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ABSTRACTS
IN THIS ISSUE

STATISTICAL ASPECT OF THE CORRELATION BETWEEN OBJECTIVE AND SUBJECTIVE MEASUREMENTS OF MEAT TENDERNESS. M. C. GACULA JR., J. B. REAUME, K. J. MORGAN & R. L. LUCKETT. *J. Food Sci.* **36**, 185–189 (1971)–The purpose of our study was to present a method of data analysis for removing sources of variation influencing objective and subjective measurements. It was found that by expressing the experimental data as a deviation from their contemporary mean, extraneous sources of variation were minimized, resulting in a substantial improvement in the degree of correlation as theoretically expected. A contemporary mean is defined as an average value derived from observations collected in the same substratum which is assumed to be homogeneous by virtue of proper experimental design.

BOVINE MUSCLE TENDERNESS AS RELATED TO PROTEIN SOLUBILITY. M. E. DIKEMAN, H. J. TUMA & G. R. BEECHER. *J. Food Sci.* **36**, 190–193 (1971)–The longissimus (modest degree of marbling) from forty beef ribs selected 48–56 hr post-mortem was used in two trials. Trial I involved A, C and E maturity ribs (10 each classification). Each rib was subjectively scored for texture (fresh) and adjacent longissimus samples were removed for the determination of protein solubility (fresh) and tenderness. Tenderness (cooked muscle) was measured with a Warner-Bratzler shear and taste panel. Protein solubilities were determined using 0.154M Krebs-Ringer-Bicarbonate buffer, 0.2M KCl + 0.01M K phosphate buffer, 1.1M KI + 0.1M K phosphate buffer, and 0.03M K phosphate buffer. Trial II involved 10 A maturity ribs. The 0.2M KCl, 1.1M KI, and 0.03M K phosphate buffers as described for trial I were used for protein extraction. Additionally, sarcomere length was measured in formalin. Multiple regression equations were developed to predict tenderness in trial II. Protein solubilities were not significantly different between the carcass maturity groups although there were trends toward increased solubility as maturity increased. Tenderness tended to decrease from A to E maturity indicating a negative relationship between protein solubility and tenderness. Several significant negative correlations between protein solubility and tenderness were found in trial I (A maturity group) and trial II. Additionally, several significant negative correlations between texture and solubility were calculated. Correlations within the C and E maturity groups were variable and showed no definite trends. Multiple regression analyses showed that a combination of protein solubilities, texture score and sarcomere length accounted for 88% of the variation in shear force and 72% of the variation in taste panel tenderness.

RELATIONSHIPS OF SERUM, MUSCLE AND SUBCUTANEOUS LIPIDS TO BEEF CARCASS TRAITS AND FLAVOR. B. E. THRALL & D. A. CRAMER. *J. Food Sci.* **36**, 194–198 (1971)–Lipid classes and fatty acid compositions of blood serum, intramuscular and subcutaneous fats were determined on 61 Hereford bull and heifer carcasses in addition to carcass measurements, cutouts and organoleptic evaluations. Differences due to sex, sire and environment were shown to be statistically significant for many of the lipid components. Phenotypic correlations of the lipid components with carcass cutout data and flavor evaluations gave indications of the lipid interrelationships and of the growth and development of the animals.

EPIMYSIAL CONNECTIVE TISSUE POLYSACCHARIDES OF BOVINE SEMIMEMBRANOSUS MUSCLE AND ALTERATIONS IN THEIR TYPE WITH AGE AND SEX DIFFERENCES. A. CORMIER, G. H. WELLINGTON & J. W. SHERBON. *J. Food Sci.* **36**, 199–205 (1971)–A significant relationship was found between age of veal, steer, heifer and aged cows and amount of polysaccharides isolated from the epimysium. 20% of the hexosamines present in the intact tissue was recovered in the isolated epimysium. Hyaluronic acid constituted 42, 19, 42 and 17% of the isolated epimysial polysaccharides of the veal, steer, heifer and cow groups. Dermatan sulfate was the major sulfated polysaccharide present in the epimysium. The ratio of hexosamine to insoluble collagen in the epimysium was associated with muscle tenderness; the higher the hexosamine: insoluble collagen ratio, the more tender the muscle.

