to adjacent absorption minima or shoulders. A perpendicular line, which was measured, was drawn through the selected wavenumber until it intersected the baseline. In system #1 the two baselines were drawn between 1700 and 1480 wavenumbers for protein and between 1110 and 800 for carbohydrate, while in system #2 the baselines were drawn between 1780 and 1480 wavenumbers and 1280 and 860. The ratios were derived by dividing the intensity of the Amide II at 1520 cm^{-1} peak into the intensity of the carbohydrate peak at 990 cm^{-1}. Figure 3 shows the procedure used for baseline drawing and peak intensity ratios computation in the case of system #1.

A linear relationship exists between the log of the ratio of the two intensities and the percent starch which corresponds directly with that of protein in both model systems. The correlation coefficients were 0.9699 and 0.9358 for system #1 and system #2 respectively. Figures 4 and 5 show the log ratios vs. composition with a plotted regression line and confidence limits at one standard deviation. Three sets of symbols appear on each graph, each representing a specific set of samples. When examining the reproducibility of the same sample mount and the performance of the ATR equipment, the two 10 spectra runs gave the following results: sample #1 (75% protein–25% starch), the mean ratio and standard deviation was 1.274 ± 0.0634; system #2 (80% protein–10% starch–10% fat), the mean ratio and standard deviation was 1.417 ± 0.08306. In both cases the standard deviation was low as it was in all spectra replications. The standard deviation as a percent of the mean (coefficient of variability) was 5.2 for system #1 and 6.2 for system #2.

The means, standard deviations and coefficients of variability calculated for the sample preparations mounted five times are shown in Tables 1 and 2. The average standard deviation for systems #1 and #2 are 0.515 and 3.707 respectively. Both the spreading trend within sets of samples (Figures 4 and 5) and the standard deviation increases as the starch content in the samples increases. An analysis of variance indicates a significant difference between samples at the .01 level but no significant difference between duplicates of samples and replications within duplicates.

Several factors may account for the less than complete correlation between the optical density data and sample composition. The preparation of the samples by mixing in a ball mill resulted in a good but less than perfect mix. The introduction of oil into the starch-protein system resulted in the coating of these components, not always with a complete coat or a uniform thickness. The starch particles were smaller in size than the protein particles in both systems. These three factors result in uneven distribution of the starch and protein particles as regarding their contact with the surface of KRS-5 prisms. The trend for the surface area contact of the starch is therefore greater. This would cause a greater attenuation for the starch intensities and a considerable variability within samples. The large difference in the average standard deviation between systems #1 and #2 is possibly due to the oil coating distortion. Since peak intensities for both starch and protein become very small as the concentration of these components approaches zero, the ratio of their peak intensities and the log of these ratios (although to a lesser extent) show a deviation from a straight line. This reduces the correlation, increases the standard deviation and is dependent on sample composition and distortions resulting from the above mentioned factors.

The band ratio technique minimizes the effects on the results of irreproducibility in surface contact area between the crystal and the sample. This problem, which is of a small significance in case of liquid samples, becomes important when analyzing solids. Using sample holders in which known or reproducible pressures can be applied on the sample, resulting in a reproducible surface contact area, in addition to control over particle size, improves the reproducibility (Wilson,

Kramer and Ben-Gera, 1969) and may be the key to the application of the ATR technique to quantitative determination of food composition. Work is presently continuing with a newly developed sample cell (Wilson, J., Kramer, A. and Ben-Gera, I., 1969, unpublished data), capable of reproducible pressure, to further test the quantitative nature of the ATR technique when actual food systems are used.

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R. G. ARNOLD and T. E. HARTUNG
Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska 68503

INFRARED SPECTROSCOPIC DETERMINATION OF DEGREE OF UNSATURATION OF FATS AND OILS

SUMMARY—Infrared spectra of various fats and oils were determined. Ratios of absorbance at 3.3μ (olefinic C—H stretching band) to absorbance of other characteristic triglyceride absorption bands were calculated. Relationships between these ratios and unsaturation, as estimated by iodine value, were determined. Analyses of 25 fats and oils showed that the ratio of absorbance at 3.3μ to absorbance at 3.5μ (aliphatic C—H stretching band) and iodine value were linearly related and exhibited a correlation coefficient of 0.98. Estimation of degree of unsaturation of 19 additional fats and oils revealed an average deviation of ± 0.97 iodine value units or ± 1.12% between measured values and values calculated from infrared absorption patterns.

INTRODUCTION

THE DEGREE OF unsaturation of food fats and oils is commonly measured by determining iodine value. A faster method of comparable accuracy would be helpful in quality control and process control applications.

Chapman (1965) suggested that infrared spectroscopy could be employed in the determination of the degree of unsaturation of fatty acid mixtures. Sinclair et al. (1952) had earlier demonstrated that a linear relationship existed between a ratio calculated from infrared absorption patterns and the number of cis-double bonds for a series of unsaturated fatty acid methyl esters. The calculated ratio was obtained by dividing the optical density of the absorption band at 3.4μ by the difference between the optical density at 3.4μ and that at 3.3μ. Holman and Rahm (1966) published the infrared spectra of a series of polyunsaturated fatty acids. These spectra revealed significant changes in the infrared patterns from 3.3—3.5μ as unsaturation increased. This region of the infrared spectrum is the characteristic C—H stretching region, with an olefinic C—H absorption band at approximately 3.3μ and aliphatic C—H absorption bands at approximately 3.4 and 3.5μ (Colthup et al., 1964).

Previous work relative to the application of infrared spectroscopy to the determination of lipid unsaturation has dealt with known fatty acids or methyl esters. The purpose of this investigation was to explore the utility of infrared spectroscopy in the determination of the degree of unsaturation of the complex triglyceride mixtures found in food fats and oils.

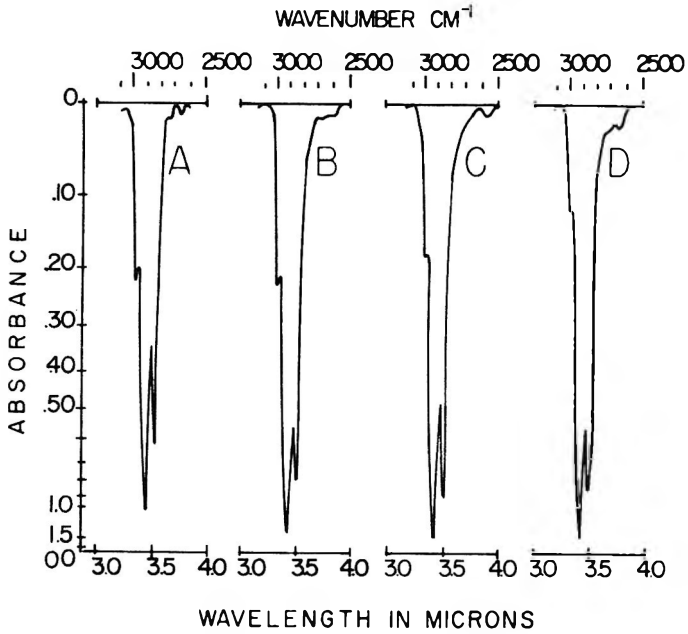

Fig. 1—Infrared absorption patterns in 3.0—4.0μ region of (A) safflower oil, iodine value 144; (B) soybean oil, iodine value 109; (C) lard, iodine value 73; and (D) milk fat, iodine value 33.

EXPERIMENTAL

Materials

Reagent grade carbon tetrachloride (J. T. Baker Chemical Co., Phillipsburg, N. J.), certified A.C.S. grade sodium thiosulfate, soluble starch and prepared Wijs iodine solution (Fisher Scientific Co., Fair Lawn, N. J.), and analytical reagent grade potassium iodide (Mallinkrodt Chemical Works, St. Louis, Mo.) were employed. Milk fat was isolated from freshly prepared butter oil by extraction with carbon tetrachloride. Corn, olive, peanut, safflower and soybean oils, various commercial blends of these oils, and lard were purchased from local retail outlets.

Equipment

Infrared absorption spectra were determined on a Beckman IR-5A infrared spectrophotometer (Beckman Instruments Co., Fullerton, California), which was operated in a double-beam, slow-scan mode. A 0.1 mm path length sodium chloride sealed cell was utilized.

Procedure

Iodine values were determined in duplicate by the Wijs method (American Oil Chemists Society, 1957). Infrared spectra of carbon tetrachloride solutions of the fats and oils were obtained coincident to iodine value determinations. Only two regions of the infrared spectrum, the C—H stretching region (approximately 3.0—4.0μ) and the carbonyl region (approximately 5.5—6.0μ), were scanned. For each sample, the ratios of the absorbance of the olefinic C—H stretching band (3.3μ) to absorbance at 3.4 and 3.5μ (aliphatic C—H stretching bands) and 5.75μ (carbonyl band) were calculated.

Preliminary analyses of various fats and oils indicated that a sample concentration of 10% in carbon tetrachloride and an instrument gain setting of 10 provided more reproducible results than those obtrained with 5 or 15% concentrations and lower gain settings. The preliminary studies also revealed that the ratio of absorbance at 3.3μ to absorbance at 3.5μ provided the best fit to a linear relationship. This ratio was therefore selected for more detailed study.

Twenty-five samples of fats and oils were subjected to duplicate iodine value determinations and triplicate infrared analyses. Nine of the samples consisted of blends of milk fat and safflower oil, with the milk fat concentration increasing from 10% to 90% w/w in 10% increments. Fats and oils described in "materials" comprised the balance of the samples. Sample means of the ratio of absorbance at 3.3μ to absorbance at 3.5μ were plotted against mean iodine values. The correlation coefficient and a regression equation relating the calculated ratios and iodine values were calculated (Steel and Torrie, 1960).

Rearrangement of the regression equation provided a prediction equation which could be used to predict iodine values of fats and oils

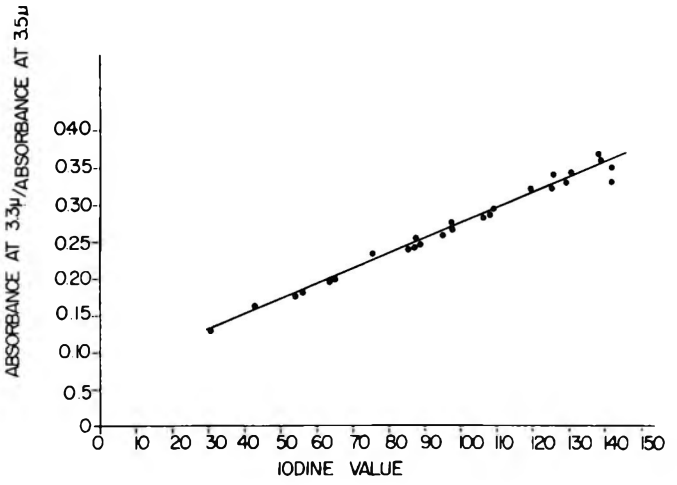

Fig. 2–Plot of iodine value vs. ratio of absorbance at 3.3μ to absorbance at 3.5μ for 25 fat and oil samples.

from the infrared ratio. This equation was applied to data obtained from duplicate infrared analyses and duplicate iodine value determinations of 21 additional samples of fats and oils. Average deviation of predicted iodine values from measured iodine values for these samples was then calculated.

RESULTS

FIGURE 1 shows the infrared absorption spectra, in the 3.0–4.0μ region, for four samples of fats and oils with iodine values ranging from 33–144. As unsaturation increases, the intensity of the olefinic C–H stretching band at 3.3μ increases, while the aliphatic C–H stretching band at 3.5μ decreases in intensity. This suggests the possibility of using the ratio of absorbances at these two wavelengths to predict unsaturation. A plot of this ratio vs. iodine value for 25 fat and oil samples is shown in Figure 2. The 25 samples range in iodine value from 31–142. Points on this graph represent average values of triplicate infrared analyses and duplicate iodine value determinations on each sample.

A summary of statistical analysis of the data represented in Figure 2 follows:

Correlation coefficient: r = 0.98

Regression equation: y = 0.00202x + 0.06965

where $y = \frac{\text{absorbance}_{3.3\mu}}{\text{absorbance}_{3.5\mu}}$

x = iodine value

Standard error of estimate: $Sy \cdot x = 0.0054$

Test of hypothesis, $\beta = 0$:

$F_{1, 23} = 3353.3^{**}$

**Significant at 1% level of probability

Rearrangement of the regression equation resulted in the prediction equation:

Iodine value = 495 (IR-ratio)–34.1

where IR-ratio $= \frac{\text{absorbance}_{3.3\mu}}{\text{absorbance}_{3.5\mu}}$

Measured iodine values and predicted values calculated from infrared absorption spectra for 21 additional samples of fats and oils are compared in Table 1. Excluding the two hydrogenated samples, the average deviation of the predicted values from measured values is ± 0.97 iodine value units or ± 1.12%. The predicted values for the two hydrogenated samples average 3.55 iodine value units or 4.42% below the measured values.