EFFECTS OF DRY AND MOIST HEAT ON SELECTED HISTOLOGICAL CHARACTERISTICS OF BEEF SEMIMEMBRANOSUS MUSCLE. H. C. REID & D. L. HARRISON. *J. Food Sci.* **36**, 206–208 (1971)–Samples of raw and heat-treated bovine semimembranosus muscle were examined to determine the effects of four heat treatments on selected histological characteristics of the muscle. Differences among heat treatments in muscle fiber width and relative proportion of straight and wavy connective tissue were not significant. However, the quantity of granular connective tissue was significantly ($P < 0.05$) greater in deep-fat fried, pressure and oven braised samples than in oven roasted samples. Intact fat cell walls were observed in both raw and heated samples.

POST-MORTEM GLYCOLYSIS IN OX SKELETAL MUSCLE: EFFECTS OF MINCING AND OF DILUTION WITH OR WITHOUT ADDITION OF ORTHOPHOSPHATE. R. P. NEWBOLD & R. K. SCOPES. *J. Food Sci.* **36**, 209–214 (1971)–Mincing caused about a three-fold increase in the rates of most of the glycolytic and associated changes, but a much greater increase in the rate of loss of nicotinamide-adenine dinucleotide. In glycolysing minces diluted with one volume of 0.16M potassium chloride lactate was produced more slowly, adenosine triphosphate (ATP) was lost faster, glycolysis stopped sooner, and the ultimate pH was higher than in undiluted minces. Inclusion of orthophosphate in the diluent stimulated lactate production and delayed the loss of ATP until the glyceraldehyde-3-phosphate dehydrogenase step became rate-limiting for lactate production and fructose-1,6-diphosphate and the triose phosphates accumulated.

POST-MORTEM GLYCOLYSIS IN OX SKELETAL MUSCLE: EFFECT OF ADDING NICOTINAMIDE-ADENINE DINUCLEOTIDE TO DILUTED MINCE PREPARATIONS. R. P. NEWBOLD & R. K. SCOPES. *J. Food Sci.* **36**, 215–218 (1971)–Addition of nicotinamide-adenine dinucleotide (NAD), at the time of dilution, to glycolysing minces diluted with one volume of 0.16M potassium chloride resulted in a somewhat faster loss of adenosine triphosphate, earlier cessation of glycolysis, and higher ultimate pH. When the diluent also contained orthophosphate, inclusion of NAD led to a reduction in both phosphorylase and phosphofructokinase activity. In addition, it prevented the accumulation of fructose diphosphate. The added NAD was lost rapidly from the diluted mince preparations, its adenine moiety being converted to inosine nucleotide.

CARROT VOLATILES. 1. Characterization and Effects of Canning and Freeze Drying. D. A. HEATHERBELL, R. E. WROLSTAD & L. M. LIBBEY. *J. Food Sci.* **36**, 219–224 (1971)–Volatiles in aqueous extracts of raw, canned, and freeze-dried carrots were investigated. Of the 23 compounds identified by GLC-MS in the raw carrot, diethyl ether, acetaldehyde, acetone, propanal, methanol, ethanol, and β-phellandrene had not been previously reported. Acetaldehyde, sabinene, myrcene, and terpinolene were considered important character-impact compounds in raw carrot aroma. Differences between canned, freeze-dried and raw carrots were mainly quantitative rather than qualitative. Ethanethiol, dimethyl sulfide and dimethyl substituted styrene compounds formed with canning. Canning resulted in an approximate 50% loss of "higher boiling" compounds; however, it produced an increase in "lower boiling" compounds, particularly methanol, which increased from 0.05–60 ppm. Freeze drying resulted in an approximate 75% loss of total volatile content.

CARROT VOLATILES. 2. Influence of Variety, Maturity and Storage. D. A. HEATHERBELL & R. E. WROLSTAD. *J. Food Sci.* **36**, 225–227 (1971)–The volatile composition of carrot varieties Imperator (Long Imperator Crookham), Nantes, Royal Chantenay, Autumn King, Oregon 4362 and Wisconsin 5 were investigated. Differences were quantitative rather than qualitative. Variation in concentration of individual terpenes and their summation (5–27 ppm) appeared to be consistent with descriptions of the flavor characteristics of different varieties. Studies on effects of maturity revealed that of the "low boiling" constituents, acetaldehyde and particularly ethanol increased in late season whereas acetone, propanal, and methanol remained relatively constant. Regarding "higher boiling" constituents, their total concentration remained relatively constant while the concentration of individual terpenes constantly changed. Mature carrots stored for 5 wk accumulated acetaldehyde and ethanol while the concentration of other volatiles did not change significantly.

EFFECT OF HEAT ON THE FLAVORING COMPONENTS OF MAPLE SIRUP: A Preliminary Study by Gas Chromatography. J. C. UNDERWOOD. *J. Food Sci.* **36**, 228–230 (1971)–Total flavor in commercial maple sirup increases in amount with additional heat treatment. It is also shown that individual flavorants change at different rates, both increasing and decreasing. The study suggests that further more detailed work should furnish data on the source of flavoring compounds in the sap and lead to control of processing factors to increase the desirable flavor components while keeping the undesirable ones at a low level.

BIOCHEMISTRY OF TEA FERMENTATION: THE ROLE OF CAROTENES IN BLACK TEA AROMA FORMATION. G. W. SANDERSON, H. CO & J. G. GONZALEZ. *J. Food Sci.* **36**, 231–236 (1971)–The carotenoid compounds present in fresh tea leaves were quantitatively extracted and separated by thin layer chromatography. Neoxanthin, violaxanthin, lutein, and β-carotene were identified and estimated by spectrophotometry. These carotenoid compounds decreased during the tea fermentation process from about 0.053% (dry weight basis) in fresh tea leaves to about 0.030% in the fermented (3 hr) leaves to about 0.026% in the fired black tea. The cause of these changes was studied first in a model tea fermentation system consisting of a crude soluble tea enzymes preparation, a tea flavanol, and β-carotene. Degradation of β-carotene took place in this model system only after oxidation of the tea flavanol and the reaction mixture was taken to dryness. β-ionone, an important constituent of black tea aroma, was identified as the major product of β-carotene degradation but several other unidentified volatile compounds were also formed. The degradation of β-carotene to β-ionone in tea leaf undergoing conversion (fermentation) to black tea was confirmed using whole tea leaf; no drying of the system was required in this case. These results have been generalized to include all of the carotenoid compounds known to be present in tea leaves. The list of products which can be expected to be formed by this mechanism includes many compounds which are known to be present in black tea aroma suggesting that the degradation of carotenoid compounds during the black tea manufacturing process is important in determining the flavor of the finished product.

NONVOLATILE ORGANIC ACIDS IN GUAVA. H. T. CHAN JR., J. E. BREKKE & T. CHANG. *J. Food Sci.* **36**, 237–239 (1971)–The nonvolatile organic acids of guava were extracted and isolated. Thin-layer chromatography of the acids showed the presence of 6 acids, 5 of the acids identified as lactic, malic, citric, ascorbic and galacturonic. Gas-liquid chromatography of the methyl esters of the guava acids also showed the presence of malic, citric and lactic acid. Quantitative determinations using succinic acid as an internal standard showed citric and malic acid to be present in almost equal amounts and lactic acid in much less amount in cultivated guavas. In wild guavas, citric acid was the predominant acid, with lesser amounts of malic and lactic acid.

ORGANIC ACID PROFILES OF THERMALLY PROCESSED, STORED SPINACH PUREE. Y. D. LIN, F. M. CLYDESDALE & F. J. FRANCIS. *J. Food Sci.* **36**, 240–242 (1971)–Spinach puree was packed in TDT tubes and processed with an Fo = 4.9 at temperatures ranging from 240–300°F with 10°F increments. Half of the pack was analyzed for organic acids, color and pH; the other half was stored for 3 months at 75°F in the dark prior to analysis. After processing, the greatest changes were noted at 240°F and only minor changes noted above 280°F. Acetic and pyrrolidone-carboxylic acid showed the most change after processing. After storage the concentration of acids, color and pH was similar for all packs. However, during storage, α-ketoglutaric acid disappeared and pyruvic, glutaric, oxaloacetic and malonic acids were formed at all processing temperatures.