DISCUSSION

RESULTS of this investigation indicate that the degree of unsaturation of naturally occurring fats and oils can be accurately determined by infrared spectroscopy. The method is based upon the direct linear relationship between iodine value and the ratio of absorbance at 3.3μ to absorbance at 3.5μ. The ratios of the 3.3μ absorbance to absorbance at 3.4 and 5.75μ also exhibited a general linear relationship to iodine value, but showed greater variability.

The concentration of fat or oil in solvent was found to be an important variable. At a 5% concentration, the olefinic C–H band (3.3μ) of fats with low iodine values was not of sufficient intensity to be detected. At 15%, the spectrum was so intense that the 3.5μ band was in the range of high absorbance readings, and could not be measured accurately. The 10% concentration ultimately selected precluded the difficulties encountered at 5 and 15% concentrations.

When the instrument was operated at the recommended gain setting of 5, the olefinic C–H band at 3.3μ was difficult to identify in the more saturated fats. This band occurs as a shoulder on the 3.4μ aliphatic C–H band. Increasing the instrument gain setting resulted in a more

Table 1–Comparison of measured iodine values and values calculated from infrared absorption data using prediction equation.

Fat or oil	IR ratio $A_{3.3\mu}/A_{3.5\mu}$	Iodine value	
		Calculated	Measured
Corn oil A	.326	127.3	125.6
Corn oil B	.320	124.3	125.0
Corn oil C	.318	123.3	124.2
Lard A	.196	62.9	63.5
Lard B	.210	69.9	70.5
Lard C	.215	72.3	73.0
Milk fat A	.136	33.2	33.5
Milk fat B	.137	33.7	32.3
Olive oil	.238	83.7	85.4
Peanut oil A	.267	98.1	97.0
Peanut oil B	.270	99.6	98.0
Safflower oil A	.337	132.7	134.0
Safflower oil B	.351	139.6	139.5
Soybean oil A	.286	107.5	108.9
Soybean oil B	.290	109.5	111.0
Commercial blends:			
Vegetable oil + animal fat A	.196	62.9	64.0
Vegetable oil + animal fat B	.196	62.9	63.0
Soybean–cottonseed	.302	115.4	115.0
Cottonseed–peanut	.258	93.6	95.0
Hydrogenated soybean oil	.242	85.7	89.4
Hydrogenated vegetable oil	.249	89.2	93.6

clearly defined 3.3μ absorption band. The maximum gain setting of 10 was necessary to reduce variability for the more saturated fats. This suggests that the resolution of the instrument employed, as influenced by the design of the instrument, gain setting, and scan speed, may affect the infrared ratio. Therefore, operational parameters and a standard curve must be established for the particular instrument employed in the analyses. The results reported in this study were obtained on an infrared spectrophotometer that is not considered to be a high resolution instrument.

With the instrument employed in this study, an iodine value of 30 was near the lower limit for accurate prediction. Infrared spectra of samples of coconut fat possessing iodine values of 10—12 did not reveal a discernible olefinic C—H band (3.3μ).

None of the 25 fats and oils analyzed in establishing the standard curve had been hydrogenated. Since trans-double bonds result in less olefinic C—H absorption (3.3μ) than cis-double bonds (Sinclair et al., 1952), it would be expected that the infrared ratio for hydrogenated oils would be reduced commensurate to the amount of cis—trans isomerization that occurred as a result of hydrogenation. The low predicted values for the two hydrogenated vegetable oil samples support this theory. A different regression equation or standard curve would have to be prepared for application to hydrogenated oils. Preliminary investigations indicate that the regression equation for hydrogenated oils is influenced by the degrees of unsaturation of both the raw material and the finished product. For this reason, a single standard curve applicable to all hydrogenated oils cannot be developed. However, it should be possible to establish a standard curve for a specific type of hydrogenated oil. Future investigations will deal with the extension of the techniques reported in this study to the determination of unsaturation of hydrogenated soybean oils.

This investigation has demonstrated that infrared spectroscopy can be used to determine degree of unsaturation of non-processed fats and oils. A potential application of the results of this study is the development of instruments specifically designed to serve as monitors of unsaturation in process control applications.

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HARVEY T. CHAN JR.[1] and H. Y. YANG
Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331

IDENTIFICATION AND CHARACTERIZATION OF SOME OXIDIZING ENZYMES OF THE MC FARLIN CRANBERRY

SUMMARY—Enzyme extracts were prepared from acetone powders with and without phenol-binding agents such as polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) and buffered PVP. The acetone-PVP combination was found most effective in reducing the polyphenolic content of the enzyme extract. Highest specific activity was obtained by using a buffered PVP extract. The pH optimum of cranberry peroxidase activity was 6.0. Heat inactivation of cranberry peroxidase was determined to follow first order kinetics. There was 90% destruction at 70, 80, and 90°C requiring 9.40, 1.60, and 0.47 min of heat treatment, respectively. Activation energy for the thermal inactivation of cranberry peroxidase was observed to be 37.2 kcal/mole. Guaicol, o-phenylene diamine (OPDA), and pyrogallol were tested for their sensitivity to cranberry peroxidase with OPDA determined as most sensitive. The pH optimum for catalse activity was found to range from 7.5 to 9.2. Kinetics for the heat inactivation of cranberry catalase was observed not to be of the first order nor zero order. Approximately 50% of the catalase activity was inactivated after heating for 17, 1.8, and 0.6 min at temperatures of 50, 60, and 70°C, respectively. The pH optimum for cranberry polyphenolase activity was determined to be 7.0. Heat inactivation of cranberry polyphenolase was found to follow first order kinetics. There was 90% destruction at 50, 60, and 70°C requiring 15.85, 7.05, and 1.37 min of heat treatment, respectively. The activation energy for the inactivation of cranberry polyphenolase was observed to be 27.7 kcal/mole.

INTRODUCTION

THE ENZYME SYSTEMS of cranberries have not been well documented. Only recently has the presence of the pectic enzymes in cranberries been confirmed in this laboratory by Arakji and Yang (1969).

The presence of peroxidase in cranberries was reported by Gorfien et al. (1955). However further information other than its presence was not given.

The presence of other enzymes such as polyphenolase, catalase, and anthocyanase has not been reported. Consequently, the purpose of this work was to confirm and further characterize the presence of peroxidase and to establish either the presence or absence of the other enzyme systems, catalase, polyphenolase, and anthocyanase.

MATERIALS & METHODS

CRANBERRIES, *Vaccinium macrocarpan* var. McFarlin, were obtained from Ocean Spray, Inc. at Markham, Washington. The washed fruit was sorted for mature, sound berries which were frozen and stored at −23°C.

Extraction of enzymes

In preliminary experiments on the extraction of enzymes from cranberries with bufferd solutions, difficulties were encountered in obtaining sufficient activity. Therefore various extraction methods were attempted to obtain an active enzyme extract. The extraction methods used were acetone powders, acetone powders in combination with either PEG (polyethylene glycol) or PVP (polyvinylpyrrolidone), and buffered extracts with PVP. The various extraction methods were then evaluated by the quantitative estimation for proteins, tannins, and peroxidase activity.

In the preparation of acetone powders, 100g of frozen cranberries were blended with 300 ml of cold acetone at −23°C in a Waring Blendor for 1 min and filtered in vacuo through a Whatman No. 1 filter paper. The procedure was repeated twice more on the residue, filtering to dryness on the last extraction. The powder was stored at −23°C.

Enzyme extracts were prepared by mixing the acetone powder with 300 ml of distilled water for 12 hr at 4°C. The slurry was squeezed through a nylon cloth and centrifuged at 9,750 × g for 30 min for further clarification.

The method for the extraction of enzymes using PVP as developed by Loomis and Battaile (1966) was applied to cranberries as follows: 100 g of frozen cranberries were powdered in a stainless steel Waring Blendor using liquid nitrogen. The frozen powdered cranberries were mixed into a paste of PVP previously prepared as follows: 25g of PVP were added to 200 ml 0.1M citrate-phosphate buffer pH 7.5, mixed and stored overnight at 4°C. The enzyme extract was expressed from the PVP paste through a nylon cloth and the residue was extracted again with 100 ml of distilled water (4°C). The extract was clarified by centrifuging at 9,750 × g for 30 min.

For the preparation of enzyme extract using acetone powder and PEG, 100g of frozen cranberries were blended with 300 ml of acetone (−23°C) and 10 ml of a 20% aqueous solution of PEG for 1 min and filtered in vacuo with Whatman No. 1 filter paper, filtering to dryness on the last extraction and stored at −23°C. Enzyme extracts were prepared from the powder as described previously.

For the preparation of enzyme extracts using acetone powder and PVP, 100g of frozen cranberries were blended with 300 ml of cold acetone (−23°C) and 2g of PVP in a Waring Blendor for 1 min and filtered in vacuo through a Whatman No. 1 filter paper. The procedure was repeated twice more on the residue, with filtering to dryness on the last extraction. The powder was stored at −23°C. Enzyme extracts were prepared from the powder as described previously.

To further concentrate the enzyme extracts an ammonium sulfate precipitation was performed on the enzyme extracts at 4°C. The enzyme extracts were made 0.65 saturated with ammonium sulfate. The precipitates formed were of two types: one floated and was removed with a spatula; the other was in suspension and was removed by centrifugation at 9,750 × g for 30 min. The precipitate was resolubilized in 40 ml of distilled water, placed into dialysis tubing and allowed to dialyze for 12 hr at 4°C.

Kjeldahl-nitrogen determination

The method used for the determination of nitrogen was the micro-Kjeldahl method as prescribed by the A.O.A.C. (1960).

Total phenolic determination

Total phenolic concentration was determined by the Folin-Denis procedure as prescribed by A.O.A.C. (1960). A standard curve was prepared for tannic acid and results reported as tannic acid equivalents.

Determination of peroxidase activity

For the quantitative estimation of peroxidase activity a modified method of Vetter et al. (1958) was used. 5 ml of enzyme extract were mixed with 0.2 ml of 1% aqueous o-phenylene diamine HCl and 0.2 ml of 0.3% H_2O_2. Absorbance at 450 mμ was measured in a Beckman DU spectrophotometer.

Peroxidase pH optimum

To determine the optimum pH of cranberry peroxidase, ½g portions of cranberry acetone powder were extracted with 100 ml of citrate-phosphate buffers of various pH's from 4.6 to 7.6 for 30 min. The liquid was expressed through a nylon cloth, centrifuged at 9,750 × g for 30 min, and analyzed for peroxidase activity as previously described.

Peroxidase heat inactivation

The dialysate of an ammonium sulfate precipitate of an acetone powder extract was used as the enzyme source for the study of the heat inactivation kinetics of cranberry peroxidase. Capillary tubes 1.5–2.0 × 10.0 mm were sealed at one end, filled approximately ¾ full with a syringe and sealed. The tubes were heated in a glycerine bath for the prescribed time, cooled in crushed ice, broken open and the contents collected. To 0.5 ml of the enzyme was added 4.5 ml of 0.1M citrate-phosphate buffer pH 6.0, and peroxidase activity was determined as previously cited.

Substrate specificity

The cranberry peroxidase activity on three commonly used "substrates" of peroxidase–guaicol, OPDA, and pyrogallol–was studied in order to determine their relative sensitivity to colorimetric measurements. The pooled frac-

[1] Present address: Hawaii Fruit Lab., USDA, 1920 Edmondson Rd., Honolulu, Hawaii 96822.

Table 1—Effect of extraction method.

Extraction method	Polyphenolic content mg tannic acid/ml	Proteins mg N/ml	Peroxidase activity/ml O.D. units
Acetone Powder	0.047	0.0374	0.284
PEG – Acetone	0.033	0.0765	0.880
PVP	0.027	0.0405	0.545
PVP – Acetone	0.020	0.0311	0.335

tions #6 through 24 from Sephadex G-75 was used as the enzyme source. To 5 ml of the enzyme extract was added 0.2 ml of 0.552M solution of substrate either OPDA, guaicol, or pyrogallol, and 0.2 ml of 0.3% H_2O_2. A 10 min reaction time at 22°C was allowed whereupon the samples were cooled in ice and to the OPDA solution was added 0.4 ml of saturated $NaHSO_3$ solution. The absorption of the solution was then read at 450 mμ for OPDA, and 420 mμ for guaicol and pyrogallol in a Beckman model B spectrophotometer.

Separation of peroxidase from cranberry proteins by gel filtration

For the further purification of the various enzymes, gel filtration with Sephadex G-75 was employed. A glass column 3.0 cm × 60 cm was equilibrated and washed with 0.1M citrate-phosphate buffer, pH 6.0 for 24 hr at 4°C. The void volume was 67 ml. 5 ml of enzyme extract having a nitrogen concentration of 0.143 mg/ml was pipetted on the gel bed. The elution was carried out using 0.1M citrate-phosphate buffer pH 6.0. 10 ml fractions were collected and assayed for protein content by measuring the absorption at 280 mμ and assayed for peroxidase, catalase, and polyphenolase activity by methods cited herein.

Catalase determination

Catalase activity was measured manometrically in a Warburg respirometer according to the conditions prescribed by Maehly and Chance (1954).

The reaction vessel contained 2.8 ml of enzyme extract and the side arm contained 0.2 ml of 0.3% H_2O_2. The flasks were equilibrated at 15°C for 10 min, tipped to mix enzyme and substrate, and shaken at a rate of 140 cycles/min. Readings were made at 3 min intervals, corrected for thermobarometric changes, and multiplied by the flask constant which expressed the results as μl oxygen evolved.

Catalse pH optimum

The pH optimum of catalase activity was determined over the pH range of 5.1 to 10.1. A PEG-acetone powder extract was used as an enzyme source. The reaction mixture consisted of 2.3 ml enzyme extract and 0.5 ml of the appropriate buffer in the main compartment and 0.2 ml of 0.3% H_2O_2 in the side arm. A blank determination consisting of a heat inactivated enzyme extract was made for each corresponding sample to compensate for an auto-oxidation. 0.2M phosphate-citrate buffers were used in the pH range 5.1–8.0 and 0.2M glycine-sodium hydroxide buffers were used in the pH range 8.5–10.1.

Catalase heat inactivation

Samples were heated in test tubes (18 × 150 mm) following the method of Sapers and Nickerson (1962). In order that heat penetration lags be minimized, 2 ml of enzyme solution were pipetted into a preheated test tube in a glycerine bath. After each heating interval the test tubes were chilled immediately in an ice bath, diluted with 8 ml of 0.2M phosphate-citrate buffer pH 7.55, and assayed for catalase activity.

Determination of polyphenolase activity

The method of Ponting and Joslyn (1948), slightly modified, was used to follow the activity of polyphenolase.

For the determination of polyphenolase activity, 5 ml of the PEG enzyme extract were incubated at 30°C with 0.3 ml of 0.02M catechol for 5 min with shaking and then cooled in ice. The absorbance was measured at 400 mμ in a Beckman model B spectrophotometer using a blank consisting of enzyme extract heated for 3 min at boiling to compensate for any auto-oxidation of the substrate.

Isolation of cranberry phenolics

For the isolation of cranberry phenolics, a modified method of Motawi (1967) was used. 100g of cranberries were macerated with 400 ml of ethanol, 95%, in a Waring Blendor for 5 min. The pulp was filtered in vacuo with Whatman No. 1 filter paper. The filtrate was concentrated to about 50 ml under reduced pressure with a Rinco rotary evaporator in a water bath at 45°C. This concentrate was washed 3 times with 100 ml of petroleum ether to remove the lipids. The washed extract was concentrated again to about 25 ml which was washed with distilled water into a 200 ml volumetric flask.

Action of cranberry polyphenolase on cranberry phenolics

To determine the action of cranberry polyphenolase on the phenolic extract of cranberries, 3 ml of the phenolic extract containing 0.76 mg tannic acid/ml were mixed with 4.0 ml of the buffered enzyme solution at 30°C with a shaking rate of 80 cycles/min. 4 ml of heated inactivated enzyme solution was used as control. The flasks were removed at hourly intervals and cooled in an ice bath. The absorbance was read in a Beckman model B spectrophotometer.

Polyphenolase pH optimum

For the determination of the effect of pH on polyphenolase activity the dialysate from the ammonium sulfate precipitate was used as an enzyme source. To 4.5 ml of enzyme extract was added 0.5 ml of buffer solution of the appropriate pH and 0.2 ml of 0.2M catechol solution. The polyphenolase activity was determined as described previously.

Polyphenolase heat inactivation

The kinetics for the heat inactivation of cranberry polyphenolase was investigated. 2 ml of enzyme solution were pipetted into a preheated test tube in a glycerine bath. After each heating interval the test tubes were chilled in an ice bath. The contents of the test tubes were pooled and 5 ml aliquots were assayed for polyphenolase activity.

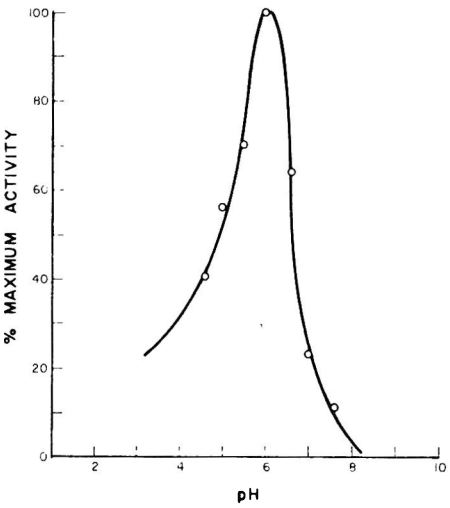

Fig. 1—Effect of pH on peroxidase activity.

Determination of anthocyanase

For the determination of anthocyanase activity a modified method of Huang (1955) was employed.

1 ml of the buffered pigment pH 4.0 extract was mixed with 5 ml of enzyme extract to give a final pigment concentration of 1.27×10^{-5}M calculated as cyanidin monogalactoside. The enzyme extracts were prepared as described previously except that a 0.1M citrate-phosphate buffer of pH 4.0 was used for the extraction. The experiments were carried out at 30°C with shaking. The absorbance was measured at 525 mμ in a Beckman model B spectrophotometer at 1 hr intervals.

Isolation of anthocyanins

The extraction and purification of anthocyanins were carried out using a modified method of Smith and Luh (1965). 200g of cranberries were macerated with 250 ml of methanolic HCl in a Waring Blendor and filtered through 2 layers of Whatman No. 1 paper. The pulp was extracted two more times. The combined extracts were concentrated in vacuo to 210 ml in a water bath at 48°C. The concentrate was filtered to remove precipitates. The filtrate was mixed with 450g of Dowex 50w × 4 cation exchange resin (100–200 mesh) in hydrogen form. After 2 hr the resin was washed and filtered twice with methanol to remove organic residues and with distilled water to remove free sugars. The pigment was eluted from the resin by successive extraction with 2% and 5% methanolic HCl, yielding approximately 6 liters of filtrate which were reduced to 200 ml by rotary evaporation.

RESULTS & DISCUSSION

Effect of extraction method on the polyphenolic content of the enzyme extract

The results of the various methods of extraction are shown in Table 1 which

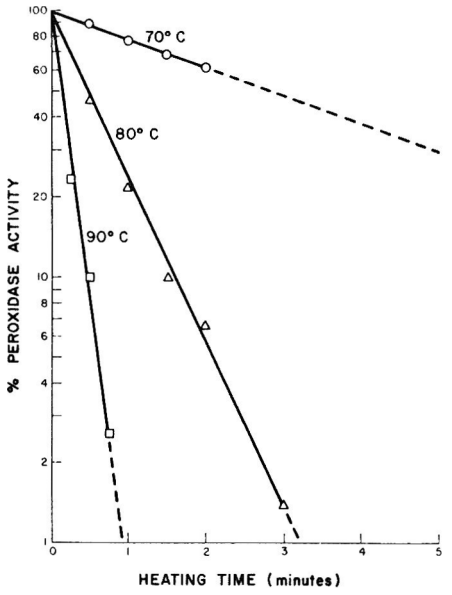

Fig. 2–Heat inactivation of cranberry peroxidase.

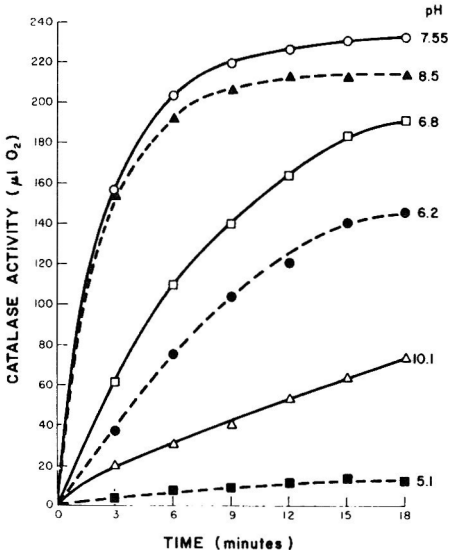

Fig. 3–Effect of pH on the decomposition of H_2O_2 by cranberry catalase versus reaction time.

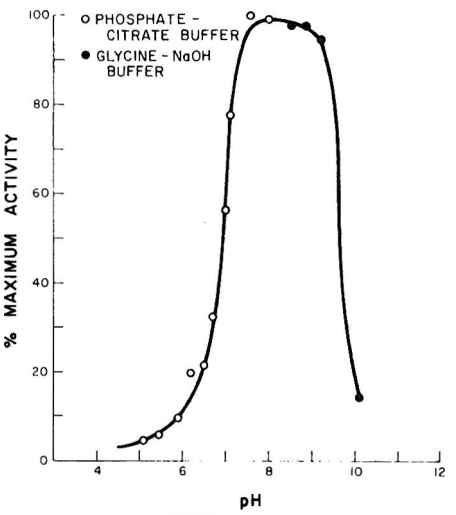

Fig. 4–Effect of pH on cranberry catalase.

expresses the amount of polyphenols present in the enzyme extract as mg tannic acid.

The combination of acetone-PVP was the most efficient phenol remover as evidenced by the low phenolic content in the enzyme extract. The next most efficient was PVP, followed by PEG-acetone, and finally acetone powder. Results were somewhat as expected since PVP is a stronger hydrogen acceptor than either PEG or acetone (Loomis and Battaile, 1966); the added presence of acetone in combination with PVP provides a greater effect than PVP alone in that acetone, besides being a good solvent for the removal of many other types of compounds, has the ability to form complexes with phenols.

Effect of extraction method on cranberry proteins

The effect of the method of extraction on the quantity of soluble proteins extracted is shown in Table 1. The greatest quantity of protein extracted was obtained using the combination of PEG and acetone. Lesser yields of protein were obtained by the other extraction methods as follows, in order of decreasing yields: PVP, acetone powder, and PVP with acetone.

Effect of extraction method on peroxidase activity

The effect of extraction method on peroxidase activity is shown in Table 1. The greatest yield of peroxidase was obtained using PEG-acetone followed in decreasing order of yield by PVP, PVP and acetone, and finally acetone powder.

The specific activity of peroxidase, O.D. units/mg N, was determined for each of the various extraction methods. The results are listed as follows: PVP 13.45, PEG-acetone 11.50, PVP-acetone 10.78, and acetone powder 7.60 O.D. units/mg N.

Although PVP did not give as high a yield in peroxidase activity or nitrogen it did give a higher specific activity. This indicates that some of the protein loss was non-peroxidase protein. The extracting method giving the next highest specific activity was PEG-acetone followed by PVP-acetone, and finally acetone. The results indicate that the PVP extraction method gave the purest enzyme extract of the four methods used in these studies.

Effect of pH on cranberry peroxidase activity

The effect of pH on cranberry peroxidase activity with OPDA is shown in Figure 1. The pH optimum for cranberry peroxidase activity is at 6.0.

Heat inactivation studies of cranberry peroxidase

Results of the kinetic studies on the heat inactivation at 70°C, 80°C, and 90°C of cranberry peroxidase are shown in Figure 2. The logarithmic decrease of peroxidase activity with increase in exposure time to heat indicates that the thermal inactivation of cranberry peroxidase follows first order kinetics. For all three temperatures 70, 80, and 90°C at which heat inactivation was carried out, first order kinetics were found to apply. The rate constants k, and D values are listed in Table 2.

Compared to peroxidase found in other sources under the same conditions, cranberry peroxidase appears to be quite heat labile. For example, Zoueil and Esselen (1958) determined that the D value for green bean peroxidase at 90°C was 10.5 min. Yamamoto et al. (1962) found two fractions of peroxidase in corn exhibiting differences in heat resistance. The heat labile fraction gave D values of 25 sec at 80°C and 16 sec at 90°C. For the heat resistant fraction the D values were 31 min at 80°C and 13.2 min at 90°C. The linearity of semilogarithmic plots for cranberry peroxidase also implies that cranberry peroxidase was homogenous in its susceptibility to heat inactivation.

Table 2–Kinetics data for heat inactivation.