EFFECT OF GAMMA IRRADIATION ON THE POSTHARVEST PHYSIOLOGY OF FIVE BANANA VARIETIES GROWN IN INDIA. P. THOMAS, S. D. DHARKAR & A. SREENIVASAN. *J. Food Sci.* **36**, 243–247 (1971)–Gamma irradiation to 20–40 krad inhibits the ripening changes in preclimacteric bananas without affecting the fruit quality. Both fruit maturity at harvest and post-irradiation storage temperature markedly influence the response to irradiation. The optimum dose and the maximum tolerable dose varied among the five varieties screened. Ability of the banana fruit to withstand higher doses of gamma irradiation depends on the physiological status of the fruit at time of irradiation. Irrespective of varietal differences, irradiation of preclimacteric bananas to doses above 50 krad resulted in severe skin discoloration and fruit splitting. Irradiation under anoxia did not markedly reduce the radiation injury, suggesting that factors other than ozone formed during irradiation in air may contribute the radiation damage. Fruits on the climacteric could tolerate up to 200 krad, but no effect on ripening was observed. Ethylene or 2,4-D could reverse irradiation-induced inhibition of ripening in bananas. Irradiation seems to decrease the sensitivity of banana fruit to the ripening action of exogenously added ethylene.

SOLUBILIZATION OF AQUEOUS SOLUTIONS IN NONPOLAR LIQUIDS. L. D. MORSE, P. A. HAMMES & C. W. EVERSON. *J. Food Sci.* **36**, 248–250 (1971)–Solubilization has occurred when surfactant micelles in water solution enclose molecules of a water insoluble substance to form a single phase. The technology deals mostly with hydrophobic matter in water. Water was solubilized at from 1%–11% in orange oil, soybean oil, benzyl alcohol, n-heptane, n-hexane and cottonseed oil, and aqueous solutions couid be enciosed by appropriate micelles and solubilized. Using dioctyl sodium sulfosuccinate, the following quantities of ascorbic acid, as 20% aqueous solution, were solubilized: 22.0 mg/g orange oil; 10.4 mg/g soybean oil; 40.0 mg/g benzyl alcohol; 15.1 mg/ml n-heptane; and 14.5 mg/ml n-hexane. Triglycerol monooleate and decaglycerol dioleate solubilized ascorbic acid in cottonseed oil at 4.8 mg/ml; caprylic acid and ethoxylated stearic acid in cottonseed oil solubilized 1.5 mg/ml. Water soluble materials can be added to liquids with which they are otherwise incompatible, and unpleasant tasting materials can be taste masked by solubilization in bland lipophilic liquids.

REDUCTION OF INTESTINAL GAS-FORMING PROPERTIES OF LEGUMES BY TRADITIONAL AND EXPERIMENTAL FOOD PROCESSING METHODS. D. H. CALLOWAY, C. A. HICKEY & E. L. MURPHY. *J. Food Sci.* **36**, 251–255 (1971)–Formation of intestinal gas is evaluated by measuring flatus passed from the rectum and the amount of bacterially formed gas present in the exhaled breath. Flatulent test meals fed in the morning cause elevation of breath hydrogen concentration, flatus volume and flatus hydrogen and carbon dioxide content about 5–7 hr later. Test responses of legumes were measured against a baseline derived from feeding a bland, low-residue formula diet. Mature, dry lima beans were found to be as high in flatulence-inducing factor(s) (FF) as are white beans. Mung beans and soybeans caused the same excretion of hydrogen in the breath as did white beans, but only about 2/3 as much flatus. Peanuts were not gas-forming. Several products processed by methods that might be expected to alter FF concentration or activity were evaluated in the same way. Soybean and mung bean sprouts appeared to retain most of the FF present in the whole bean. Soybean curd (tofu) had little residual FF but another high-protein food, MPF, made from toasted soy grits, caused as much gas formation as an equal weight of soybean carbohydrate fed as whole beans. Enzymatic treatment of comminuted soybeans, designed to hydrolyze the constituent oligosaccharides, raffinose and stachyose, frequently cited as gas-formers, had only negligible effect on production of intestinal gas. Tempeh, made from soybean grits by mold fermentation, did not increase gas production over baseline values and caused a significant delay in the time of gas formation, suggestive of temporary suppression of intestinal bacteria. Ethanolic extraction of whole white beans reduced but did not eliminate their gas-forming quality.

ABSTRACTS:
IN THIS ISSUE

EVALUATION OF CERTAIN PHYSICAL PROPERTIES OF MEAT USING A UNIVERSAL TESTING MACHINE. D. W. STANLEY, G. P. PEARSON & V. E. COXWORTH. *J. Food Sci.* **36**, 256–260 (1971)–The Instron tester served to evaluate physical properties of uncooked rabbit and beef muscle including work of rupture, breaking strength, break elongation elasticity and stress relaxation. These methods measure variations in muscle type, aging and post-mortem treatments comparably with shearing instruments. Shank showed higher tensile properties than tenderloin, less elasticity and lost more applied stress. With rabbit, the breaking force of longissimus dorsi unrestrained during rigor was .237 lb/g ± 7.5% for samples 5.0 cm by 0.2–0.5 cm^2 while restrained muscle gave .168 ± 9.9% and also exhibited higher elasticity and break elongation. Post-mortem aging decreased tensile properties and elasticity. Psoas muscle, characterized by more coextensive fibers, had higher tensile properties than longissimus dorsi.

EFFECTS OF TYPES AND LEVELS OF FAT AND RATES AND TEMPERATURES OF COMMINUTION ON THE PROCESSING AND CHARACTERISTICS OF FRANKFURTERS. W. E. TOWNSEND, S. A. ACKERMAN, L. P. WITNAUER, W. E. PALM & C. E. SWIFT. *J. Food Sci.* **36**, 261–265 (1971)–Frankfurter emulsions containing either 25% or 35% beef fat, pork fat, or cottonseed oil were prepared by comminuting at 1500, 2500, or 5000 rpm to temperatures ranging from 45°–85°F. Data were obtained on the viscosities of the emulsions; except for initially high viscosities for which unmelted fat was responsible, the viscosities of emulsions containing the fats, or oil, were similar: viscosities tended to decrease with increasing time and temperature of chopping. The frankfurters were stuffed, smoked, and cooked, and data were obtained on shrinkage, fat retention, ease of peeling, specific gravity, and texture. Shrinkage was inversely related to content of fat. Fat separation mainly occurred in processing frankfurters containing beef fat; the data suggest that emulsions containing beef fat should be comminuted to 65°–75°F to avoid possible under or overchopping: the results show that optimum conditions were time as well as temperature dependent. The air content of frankfurters varied inversely with the maximum temperature attained during comminution. Frankfurter skin strength was lessened on increasing the temperatures to which emulsions were comminuted; elasticity, the equivalent of rubberiness, decreased under these conditions.

EFFECTS OF TYPES OF FAT AND OF RATES AND TEMPERATURES OF COMMINUTION ON DISPERSION OF LIPIDS IN FRANKFURTERS. S. A. ACKERMAN, C. E. SWIFT, R. J. CARROLL & W. E. TOWNSEND. *J. Food Sci.* **36**, 266–269 (1971)–The effect of time, temperature and rpm of comminution of emulsions was determined on the dispersion of approximately 25% of beef fat, pork fat or cottonseed oil in frankfurters. The numbers of lipid particles 5 μ or less in diameter increased in frankfurters containing either beef or pork fat as comminution was continued to higher temperatures, with pork fat dispersed more thoroughly. Fat tended to separate from frankfurters containing beef fat in particles 200 μ or more in diameter. In contrast, no specific degree of dispersion of particles 5 μ or less in diameter consistently indicated emulsion stability, or its lack. Increased rpm during comminution produced an increased dispersion of beef or pork fat. Under given conditions pork fat was more finely dispersed than beef fat. Dispersion of cottonseed oil produced particles smaller and more closely packed than could be studied using light microscopy. Electron microscopy revealed many particles 1 μ or less in diameter.

EFFECT OF NEAR INFRARED ENERGY ON RATE OF FREEZE DRYING OF BEEF. 1. Chamber Pressure and Radiation Intensity. F. BURGHEIMER, M. P. STEINBERG & A. I. NELSON. *J. Food Sci.* **36**, 270–272 (1971)–The effects of vacuum chamber pressure and intensity of near infrared energy on freeze drying rate for 1-in.-thick slices of eye round were investigated. The radiation source was a 500-w quartz iodine lamp. Decreasing the chamber pressure increased the freeze drying rate, especially during the early stages of the drying cycle corresponding to the initial and constant rate periods. The critical moisture content seemed to be about 43%. Different energy intensities of the same radiation characteristics were obtained by varying the distance between infrared heater and product. For distances of 9, 13.5 and 18 in., the inverse-square law was not followed; the drying rate was faster than predicted and appeared to vary linearly with distance.