Temp °C	Rate Constant min^{-1}		D value min	
	Peroxidase	Polyphenolase	Peroxidase	Polyphenolase
50	–	0.145	–	15.85
60	–	0.327	–	7.05
70	0.245	1.746	9.40	1.32
80	1.435	–	1.60	–
90	4.920	–	0.47	–

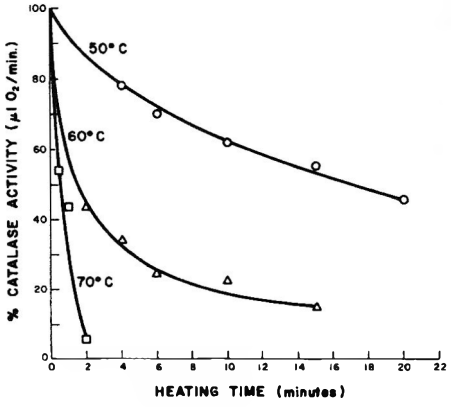

Fig. 5–Heat inactivation of cranberry catalase.

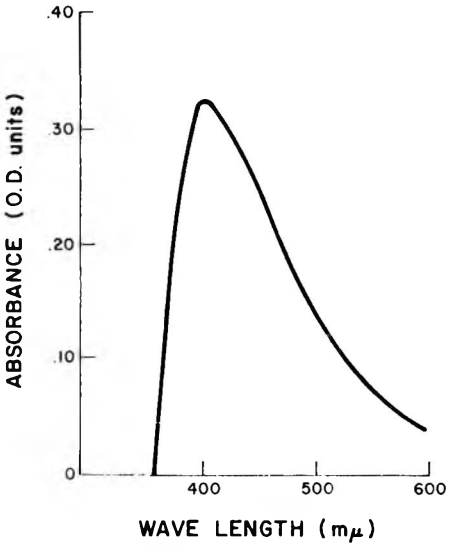

Fig. 6–Absorption spectrum on the product of cranberry polyphenolase on catechol.

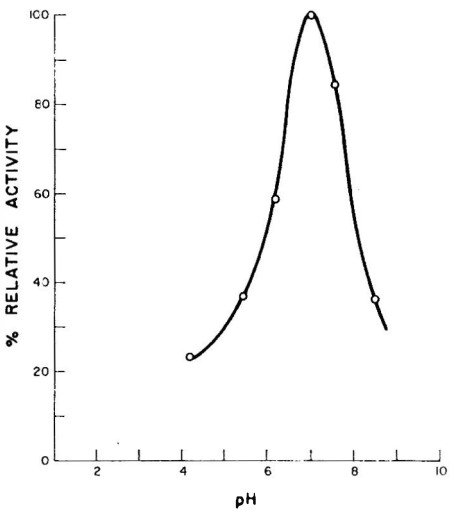

Fig. 7–pH optimum of cranberry polyphenolase.

The activation energy for the thermal inactivation of cranberry peroxidase was determined from an Arrhenius plot. For cranberry peroxidase the activation energy for the thermal inactivation of its activity was observed to be 37.2 kcal/mole.

Sensitivity of cranberry peroxidase to three commonly used substrates

The sensitivity of cranberry peroxidase from the purified fractions of Sephadex G-75 to 3 common hydrogen donors was measured. Results show OPDA to be the most sensitive followed by pyrogallol and the guaicol. These results agree with that of Reddi et al. (1950) who in measuring apple peroxidase with the same 3 hydrogen donors, namely guaicol, pyragallol, and OPDA, found that OPDA was the most sensitive.

Effect of time and pH on catalase activity

The course of the catalase reaction with time as measured manometrically by the evolution of oxygen is shown in Figure 3. As demonstrated by the curve the amount of oxygen evolved increases with reaction time but decreases markedly after 6 min. The decrease in activity cannot be attributed exclusively to depletion of substrate concentration as calculations show that only about 10% of the H_2O_2 had undergone decomposition.

As shown in Figure 3, pH has a greater effect on the initial rate of reaction. For example, after 3 min of reaction the differences in the amount of oxygen evolved between pH 7.55 and pH 6.8 are great, but as the reaction time increases the amount of oxygen evolved at pH 6.8 approaches that evolved at pH 7.55 indicating that pH has its greatest effect on the initial rate.

Therefore the amount of oxygen evolved after 6 min was used to determine the rate of reaction and the results were expressed as μl oxygen evolved per min.

The effect of pH on cranberry catalase activity is shown in Figure 4. Catalase activity was determined from the amount of oxygen evolved for the first 6 min of the reaction and the results were expressed as μl oxygen evolved per min.

Catalase activity increased rapidly with a rise in pH up to a pH optimum of 7.55 whereupon further increases in pH resulted only in a slight decrease in activity up to pH 9.2; thereafter catalase activity decreased sharply with increase in pH.

Heat inactivation of cranberry catalase

The results of the heat inactivation of cranberry catalase at 50, 60 and 70°C are shown in Figure 5. The heat inactivation of cranberry catalase is a non-linear relationship to heating time, under the conditions tested. Neither was there a linear relationship when plotted on semilogarithmic paper. Hence, the heat inactivation of catalase follows neither zero nor first order kinetics.

As expected, the rate of heat inactivation was greater with increase in temperature. Approximately 50% of the catalase activity can be expected to be inactivated after heating for 17, 1.8, and 0.6 min, at temperatures of 50, 60, and 70°C, respectively.

Absorption spectrum of action of cranberry polyphenolase on catechol

The absorption spectrum for the product of the reaction between catechol and cranberry polyphenolase is shown in Figure 6. The absorption maximum occurs at 400 mμ. The absorption band at 400 mμ which is characteristic of the oxidation product of o-diphenol, o-quinone indicates that cranberry polyphenolase possesses catecholase-like activity. An attempt was made to determine if cresolase activity was also present in cranberries by using p-cresol as a substrate in the reaction in place of catechol. No reaction with p-cresol was observed indicating that cranberry polyphenolase is of the catecholase type.

Effect of pH on cranberry polyphenolase

The effect of pH on cranberry polyphenolase activity is shown in Figure 7 where the pH optimum occurs at pH 7.0. The pH curve is characterized by a decrease in polyphenolase activity with pH values less than or greater than 7.0.

Heat inactivation of cranberry polyphenolase

The heat inactivation of cranberry polyphenolase was carried out at 50, 60, 70°C and the results of these studies are shown in Figure 8. When the results are plotted with the logarithm of the residual activity versus the heating time, a linear relationship exists indicating that the heat inactivation of cranberry polyphenolase follows first order kinetics.

The kinetics data for the heat inactivation of cranberry polyphenolase which include the rate constant, k, and D value are shown in Table 2. The D values indicate that 90% of the polyphenolase is inactivated by heating for 1.32 min at 70°C, 7.05 min at 60°C, or 15 min at 50°C.

From the rate constants for the heat inactivation of cranberry polyphenols an Arrhenius plot was constructed and the activation energy for the heat inactivation of cranberry polyphenolase was observed to be 27.7 kcal.

Action of cranberry polyphenolase on cranberry phenolic extract

In order to demonstrate the presence

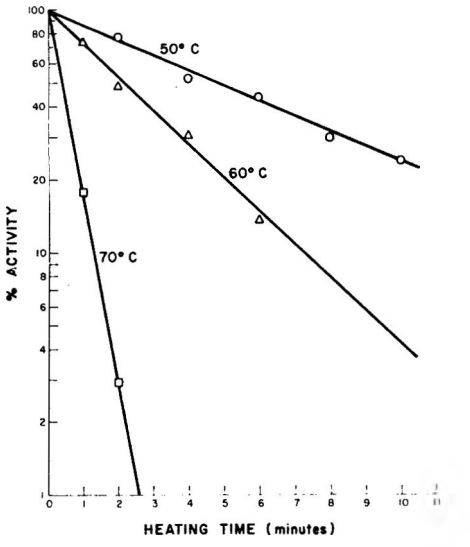

Fig. 8–Heat inactivation of cranberry polyphenolase.

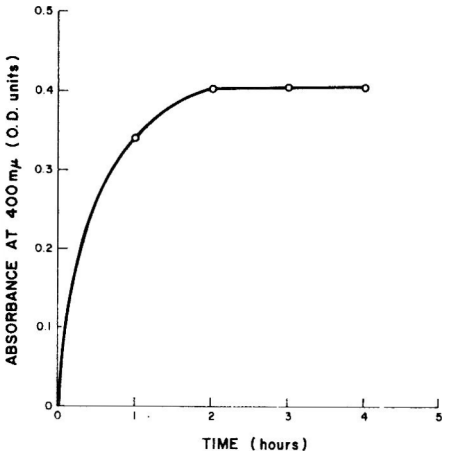

Fig. 9–Action of cranberry polyphenolase on cranberry phenolic extract versus time.

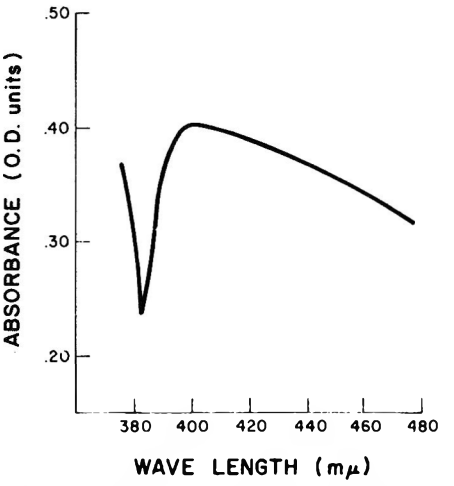

Fig. 10–Absorption spectrum for the reaction of cranberry polyphenolic extract and cranberry enzyme extract.

of both enzyme and substrate in cranberries the cranberry phenolic extract was reacted with cranberry polyphenolase. The results are shown in Figure 9 where activity was measured by absorption at 400 mμ after incubation for 1, 2, 3, and 4 hr. The results show the polyphenolase activity rises sharply and reaches its maximum activity after 2 hr of incubation at 30°C. The absorption spectrum for the sample incubated for 2 hr and measured on a Beckman DB spectrophotometer is shown in Figure 10. The absorption spectrum for the product of the reaction between that of the cranberry polyphenolic extract and cranberry polyphenolase was similar to the spectrum obtained using catechol as a substrate (see Fig. 6) in that the absorption maxima for both substrates were 400 mμ. However, the spectrum for the polyphenolic extracts differs from that for catechol in that instead of decreasing in absorption at 385 mμ, the absorption increases again. This increase may be due to the presence of endogenous substrates other than polyphenols in the polyphenolic extract of cranberry.

Both oxidized catechol and oxidized polyphenolic extracts showed absorption peaks of 400 mμ. This agrees fairly well with the results of Bedrosian et al. (1960) obtained when apple polyphenolase reacted with catechol and apple polyphenolics.

Anthocyanase studies

Cranberry enzyme extracts prepared by the various methods previously decribed were mixed with cranberry anthocyanins. The samples were incubated at 30°C with shaking. No decreased absorption at 525 mμ was observed over a 6-hr incubation period. Therefore no anthocyanase activity could be detected by this method.

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J. W. DONOVAN and L. U. HANSEN
USDA Western Regional Research Laboratory, ARS, Albany, California 94710

THE β-N-ACETYLGLUCOSAMINIDASE ACTIVITY OF EGG WHITE. 1. Kinetics of the Reaction and Determination of the Factors Affecting the Stability of the Enzyme in Egg White

SUMMARY—Enzymatic activity of β-N-acetylglucosaminidase, which occurs naturally in chicken egg white, was characterized to establish conditions suitable for routine assay for this enzyme in egg products. A variation in enzyme content of approximately 3-fold was observed in individual fresh eggs. The enzyme has a pH optimum between 3.0 and 3.4, and a K_m of approximately 0.6 mM for the substrate p-nitrophenyl-N-acetyl-β-D-glucosaminide. Activation energy for hydrolysis of this substrate is 10.7 ± 0.8 kcal/mole. The enzyme is stable for at least several hours at ambient temperature from pH 6.8 to approximately 8.8. Above pH 8.8, inactivation is first order with respect to time. Enzyme activity in shell eggs decreases fairly rapidly at ambient temperature; loss of activity probably results from increase in pH of egg white, which occurs normally upon loss of carbon dioxide. Eggs held at 4°C retain activity much longer.