EFFECT OF NEAR INFRARED ENERGY ON RATE OF FREEZE DRYING OF BEEF. 2. Spectral Distribution. F. BURGHEIMER, M. P. STEINBERG & A. I. NELSON. *J. Food Sci.* **36**, 273–276 (1971)–Infrared radiation was extensively investigated as a heat source for freeze drying 1-in.-thick slices of beef. Two approaches were used to study the effect of spectral regions on the drying rate. First, filters which transmitted definite wavebands in the near infrared were interposed between the heaters and the product. Secondly, different spectral distributions were obtained by varying the voltage applied to the heaters while keeping the total radiating power constant. From the work with filters it was concluded that the short wavelengths, 1 μ or less, gave the most rapid drying. Similarly, the work with voltage variation showed that drying rate was improved by increasing intensity and decreasing wavelength to about 0.95 μ. The shortest complete drying cycle using infrared heating was 7.0 hr as compared to 11 hr for the conventional control. Samples were evaluated for surface appearance, rehydration characteristics and organoleptic quality of cooked meat. The quality of samples produced with infrared radiation of short wavelengths predominantly at about 1 μ was judged to be similar to that obtained with conventional heating.

HEAT PASTEURIZATION OF CRAB AND SHRIMP FROM THE PACIFIC COAST OF THE UNITED STATES: PUBLIC HEALTH ASPECTS. P. LERKE & L. FARBER. *J. Food Sci.* **36**, 277–279 (1971)–While pasteurization for 1 min at 180°F killed large inocula of staphylococci and salmonellae placed into packages of crab and shrimp meat, heating for 5 min at 180°F failed to completely destroy a 1,000-spore inoculum of *Clostridium botulinum* E. Both meats were good substrates for the growth of all organisms studied. Storage at 40°F prevented the growth of inoculated staphylococci, salmonellae and *Cl. botulinum* A and proteolytic B but not of E, which produced toxin in crab meat after 30–40 days at that temperature. For complete safety of the pasteurized product a holding temperature of 36°F or lower would be required but would likely not be attainable in commercial channels.

COMPUTER-AIDED PREDICTIONS OF FOOD STORAGE STABILITY: OXIDATIVE DETERIORATION OF A SHRIMP PRODUCT. I. B. SIMON, T. P. LABUZA & M. KAREL. *J. Food Sci.* **36**, 280–286 (1971)–A study was undertaken to develop a method for predicting the storage stability of dry foods stored in semipermeable containers and deteriorating through oxidation of lipids. A shrimp bar was the test food in which organoleptic deterioration was correlated with absorption of oxygen and oxidation of carotenoid pigment. Rates of oxidation were studied as a function of oxygen concentration, and permeability characteristics of materials used to package the bars were determined. A mathematical model for prediction of storage life of the product was formulated using the kinetic data for shrimp oxidation and the permeability data. Predictions of storage life based on this model were compared with actual storage tests and found to give good results.

STORAGE TEMPERATURE EFFECTS ON THE PROTEOLYTIC ACTIVITY OF RADIATION-SURVIVING BACTERIA IN OYSTERS. J. A. LIUZZO, M. K. FARAG & A. F. NOVAK. *J. Food Sci.* **36,** 287–288 (1971)–The activity of 2 radiation-surviving and strongly proteolytic strains of *Pseudomonas* and *Achromobacter* were compared to the activity of 2 lesser active strains of *Neisseria* and *Bacillus* in fresh oysters during iced (32°F) and refrigerated (40°F) storage for 15 days. Radiation doses used for the oysters were 100 and 800 krad. The activity of the former bacteria was higher than that of the latter 2 at both temperatures and radiation doses. Storage at 40°F resulted in more bacterial growth and higher activity than at 32°F even in the 100 krad-irradiated oysters. Results showed a strong relationship between low-dose radiation and storage temperature in oysters.

A SYSTEM FOR CONTINUOUS THERMAL PROCESSING OF FOOD POUCHES USING MICROWAVE ENERGY. E. M. KENYON, D. E. WESTCOTT, P. LA CASSE & J. W. GOULD. *J. Food Sci.* **36,** 289–293 (1971)–A system for continuous microwave sterilization of foods packaged in plastic pouches has been designed, constructed and tested. Air pressure was used to prevent pouch rupture. Pouches containing food for use in military rations were sealed and introduced through an airlock on to a conveyor inside a plastic pipe within a microwave cavity. Microwave energy was supplied up to 10 kw at 2,450 MHz. Conveyor speed and power were regulated to provide the process time and temperature. Pouches were cooled in a water bath before releasing the pressure to prevent bursting. Pouches were overpackaged in a foil laminate for additional protection. Two food products were processed. Total process times of 9–14 min were achieved.