INTRODUCTION

THE U.S. Food and Drug Administration (1968) now requires that all egg products shipped in interstate commerce be free of viable Salmonella. All USDA-certified egg products must be pasteurized by a suitable method (USDA Consumer and Marketing Service, 1969). We have attempted to find a substance normally present in eggs which would be so modified by the pasteurization process that the alteration in this substance could be used as an indication that an egg product had received the recommended pasteurization treatment. In food processing, thermal inactivation of enzymatic activities is often used to estimate proper heat treatment. The heat inactivation of alkaline phosphatase (Kay and Graham, 1935) present in milk is widely used as an indicator of heat treatment given milk products. In Great Britain, an enzymatic test for α-amylase (Brooks, 1962) is used to assess the heat treatment given whole egg (Statutory Instruments—Great Britain, 1963). However, pasteurization temperatures ordinarily employed in the United States are less severe (USDA, Agricultural Research Service, 1969), and the α-amylase is not destroyed.

The enzyme β-N-acetylglucosaminidase in chicken egg white (Lush and Conchie, 1966) is heat inactivated when exposed to temperatures near 60 degrees (Henderson and Robinson, 1969). The possibility of using the heat inactivation of this enzyme as an indicator of heat treatment given egg white during pasteurization at pH 7 (Cunningham and Lineweaver, 1965) was investigated. The characterization of the enzyme activity in egg white is the subject of this paper (paper 1); the accompanying paper (2) presents experimental results on the heat inactivation of the enzyme and considers the requirements necessary for an enzymatic test for pasteurization treatments.

MATERIALS & METHODS

CLEAN, but unoiled and unwashed eggs from 1-year-old white leghorn (Kimber No. 137) chickens were stored in a cold room (4°C) in a standard corrugated egg crate (30-dozen size) unless otherwise stated. These eggs had been laid within 24 hr of receipt. They were broken out normally immediately upon receipt, but in no case later than 5 days after receipt. When broken out, the pH of the egg white of different lots of eggs was determined to be between 8.3 and 8.9. The egg white was separated from the yolk and chalazae, and pooled. (Customarily, sufficient eggs were broken out so that a pool of 750 ml of egg white was obtained. This volume of egg white could be blended conveniently in a Waring Blendor without producing much foam.) The pooled egg white was blended in the cold room until the viscosity measured at 4°C as flow time through a Zahn No. 2 viscometer was approximately 17 sec.

The substrate p-nitrophenyl-N-acetyl-β-D-glucosaminide was obtained from Sigma Chemical Co. Eastman p-nitrophenol was recrystallized twice from acetone and water by A.K. Balls. Other chemicals used were reagent grade.

A stabilizer solution (Cunningham and Lineweaver, 1965) consisting of 0.0625 g of $Al_2(SO_4)_3 \cdot 18\ H_2O$ per ml of 25% lactic acid was used for the pH adjustment of egg white in some experiments. Presence or absence of aluminum sulfate appeared to have no effect on activity of the enzyme.

Standard assay for β-N-acetylglucosaminidase activity in egg white

The release of p-nitrophenol from p-nitrophenyl- N-acetyl-β-D-glucosaminide was followed by a modification of the procedure of Lush and Conchie (1966). The incubation mixture, containing 0.01 ml of egg white, 260 μg (0.76 μmole) of substrate in 0.30 ml of 0.1 M pH 3.0 citrate buffer and 0.19 ml of water, was incubated at 37°C for 25 min. The reaction was stopped by addition of 0.66 ml of 0.2 M Na_2CO_3 to the incubation mixture. The absorbancy of the resulting solution was measured in a narrow absorption cell (1-cm light path) in a Beckman DU spectrophotometer at 420 mμ against a water reference. The absorbancy of the blank (egg white added after Na_2CO_3) was subtracted from that of each solution. The amount of p-nitrophenol released was calculated from a calibration curve for p-nitrophenol determined separately. A change in absorbancy of 1.0 corresponds to the hydrolysis of approximately 6% of the substrate.

RESULTS

Optimum pH for β-N-acetylglucosaminidase assay in egg white

The enzyme exhibits at least 20% of maximum activity over the pH range 1.5–5.5. The optimum pH is 3.0 (Fig. 1). Citrate buffer, pH 3.0, was chosen for the standard assay since its pK' is close to the pH optimum of the enzyme.

Enzymatic activity as a function of substrate concentration

At p-nitrophenyl-N-acetyl-β-D-glucosaminide concentrations greater than 2 mM the enzymatic activity is nearly independent of substrate concentration (Fig. 2A). In the standard assay, 0.76 μmole of substrate was present in 0.5 ml of reaction mixture to give a concentration of 1.5 mM. Use of this concentration conserved substrate but essentially retained the zero-order kinetic behavior.

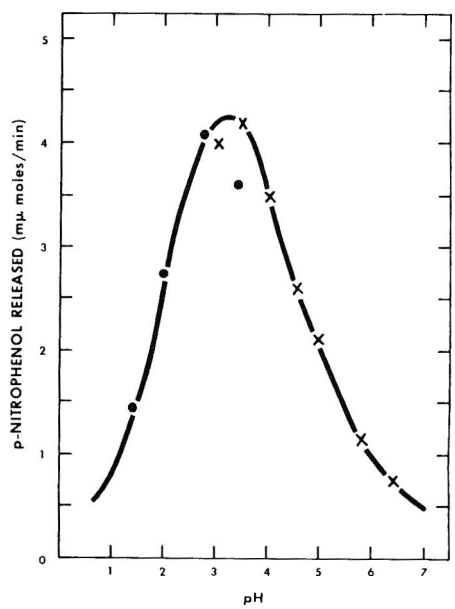

Fig. 1—Effect of pH on β-N-acetylglucosaminidase activity in egg white. p-Nitrophenol released by 0.01 ml of egg white was measured after a 10-min incubation period at 37°C; ●, 0.1M glycine-HCl; X, 0.1 M citrate.

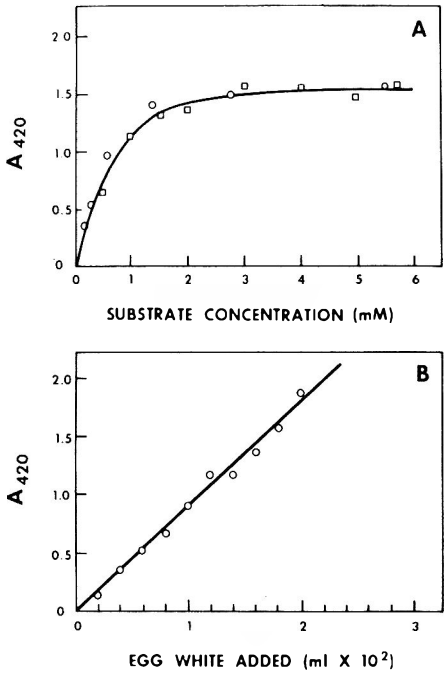

Fig. 2—Release of p-nitrophenol as a function of substrate and enzyme concentrations. A, effect of substrate concentration for standard assay conditions (except for variation in substrate concentration)—2 experiments with different enzyme concentrations and incubation times; B, linearity between amount of color produced and amount of enzyme added—standard assay conditions (except for variation of amount of egg white added), 25-min incubation time.

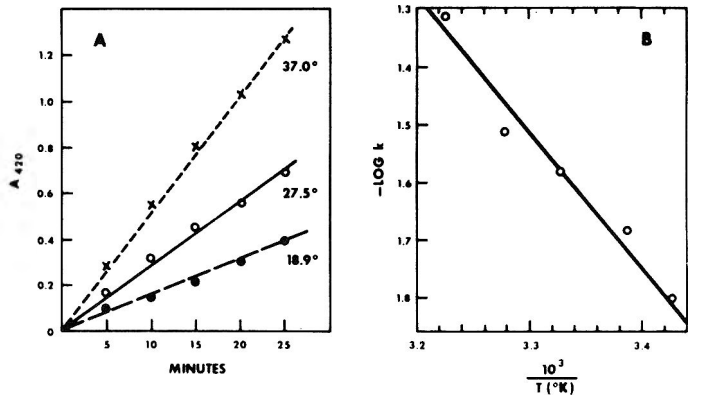

Fig. 3—Temperature dependence of the rate of release of p-nitrophenol. A, release of p-nitrophenol as a function of time at 3 incubation temperatures (°C)—standard assay conditions used; B, temperature dependence of the zero-order rate constant.

Linearity between p-nitrophenol released and enzyme in egg white

Figure 2B shows that the color produced at the fixed time of incubation is proportional to the amount of egg white added to the incubation mixture in the range 0.002—0.020 ml.

Enzymatic activity as a function of incubation temperature

The zero-order nature of the release of p-nitrophenol over a wide temperature range is shown in Figure 3A. The dependence of the logarithm of the zero-order rate constant upon reciprocal temperature is linear (Fig. 3B). The activation energy for the hydrolysis of the substrate, calculated from a linear least-squares fit of the data of Figure 3B, is 10.7 ± 0.8 kcal/mole. Since the enzyme is about 3/4 saturated with substrate under these experimental conditions, this activation energy reflects, in part, the binding of substrate to enzyme.

Stability of the enzyme as a function of pH

Samples of egg white were held at various pH values for 2 hr at room temperature (24°C) and then assayed for enzymatic activity. If the pH of the egg white is lowered much below pH 6.8, some of the egg white proteins precipitate. Figure 4 shows that the enzymatic activity is stable at room temperature for 2 hr in the pH range 6.8—9.0. When fresh egg white was adjusted to pH 9.6 with 1 M NaOH, a rapid inactivation of the enzyme resulted. Loss of activity followed first-order kinetics, as shown in Figure 5.

Variation in β-N-acetylglucosaminidase activity in egg white

Eggs received in this laboratory over a 1-year period have been assayed for enzymatic activity, using the standard assay. The activities in pooled white from 15 different lots had a mean value of 4.12 mμmoles/min with a standard deviation of a single measurement of 0.50 mμmoles/min. No seasonal variations in activity were observed.

The distribution of activities in individual eggs from one lot, freshly broken out, after homogenization of the white, is shown in Figure 6. The enzymatic activity had a mean value of 4.50 mμmoles/min, with a standard deviation for a single egg of 0.91 mμmoles/min.

Effect of storage

Unoiled, unwashed eggs stored at 4°C for 2 months (Fig. 7A) showed a slow decrease in activity (1 to 6 X 10^{-2} mμmoles/min/day of storage) and a slow rate of increase in pH (0.0087 unit/day of storage after the initial rapid increase in pH which occurs in the first few days). The rise in pH, associated with loss of carbon dioxide, is dependent on storage temperature and has been extensively studied [see Romanoff and Romanoff (1949) for review]. Eggs stored at 22°C for 7 days showed a more rapid loss of activity, initially 7.5 x 10^{-1} mμmoles/min/day of storage (Fig. 7B). The rate of increase in pH was much greater, averaging 0.17 pH unit/day of storage after the initial rapid rise in pH. To ensure that small changes in enzymatic activity with time would be detected in these experiments, aliquots of egg white at each storage time were incubated for 4 different time periods (max 30 min) and the rate constant for liberation of p-nitrophenol determined by least-squares fit of A_{420} to incubation.

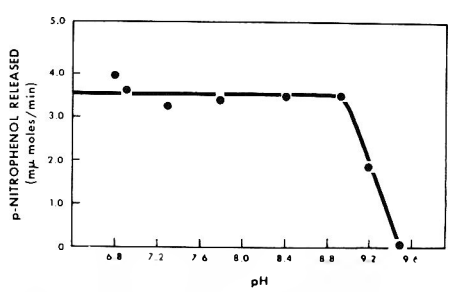

Fig. 4—Effect of pH on the stability of the enzyme activity in egg white. Egg white (pH 8.9) adjusted to higher pH with 1 N NaOH or to lower pH with stabilizing solution (see text), then held at 24°C for 2 hr prior to standard assay.

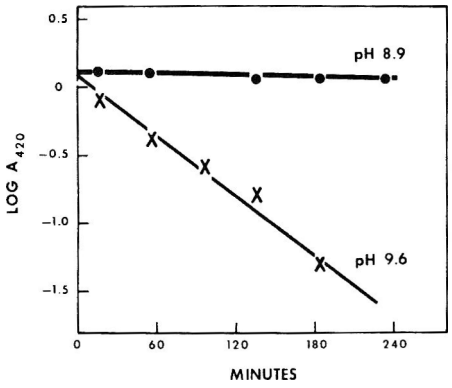

Fig. 5—First-order plot of the inactivation of the β-N-acetylglucosaminidase activity of egg white at 21.5°C.

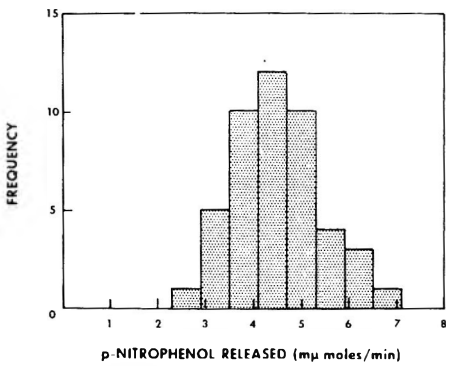

Fig. 6—Distribution of β-N-acetylglucosaminidase activities in egg white from 46 individual eggs. Data obtained by means of an automated β-N-acetylglucosaminidase assay.