HEAT AND MASS TRANSFER IN A BATCH DRY RENDERING COOKER. L. S. HERBERT & T. E. NORGATE. *J. Food Sci.* **36,** 294–298 (1971)–Heat transfer measurements have been made during batch dry rendering runs using inedible (mainly sheep) offal in a full scale Laabs type cooker with steam heated shaft. Data were obtained at 6 min intervals throughout several cycles and heat and mass balances obtained for the complete cycles. It is proposed that changes in heat transfer coefficients occurring during the cycle are caused by the inversion of the tallow/water emulsion initially present as the liquid phase in the cooker, to a water/tallow emulsion. Water droplets are progressively evaporated, until in the last part of the cycle, drying of the solid particles is taking place in an environment of anhydrous tallow.

POPULATION DISTRIBUTION OF HEAT RISE CURVES AS A SIGNIFICANT VARIABLE IN HEAT STERILIZATION PROCESS CALCULATIONS. D. H. HERNDON. *J. Food Sci.* **36,** 299–305 (1971)–Sterilization levels found in inoculated test packs are commonly in disagreement with predicted values. This study was made to determine if predicted sterilization values would be closer to actual test values if the population distribution of the slope indices from a sample of heat rise curves were used instead of the traditional slowest or mean single value of slope index in the sterilization calculations. A computer was programmed to calculate from the basic equations of thermal death times and heat penetration, the amount of sterilization achieved at designated time intervals in a population of food packages. Means and standard deviations of slope indices from both real and postulated heat penetration tests were fed into the computer together with specified processing conditions. Predicted spoilage levels were very close to those obtained from actual inoculated test packs. From input of postulated heat penetration values, it was demonstrated that the larger the standard deviation, the greater the error will be if only a single value of the slope index is used. Manual procedures are given for an accurate determination of the minimum process time required for sterilization. Methods are also given for data expansion to show a curve illustrating the complete relationship between process time and food sterility.

THEORETICAL FORMULAS FOR TEMPERATURES IN CANS OF SOLID FOOD AND FOR EVALUATING VARIOUS HEAT PROCESSES. K. HAYAKAWA & C. O. BALL. *J. Food Sci.* **36,** 306–310 (1971)–A general solution is obtained for transient temperature distributions in a finite cylinder by applying several integral transformations to a heat conduction equation when it is subjected to time variable surface temperatures. From this general solution, various formulas for temperature distributions are derived for five different surface temperature-processing time relationships. By using these derived formulas, we obtain formulas for these two parameters: slope indices and intercept coefficients of heating or cooling curves of cylindrical cans of conductive food. Expressions are also derived for estimating sterilizing values during a come-up period of the heat process and also during the sinusoidal fluctuation of retort temperature.

EFFECTIVENESS OF PNEUMATIC CONVEYING SYSTEMS FOR COOLING SPRAY-DRIED FOOD PRODUCTS. D. R. HELDMAN, P. Y. WANG & A. C. CHEN. *J. Food Sci.* **36,** 311–314 (1971)–To ensure maximum product quality, dry food products must be cooled rapidly after leaving the spray dryer or similar drying operation. This investigation was initiated to measure the effectiveness of different types of systems for cooling during pneumatic conveying of the product. Mathematical expressions which predict the temperature of product and air as a function of distance from the point of mixing have been developed and presented. These predictions have been verified by experimental temperature measurements in a conveying tube. A comparison of results obtained from various conveying tube wall conditions indicated that effectiveness of product cooling by a water spray was 4 times greater than natural air circulation and double that with forced-air circulation over the tube exterior.

THE CENTRIFUGAL FLUIDIZED BED. 2. Drying Studies on Pieceform Foods. M. E. LAZAR & D. F. FARKAS. *J. Food Sci.* **36,** 315–319 (1971)–Drying food pieces in a centrifugal fluidized bed with relatively high air flows may be self-limiting, even when only partial drying is desired. Rate increases obtained in early stages of drying may be more than offset through rate-retarding effects of a skin-like layer of collapsed surface tissue which forms on the pieces. This skin layer becomes increasingly resistant to heat and moisture transfer. Rate data and piece temperature patterns for potato, apple and carrot confirm visual observations that skin barriers 0.2–1.0 mm thick form in the first 8–10 min of drying with high air velocities and moderate temperatures. When air temperatures were 250°F with velocities of 15 fps, 1.2-cm cubes of potato could be blanched in only 6 min, suggesting a procedure for blanching some types of food products without leaching.