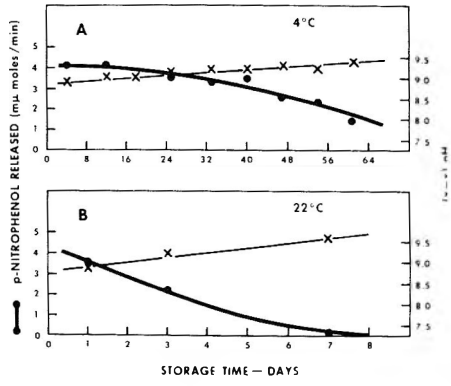

Fig. 7—Effect of storage time and temperature on β-N-acetylglucosaminidase activity of egg white. A, storage at 4°C; B, storage at 21.5°C. Age of eggs is approximately 1 day greater than storage time indicated.

DISCUSSION

THE β-N-acetylglucosaminidase of egg white differs from that obtained from other sources in its optimum pH (Table 1). The temperature dependence of the rate of release of p-nitrophenol is reported here (Fig. 3) because incubation at other temperatures (for example, at room temperature) may be convenient in practical applications of this assay. The observed activation energy (Table 1) is similar to that reported for the enzymes from pinto beans (Agrawal and Bahl, 1968), snail tissue (Neuberger and Pitt Rivers, 1939) and ox liver (Watanabe, 1936).

The amount of β-N-acetylglucosaminidase activity found in pooled fresh chicken eggs varies severalfold. Lush and Conchie (1966) found a 2-fold variation in the amount of this enzyme in single fresh eggs from 6 to 9 different chickens of each of several breeds and strains including brown leghorn, white leghorn and Rhode Island red. However, Henderson and Robinson (1969) have found a 6-fold variation in the β-N-acetylglucosaminidase activity of samples of fresh egg white. A 3-fold variation in enzymatic activity in individual eggs was observed in this laboratory (Fig. 6). A smaller range of activities was found for samples of pooled egg white, an expected result of pooling. Variation in the amount of enzyme found in fresh eggs presents problems in using this enzyme assay to determine extent of heat treatment of egg products. However, the assay can be used for this purpose as long as some enzyme activity remains (see paper 2).

Upon storage, particularly at ambient temperatures when the pH of the egg white exceeds pH 9, the enzyme is gradually inactivated (Fig. 5 and 7B). However, the amount of the enzyme present in fresh eggs remains high for a considerable length of time if the eggs are stored under refrigeration (Fig. 7A). Since unoiled eggs were used in these experiments, oiled eggs, when refrigerated, should retain their enzyme activity longer than indicated by Figure 7. Unfortunately, since the amount of enzyme present in a fresh egg is variable, an enzyme assay, by itself, cannot be used to determine either the age or the history of the storage of the egg.

The measurement of the pH of the egg white (Romanoff and Romanoff, 1949) and the Haugh index are commonly used as indicators of the quality of eggs. Both these quality factors have their merits and disadvantages (see Wells, 1968, for a review). The results presented here indicate that the measurement of the β-N-acetylglucosaminidase activity might also serve as a test of egg quality, as suggested by Lush and Conchie (1966). Since this test is more complicated than the other indices of quality, we do not envision its general use for this purpose except under circumstances in which the other tests prove inapplicable.

This assay for β-N-acetylglucosaminidase is simple and rapid enough to be carried out using automated equipment (Hansen, in preparation). On the other hand, simplifications can be made in the assay, as here described. The yellow color developed upon addition of Na_2CO_3 could be measured with a colorimeter, or against standard filters.

The heat denaturation of this enzyme

Table 1—Enzymatic properties of β-N-acetylglucosaminidases from various sources.

Source	pH optimum	K_m (mM)	Activation energy (kcal/mole)
Chicken egg white[a]	3.0–3.4	0.59 ± 0.06	10.7
Calf brain[b]	5.2	0.72	—
Rat kidney[c]	4.3	1.78	—
Pig epididymis[d]	4.2	1.8–2.1	—
Rat testis[e]	4.5	1.2	—
Human spleen[f]	4.5–5.0	0.67	—
Pinto beans[g]	4.6–4.8	0.47	9.8
Ox liver[h]	3.8–4.1	3.0	12.2
Beef liver[i]	4.5	1.2	—
Snail liver and digestive tract[j]	3.6	—	10.6

[a]This paper. K_m and its standard deviation obtained by unweighted linear least-squares analysis of a double reciprocal plot.
[b]Frohwein and Gatt, 1967.
[c]Pugh et al., 1957.
[d]Findlay and Levvy, 1960.
[e]Woollen et al., 1961.
[f]Robinson and Stirling, 1968.
[g]Agrawal and Bahl, 1968.
[h]Watanabe, 1936.
[i]Langley and Jevons, 1968.
[j]Neuberger and Pitt Rivers, 1939.

in egg white and the applicability of this test as an indicator of proper heat treatment of egg white and whole egg is the subject of the accompanying paper (paper 2).

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J. W. DONOVAN and L. U. HANSEN
USDA Western Regional Research Laboratory, ARS, Albany, California 94710

THE β-N-ACETYLGLUCOSAMINIDASE ACTIVITY OF EGG WHITE. 2. Heat Inactivation of the Enzyme in Egg White and Whole Egg

SUMMARY—The kinetics of the heat inactivation of β-N-acetylglucosaminidase in egg white and whole egg at neutral pH is first order in the temperature range 58–62°C. Rate constants for enzyme inactivation by heat are reported for both egg white and whole egg. The activation energy for heat denaturation is 91 kcal/mole in egg white and 73 kcal/mole in whole egg. Reduction of enzymatic activity upon heating can be used as an indication of whether egg white or whole egg has been maintained at the time-temperature combinations approved for pasteurization by the U.S. Department of Agriculture. This test should be useful as a process control tool.

INTRODUCTION

THE β-N-ACETYLGLUCOSAMINIDASES from various sources are heat-denatured at temperatures near 60°. We have found that this enzyme, which occurs naturally in egg white, Lush and Conchie (1966), is also denatured at 60° at neutral pH. Accordingly, a test for this enzyme might serve to determine whether egg products have been given the recommended pasteurization treatment. The properties of this enzyme were presented in the preceding paper (paper 1), and form the basis for the work presented here on the destruction of the enzymatic activity upon heating.

MATERIALS & METHODS

EGG WHITE was prepared and stabilized with aluminum sulfate and lactic acid as previously described (paper 1). Whole egg was prepared from eggs from the same source by blending the contents of broken-out eggs for the same time as egg white had been blended. No pH adjustment was made for whole egg. Both egg white and whole egg were heat-treated as follows: aliquots of 0.10 ml were added to centrifuge tubes (Corning 8120, 17 by 120 mm) 30 min after the tubes were inserted in a constant temperature water bath (± 0.03°C). At definite time intervals, the tubes were withdrawn from the water bath and immersed in ice water. The samples were then held at refrigerator temperature (5°C) until an aliquot was assayed at 1 incubation time period for enzyme activity as previously described (paper 1). For whole egg, the incubation mixture, after addition of sodium carbonate, was centrifuged 10 min in a small clinical centrifuge to reduce turbidity. The average blank (sample added after sodium carbonate) was 0.015 absorbancy unit (420 mμ) for egg white and 0.090 for whole egg. All rate constants were determined by unweighted linear least-squares fit of logarithm of activity remaining (log A_{420}) vs. time. The time required for the sample to attain the bath temperature did not affect the determination of the rate constant, since the rate constant was obtained from the slope, not the intercept, of the least-squares line. 5 to 8 heating time periods were used to determine the rate constant at each temperature and pH. The standard error in the rate constant averaged 1/10th of the magnitude of the rate constant. The dependence of the logarithm of the rate constant upon pH or reciprocal temperature was calculated by a weighted linear least-squares procedure. The weighting term was the inverse of the standard error of the rate constant.

Some samples of egg white and whole egg were heat treated in a continuous pasteurizer built by K. Ijichi and V. Kaufman of this laboratory. In this apparatus, an adaptation of the slug-flow heat exchanger described by Stroup et al. (1969), the time required for the sample to attain the nominal temperature was of the order of a few seconds.

RESULTS

Egg white

In the temperature range 58–62°C, the loss of the β-N-acetylglucosaminidase activity of egg white is first order with respect to time (Fig. 1). First-order rate of inactivation is also observed when whole egg is heated. The temperature dependence of the rate of heat inactivation of the enzyme in egg white is shown in Figure 2. The linearity of the plot indicates the activation energy for heat inactivation is approximately constant over this rather narrow temperature range. The activation energy calculated by use of the Arrhenius equation, $E_a = -R\ d(\ln k)/d(1/T)$ and the slope of the line in Figure 2, as given by a weighted linear least-squares analysis is 91.1 ± 8.9 kcal/mole. Henderson and Robinson (1969) have reported an activation energy for heat denaturation of this enzyme in egg white (no pH specified) of 63 kcal/mole.

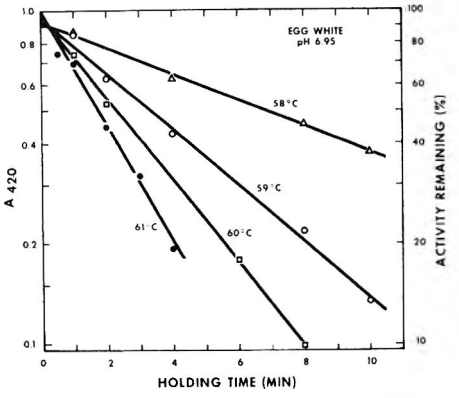

Fig. 1—Kinetics of the thermal inactivation of β-N-acetylglucosaminidase in egg white. The absorbancy at 420 mμ (left ordinate) is proportional to the amount of p-nitrophenol liberated upon assay after heat treatment. The right ordinate shows, approximately, the activity remaining in the egg white samples.

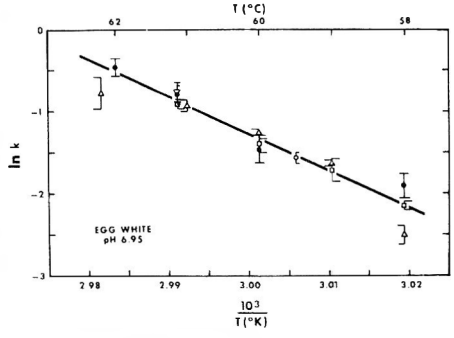

Fig. 2—Temperature dependence of the first-order rate constant for thermal inactivation of β-N-acetylglucosaminidase in egg white. The natural logarithm of the rate constant (min^{-1}) is plotted against reciprocal absolute temperature. Different samples of egg white are represented by different symbols. The vertical bars are 2 standard deviations in length.

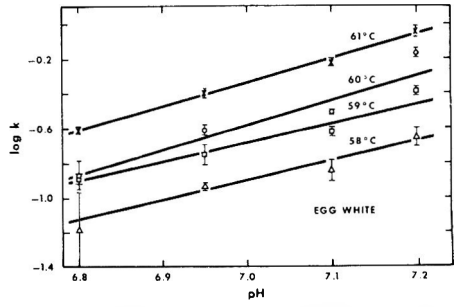

Fig. 3—Dependence of the common logarithm of the first-order rate constant for heat inactivation of β-N-acetylglucosaminidase in egg white upon pH. Vertical bars are 2 standard deviations in length. Lines are calculated (weighted linear least squares).

The first-order rate constant, k, for enzyme inactivation increases with pH (Fig. 3) in the pH range recommended for pasteurization of aluminum-stabilized egg white (USDA, 1969). The change in k with pH was independent of temperature between 58 and 61°C, since the lines in Figure 3 are parallel, within experimental error. The average value of d(log k)/d pH was 1.22, with an average standard error of ± 0.22. Since the change in k with temperature is constant over this limited pH range, the activation energy for enzyme inactivation must also be constant over this pH range.

To determine whether age of eggs had an effect on rate of inactivation of the β-N-acetylglucosaminidase, the rate constant for heat inactivation was determined for samples of egg white obtained from eggs stored at 4°C for 10 time periods up to 60 days. Within experimental error, the rate of enzyme inactivation did not vary with time of storage, although the initial activity in the unheated egg white varied from sample to sample (see paper 1).

Figure 4 shows the amount of enzyme inactivation produced by heating egg white for a constant time period, as a function of temperature. Approximately 2/3 of the β-N-acetylglucosaminidase activity is destroyed by heating for 3.5 min at 60°C, minimum conditions recommended for pasteurization of whole egg and of aluminum stabilized egg white, USDA (1969). There appears to be a 10–30% increase in enzyme activity when egg white is heated at 45–50°C and then cooled for assay. For the present purposes, the amount of enzyme activity present in the untreated egg white has been taken as that activity found in unheated egg white.

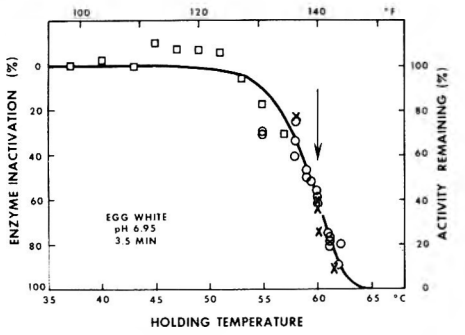

Fig. 4—Inactivation of β-N-acetylglucosaminidase produced by heating egg white for 3.5 min. Points calculated from rate constants of separate kinetic experiments such as those shown in Figure 1, O; measured activity after a single sample was heated for 3.5 min plus time required for sample to attain bath temperature (30 sec), □; continuous slug-flow pasteurizer used, ×. The curve shows the calculated amount of activity remaining after 3.5 min, assuming that the equation for the line in Figure 2 applies over the entire temperature range. The arrow indicates the temperature recommended for pasteurization of whole egg and of aluminum-stabilized egg white at the holding time of 3.5 min (USDA, 1969).

Whole egg

Separated egg yolks plus membranes, after washing with distilled water, were blended and assayed for the enzyme. The activity observed was less than 1% of that found in an equal volume of egg white. Accordingly, the activity observed in blended whole egg is essentially that present in the egg white. It is possible that all activity observed in yolk prepared in this way was due to traces of egg white still adhering to the yolk membrane.

The temperature dependence of the rate constant for heat inactivation of the enzyme in whole egg is shown in Figure 5. The activation energy is 72.5 ± 6.5 kcal/mol in the temperature range shown. The enzyme is inactivated more rapidly in whole egg than in egg white (compare Fig. 2 and 5). Preliminary experiments carried out at 60°C over the pH range 3.5–8.5 showed that in whole egg, the enzyme was most resistant to heat inactivation between pH 6 and 7.

Figure 6 shows the amount of enzyme inactivated by heating whole egg for a constant time period, as a function of temperature. Approximately 80% of the enzyme activity is destroyed by heating at 60°C for 3.5 min, minimum conditions recommended for pasteurization of whole egg (USDA, 1969).

DISCUSSION

BEFORE considering the applicability of these results to the testing of heat-treated egg products, there are several points which should be made regarding our experimental methods and results. First among these was the choice of heating procedure used for the determination of the kinetics of heat inactivation. Attempts were made to heat egg white samples in long, thin-walled glass capillary tubes, both to reduce come-up time (time required for the sample to attain bath temperature) and to reduce loss of carbon dioxide, which loss results in a rise in pH of the sample. These experiments, for reasons we were unable to determine, gave both poor reproducibility and poor precision in determination of the rate constants for heat inactivation. The method actually used (see Methods) gave a come-up time in the range of 30 sec. Since all rate constants were determined from slopes of first-order kinetic plots, the come-up time did not affect the accuracy with which the rate constants were determined. Of somewhat greater consequence was the increase in pH of the small samples heated in relatively large centrifuge tubes. For example, at 61°C, for egg white with an initial pH of 7.0, the pH rise was 0.15 pH unit after a 4-min heating time, and 0.25 pH unit after 8 min. Since most of the kinetic data were obtained in much less than 8 min, and since no curvature was detected in the first-order kinetic plots, the pH rise did not appear to have an appreciable effect upon the results obtained near 60°C. At temperatures below 55°, where much longer heating times were required to produce significant enzyme inactivation, the increase in pH was greater, and the rate constants determined were probably too large. Accordingly, they have not been reported here. For the experiments with whole egg, the change in pH on heating was no greater than that given for egg white. Since the rate constants in whole egg were, on the average, greater than those in egg white, errors produced

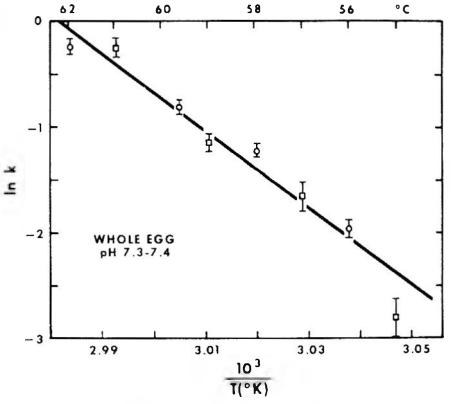

Fig. 5—Temperature dependence of the first-order rate constant for thermal inactivation of β-N-acetylglucosaminidase in whole egg, pH 7.3 to 7.4. (See legend to Figure 2.)

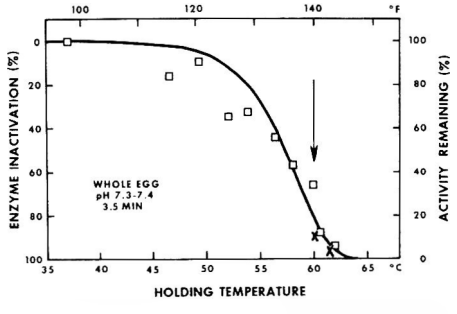

Fig. 6—Inactivation of β-N-acetylglucosaminidase produced by heating whole egg for 3.5 min. (Symbols are the same as in Figure 4.) The curve shows the calculated activity remaining after 3.5 min, assuming the equation for the line in Figure 5 applies over the entire temperature range. (Refer to legend to Figure 4.)

Table 1—Heat inactivation of β-N-acetylglucosaminidase compared to Salmonella destruction in egg products.

Conditions	D[a] at 60° (min)	Z[b] (°C)	E_a[c] (kcal)
In egg white at pH 7.3[d]			
Salmonella typhimurium Tm-1[e]	0.20	4.2	114.
β-N-acetylglucosaminidase[f]	3.0	5.6	91.
In whole egg			
Salmonella typhimurium Tm-1[e] pH 7.5	0.27	4.3	111.
β-N-acetylglucosaminidase, pH 7.3–7.4	4.7	7.1	73.

[a] $D = t_{0.9} = 2.303/k$.
[b] Z = temperature increase required for 10-fold increase in k.
[c] $E_a = -R\ (d \ln k)/d\ (1/T)$.
[d] Stabilized with aluminum and lactic acid.
[e] Garibaldi et al., 1969a.
[f] Extrapolated from data obtained between pH 6.8 and 7.2.

Table 2—Amount of active enzyme remaining after equivalent pasteurization treatments.[a]

Pasteurization treatment		Enzyme activity remaining (%) at pH			
		Egg white			Whole egg
Temperature (°C)	Holding time (min)	6.80	7.00	7.20	7.3–7.4
56	28.	40	20	7	3
58	9.8	48	27	12	9
60[b]	**3.5**[b]	**54**	**34**	**17**	**18**
62	1.25	61	42	24	31
64	0.44	68	50	33	45

[a] Equivalent pasteurization treatments are those time-temperature combinations which give equivalent kill of Salmonella Tm-1 (Garibaldi et al., 1969a). This table was compiled from the results of 40 kinetic runs comprising about 250 enzymatic assays.
[b] Proposed standard conditions for pasteurization of egg white (pH 7) and whole egg (USDA, 1969).

by pH changes on heating were smaller for whole egg.

The activation energy observed for heat inactivation of the enzyme in egg white, 91 kcal/mole, is significantly different from that observed in whole egg, 73 kcal/mole. We have not tried to determine why there is a difference in activation energies when the enzyme is heated in these 2 different egg products at approximately the same pH. Both activation energies are greater than the 63 kcal/mole reported by Henderson and Robinson (1969) for inactivation of the enzyme in egg white. Although Henderson and Robinson do not state the pH of their egg white samples, from the context of their paper we deduce that the pH was probably near 9. This difference in pH might account for the discrepancy in the activation energies for inactivation of the enzyme in egg white.

In considering those properties of the β-N-acetylglucosaminidase of egg white that pertain to its use in a test for compliance with pasteurization conditions, the following criteria must be applied. These criteria have been given in abbreviated form by Brooks (1962), and as given here, approach the ideal criteria for such a test:

1. The test substance (e.g., enzyme) occurs naturally in egg white.
   a) A constant amount is present in all eggs.
   b) The amount present does not change upon storage of the eggs.
2. The substance is changed or destroyed at pasteurization temperatures.
   a) A first-order rate of change is most convenient, since the destruction of microorganisms by heat obeys the first-order rate law.
   b) The rate of alteration produced in the test substance by heat should not be a function of the storage conditions under which the material to be pasteurized is held prior to pasteurization.
   c) At any temperature, the amount of alteration of the test substance should be smaller than the destruction of microorganisms by at least a factor of 10^3 to 10^5. This large difference in rate is required because, ideally, the test substance should be completely altered (i.e., 99% changed, a factor of 10^2) in the same time period that the viable bacteria count is reduced by a much larger factor (i.e., $10^5 - 10^8$).
   d) The temperature dependence of the rate constant (the activation energy) characteristic of alteration of the test material should, ideally, be the same as that for the destruction of microorganisms.
3. A quantitative assay for the test substance should be convenient, and relatively simple.

We feel that it is important to emphasize that such a test for compliance with pasteurization conditions can only determine whether the product has been held under the minimum conditions of time and temperature prescribed for pasteurization. It is not a test for absence of viable pathogens, particularly since post-pasteurization contamination appears to be the major cause of the presence of viable Salmonellae in egg products (Garibaldi et al., 1969b).

It is clear from the data presented above and in paper 1 that the β-N-acetylglucosaminidase of egg white meets many, but not all, of the criteria listed above. A quantitative comparison of the effect of heat on Salmonellae and the enzyme is given in Table 1. At pH 7.3, at 60°C, it takes 14.7 times as long to inactivate 90% of the enzyme in egg white as it does to kill 90% of the Salmonellae. Thus, in the same time that the active enzyme content is reduced by a factor of 10, the viable Salmonellae count is reduced by a factor of $10^{14.7}$. Such a large difference in rates allows some active enzyme to remain as a measure of the heat treatment used to destroy Salmonellae. Table 2 shows the calculated fractional activity remaining for equivalent pasteurization conditions (time-temperature combinations giving equal kill of Salmonellae Tm-1). The variation in activity remaining at different temperatures results from the inequalities in activation energies for destruction of Salmonellae and the enzyme activity (Table 1). A smaller amount of enzyme activity remains after equivalent heat treatment at the lower temperatures (Table 2).

The most serious deficiency of the β-N-acetylglucosaminidase, as a test for heat treatment, is its instability when eggs are stored at ambient temperatures. Under these conditions, the increased pH of the egg white leads to inactivation of the enzyme (paper 1). Although storage of eggs at ambient temperature is not recommended, it is frequently observed in retail stores. Since returns (eggs unsold by a certain time limit) from retail stores are often broken out for use as whole egg or separated egg products, these products have considerably lower enzyme activity than fresh eggs. However, the loss of β-N-acetylglucosaminidase activity can still be used as a measure of adequacy of pasteurization for eggs which have been stored under adverse conditions, provided either: a) some activity remains in the unheated egg product or b) some fresh egg white which has appreciable enzyme activity is added to the product before

pasteurization. Either case requires that the amount of activity observed after heat treatment be compared with that present before heat treatment. The variations in activity we have observed in different lots of fresh eggs (see paper 1) will also be compensated for by carrying out the assay both before and after pasteurization.

There is a simple way to use this assay to test for compliance with pasteurization regulations. For example, egg white pasteurized at 60°C for 3.5 min at pH 7.0 should have only 1/3 of its original enzyme activity remaining (Table 2). Thus, the color obtained upon assay of 1 volume of properly heat-treated egg white should be equal to or less than that obtained from 1/3 volume of the untreated egg white. Since many processors normally maintain pasteurizers at slightly higher temperature than required (often 1°F higher—K. Ijichi, personal communication), 1 volume of heated egg white may show less color upon assay than 1/3 volume of unheated egg white. All of a limited number of comparisons we have made of "before" and "after" samples of pH 7 egg white which had been pasteurized in commerce showed that the heat-treated sample produced less color than that indicated in Table 2.

We have not yet attempted a detailed study to determine whether this enzyme assay is suitable for monitoring the pasteurization of egg white at pH 9. At this pH, untreated egg white requires heating for 3.5 min at 56.7°C and egg white pasteurized by the Armour process is treated with peroxide and heated at 53°C (see USDA, 1969, for a review of these pasteurization treatments).

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

R. A. SAVAGE[a] and C. R. STUMBO
Department of Food Science and Technology, University of Massachusetts, Amherst, Massachusetts

CHARACTERISTICS OF PROGENY OF ETHYLENE OXIDE TREATED Clostridium botulinum TYPE 62A SPORES

SUMMARY—Spores of Clostridium botulinum *Type 62A were exposed to a mixture of 12% ethylene oxide (ETO) and 88% dichlorodifluoromethane to determine if the ETO resistance, toxin producing ability and spore producing ability would be retained in progeny of successive generations exposed to ETO. D values for the spores exposed to 700 mg per liter of ETO, at 40°C and 47% relative humidity, showed that there was no significant difference in ETO resistance through four successive generations of survivors. Toxin producing ability of the various generations was shown to be qualitatively similar by injecting mice intraperitoneally with culture supernatants. Microscopic examination of the different generations revealed no difference in rate or extent of spore production. Therefore, according to these criteria, there appeared to be no adaptations or mutations caused by four ETO exposures.*

INTRODUCTION

NUMEROUS STUDIES during the past 30 years have demonstrated that gaseous ethylene oxide (ETO) is a very effective microbicide. Many of these studies have indicated potential for its widespread use in the food industry. To date it has been successfully applied in the sterilization of quite a large number of dry or semi-dry foodstuffs, including grain, spices, starches and flours.

Many of the materials to which it is now being applied and may be applied in the future, to reduce microbial contamination, are used in the formulation of low-acid food items which subsequently may or may not be given heat processes sufficient to free them of the most dangerous food poisoning organism, *Clostridium botulinum.*

It is a well known fact that heat pasteurization of some foods often provides conditions more favorable for the subsequent growth and toxin production of *C. botulinum.* This is accomplished by the reduction of the numbers of other organisms which normally would grow competitively in the foods and thereby suppress the growth of *C. botulinum.* It is conceivable that inadequate ETO treatment of food ingredients could create similar circumstances in formulated low-acid food products. The fact that Kuzminski et al. (1969) demonstrated that spores of *C. botulinum* Type A (strain 62) are almost as resistant to ETO as are the spores of *Bacillus subtilis* (thus far the most resistant species found) emphasizes the importance of this consideration. It also emphasizes the importance of knowledge relating to whether repeated ETO treatment of *C. botulinum* progeny of spores previously surviving ETO treatments might cause strain or species mutations or adaptations, particularly with respect to toxin producing ability and ETO resistance. Treatment with alkylating agents, including ETO, have been observed to cause mutations (Auerback, 1946, 1958; Kolmark and Kilbey, 1968).

Therefore, this study was undertaken to determine if certain characteristics of *C. botulinum* would be retained (or altered) in progeny of successive generations exposed to ETO. Characteristics studied were ETO resistance, toxin producing ability, and rate and extent of sporulation of the progeny of spores surviving ETO treatments.

MATERIALS & METHODS

Test organism

Clostridium botulinum Type A (strain 62) was chosen as the test organism to be used throughout this study.

Sporulation medium

Beef heart casein broth prepared as described by Stumbo (1965) was used throughout the study.

Recovery medium

Pork infusion agar as described by Stumbo (1965) was used throughout the study.

Preparation of parent spore suspension

Ten screw cap tubes containing 20 ml of beef heart casein broth and about 1 in. of beef heart particles each were sterilized by autoclaving at 121°C for 15 min. 1 ml of a stock spore suspension was heated at 80°C for 15 min and a loopful of it then used to inoculate each of the 10 tubes of beef heart casein broth. (Prior to inoculation, the broth was exhausted of air by autoclaving at 5 psig for 15 min and allowed to cool.) After inoculation, the tubes were incubated at 30°C until maximum vegetative growth was reached as determined by wet mount examination under the phase contrast microscope. When maximum vegetative growth was reached, 1 ml of broth from each of the 10 tubes was used to inoculate larger amounts of beef heart casein broth in dilution bottles. The bottles were then incubated at 30°C until maximum sporulation was reached, as determined by microscopic examination of wet mounts and malachite green spore stains. (Usually about 1 wk was required to obtain maximum sporulation.)

When maximum sporulation was reached, the broth was filtered through sterilized milk filters to remove the meat particles. The supernatant was collected in sterile polyethylene centrifuge bottles and centrifuged at 7000G for 15 min at 2°C. Following centrifugation, the supernatant was decanted and the spore pellets resuspended in cold sterile distilled water and recentrifuged. This procedure was repeated 7 times to reduce extraneous materials that might interfere with the resistance of the spores to ETO. Following the last centrifugation and decanting process, the spores were resuspended in 20 ml of sterile, distilled water and collected in a sterile dilution bottle containing about 1 in. of No. 2 glass beads. The spore suspension was then stored at 2°C.

Preparation of test samples

An appropriate dilution was made from the prepared stock spore suspension to obtain a suspension containing approximately 10^7 spores per ml. 1 ml from this suspension was heated at 80°C for 15 min to kill any remaining vegetative cells and to activate the spores for germination. Sterile absorbent paper disks (3/8 in. diameter), which were sterilized in petri dishes, were each inoculated with 0.01 ml of the heat shocked spores. The test samples were then held in a desiccator for humidity equilibration for 24 hr. The desiccator contained a solution of 159.9 ml of sulfuric acid and 340 ml of water which according to Stokes and Robinson (1949), gave a relative humidity in the desiccator atmosphere of approximately 47%.

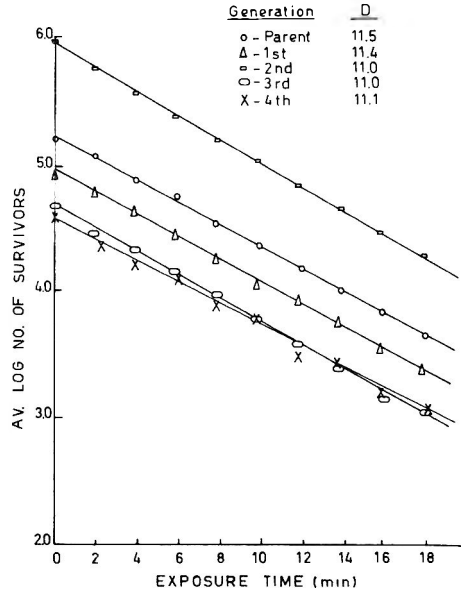

Fig. 1—Survivor curves for spores of successive generations of ETO treated C. botulinum.

[a]Present address: The Great Atlantic and Pacific Tea Co., Inc., Horseheads, N.Y.

Exposure conditions

A mixture of 12% ETO and 88% dichlorodifluoromethane by weight, was employed as the sterilant gas. All exposures were made at a gas temperature of 40°C. Relative humidity within the exposure chamber was maintained at 47% by injection of 0.11 ml of sterile, distilled water through a rubber septum prior to the introduction of the sterilant gas. The relative humidity was monitored throughout the exposure procedure via an electric hygrometer. Pressure within the exposure chamber was maintained at 5 psig to obtain a continuous ETO concentration of 700 mg per liter.

Exposure procedure

The stepwise procedure employed throughout the study was essentially the same as employed by LIU et al. (1968) and Kuzminski et al. (1970).

Determination of survivors and D values

After the exposure treatment, each paper disk was transferred to a sterile screw cap culture tube containing 10 ml of 1% Darvan solution. The tubes were agitated on a Vortex Genie Mixer until the paper disks were pulped. After appropriate dilutions were made in sterile, distilled water, 1 ml from each dilution was transferred to each of three tubes of recovery medium and sealed with a paraffin, vaseline and mineral oil sealing mixture (Stumbo, 1965). All tubes were incubated at 30°C for 2–3 days, after which colony counts were made. D values were then determined from a plot of logarithms of the average numbers of surviving organisms versus the times of exposure. (D equals time in minutes required to destroy viability of 90% of the population.)

Development of new spore suspensions from ETO survivors

The equation t = D(log a – log b), Stumbo (1965), was employed to determine the amount of time (t) which should reduce the population of viable spores to approximately 50 per disk. Since after treatment each disk was placed in 10 ml of Darvan solution, the predicted number of viable spores remaining per ml was approximately 5, the number of colonies which should arise per tube of medium receiving 1 ml of the solution. Following the exposure of the spores to the calculated time of ETO treatment, the inoculated tubes were incubated for 3 days at 30°C for colony development. The agar was then removed from a tube and transferred to a sterile petri dish. The colonies were picked and wet mounts, gram stains and malachite green stains made. Growth from a colony was then used to inoculate 1 or 2 tubes of sporulation broth if microscopic examination indicated the colony to be that of *C. botulinum*. The same procedure was then employed to produce spores from this generation as was used to produce spores from the parent culture.

Test for toxin production

To verify that cells of *C. botulinum* Type A were being isolated for each generation of ETO survivors, identification of the Type A toxin of *C. botulinum* was necessary. Also, any qualitative changes in the toxin producing ability of the organisms surviving ETO exposure could be observed. Sterile polypeptone broth was inoculated with 0.5 ml of heat shocked spores produced for each of the different generations. The inoculated polypeptone broth was incubated at 30°C for 4 days. The broth was then centrifuged at 7000G for 15 min at 2°C to pelletize the cells. The supernatant was decanted and used for the toxin assay. A 1:4 dilution of the supernatant was made in sterile distilled water and held in an ice bath until needed. One dilution from each generation was autoclaved at 121°C for 15 min to sterilize it and inactivate the toxin. These samples were then used as controls throughout the experiment. Each of a pair of male white mice, 4–6 wk old and weighing about 20 gm, was injected intraperitoneally with 0.2 ml of bivalent Type AB Antitoxin. After 30 min each was injected with 0.25 ml of unheated culture supernatant (1:4 dilution). Each of another pair of mice was injected with 0.2 ml of Type B Antitoxin and, after a similar waiting period, with 0.25 ml of unheated culture supernatant. Each of a third pair of mice was injected with 0.2 ml of heated culture supernatant. Following the injection, all mice were observed periodically for 48 hr for symptoms of botulism or time of death.

Table 1—Reactions[1] of mice injected with heated and unheated culture supernatant and antitoxins.

Material Injected	Generation 1st	2nd	3rd	4th	5th
Heated culture	++	++	++	++	++
Unheated culture	–	–	–	–	–
AB Antitoxin and Unheated culture	++	++	++	++	++
B Antitoxin and Unheated culture	–	–	–	–	–

[1] + = Lived; - = Died

RESULTS & DISCUSSION

THE SURVIVOR curves for the parent spore suspension of *C. botulinum* and for the progeny of successive generations from surviving spores appear in Figure 1. The curves as given are the straight lines of best fit as determined by the least squares method. The resulting regression coefficients were used to determine the D values. A D value of 11.5 min was obtained for the parent spore suspension. The equation of the survivor curve, t = D(log a – log b), was then used to approximate the time of exposure to the sterilant required to reduce the population of the parent suspension to about 50 spores per ml. After exposure and culturing as described above, cells from one colony were used to produce another generation. Spores produced were then exposed at 40°C and 47% relative humidity to the gaseous mixture containing 700 mg of ETO per liter. Four successive generations were produced and exposed in this manner. D values obtained for death of spores produced by the successive generations were 11.4, 11.0, 11.0, and 11.1 min respectively. It was found, by subjecting the D values to a Student's "t-test," that no statistical difference at the 1% confidence level existed between any of the D values for the different generations and the D value for the parent spore suspension. Therefore, it can be concluded that within the limitations of this experiment, no adaptation or mutation occurred which influenced ETO resistance.

Similar results were found by Church et al. (1956), who, by selective subculturing survivors of *Bacillus cereus* spores, following a 60 min exposure to liquid ETO and re-exposing to ETO, determined that the final spore population behaved in a manner identical to that of the original unselected culture.

Reporting on the mechanism of action of ETO on spores of *B. subtilis*, Shull (1963) stated that with increasing ETO treatment, the rate of germination of and outgrowth from the spores decreased. It was noted in this study that *C. botulinum* spores surviving longer times of ETO treatment, compared to untreated spores, required an additional day of incubation to produce visible colonies.

To identify the various isolates obtained following ETO exposure as being *C. botulinum* Type A, mice were injected intraperitoneally as described above. The findings regarding death and survival are given in Table 1. It is apparent from the results that each generation of spores was *C. botulinum* Type A. All mice injected with unheated culture supernatant alone and with culture supernatant plus B Antitoxin died within 3–6 hr after injection. The similarities in times of death for the mice indicated that there were no appreciable changes in the ability of *C. botulinum* of the different generations to produce toxin.

Throughout the study no detectable differences among the different generations, with respect to either the rate or extent of spore production, was noted.

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