FREEZE CONCENTRATION BY DIRECTIONAL COOLING. A. KRAMER, K. WANI, J. H. SULLIVAN & I. SHOMER. *J. Food Sci.* **36,** 320–322 (1971)–It is generally assumed that as food materials are frozen, soluble solids move ahead of the "ice front." Under conditions tested in these studies the above was true only when the ice front moved in a descending direction. Thus, when foods were frozen in an ascending direction (as on a plate freezer) there was little, if any, movement of solids. When the freezing surface was placed above the material to be frozen, there was a rapid downward movement of solids. This "solids descent" was most apparent in true solutions such as drinks, and less apparent in structural cellular foods such as pieces of meat or potatoes. It is suggested that this phenomenon may be utilized for more efficient freeze drying or concentrating, or for the simultaneous production of low-solids and concentrated foods, particularly beverages.

HEAT AND CALCIUM TREATMENTS FOR FIRMING RED TART CHERRIES IN A HOT-FILL PROCESS. R. L. LaBELLE. *J. Food Sci.* **36,** 323–326 (1971)–Preheating of freshly pitted cherries at about 140°F for 5–20 min promoted firming sufficiently to permit pasteurizing the fruit in bulk without excessive tearing. Improved texture from this pretreatment was demonstrated in terms of increased extrusion force, drained weight and bulk volume. Addition of calcium up to .04% of final product led to 50% greater firmness. Hot-fill temperatures of 180°F or higher were required to protect color and flavor from enzymatic degradation, even though product character was adversely affected thereby. While firmness or chewiness of the fruit was notably greater in the pretreated, hot-filled product, the conventional cold-fill, exhaust and retort process provided superior drained weight and bulk, with about equal color.

DIFFUSIONAL PROCESS IN THE DRYING OF TAPIOCA ROOT. J. CHIRIFE. *J. Food Sci.* **36,** 327–330 (1971)–Experimental results obtained in the through-circulation drying of tapioca root slices were analyzed in order to obtain the effective diffusivity in the material. They are in good agreement with the theoretical solution which assumes the diffusivity to be constant, though, for a better comparison, a model with variable diffusivity appears to be necessary. Also, it can be seen that as temperature increases, the constant diffusivity model represents closely the experimental results. The assumption that water migrates within tapioca root by a process of liquid diffusion is also confirmed by the Arrhenius type temperature dependence of calculated values of the effective diffusivity.

ABSTRACTS:
IN THIS ISSUE

MONILINIA AND RHIZOPUS DECAY CONTROL DURING CONTROLLED RIPENING OF FREESTONE PEACHES FOR CANNING. J. M. OGAWA, S. LEONARD, B. T. MANJI, E. BOSE & C. J. MOORE. *J. Food Sci.* **36**, 331–334 (1971)–Decay was controlled when freestone peach fruit harvested at various stages of maturity ranging from green to straw-color, were dipped in 50% 2,6-dichloro-4-nitroaniline (DCNA) at 1-1/2 and 4 lb/100 gal water and ripened for 5–17 days at 20°C and 90% R.H. Treated fruit had less than 10% decay while untreated fruit developed as much as 61% decay during this period. Regardless of treatment, straw-blush and full-blush fruit held for only 3 days did not develop decay. The most commonly occurring fungus pathogens, *Monilinia fructicola* and *Rhizopus stolonifer*, were controlled with these treatments. Postharvest DCNA plus captan dip treatments gave more effective decay control than from preharvest field sprays. Concentrations of DCNA or DCNA + captan required for *Monilinia* decay control, suggested by preliminary laboratory tests on fruit, were verified by these commercial-size experiments.

COMBINED EFFECT OF HEAT AND ALKALI IN STERILIZING SUGARCANE BAGASSE. Y. W. HAN, H. A. SCHUYTEN JR. & C. D. CALLIHAN. *J. Food Sci.* **36**, 335–338 (1971)–Bacterial spores contained in sugarcane bagasse were subjected to various combinations of heat exposure and alkali concentration and the rate of destruction determined for each set of conditions. A series of survival, thermal death time and alkaline destruction curves revealed a different mode of death by heat exposure than alkali treatment. Addition of alkali into heating menstruum caused the death rates of bacterial spores to be much greater than with heat alone at a given temperature. From a series of thermal destruction and alkaline destruction curves, an empirical equation expressing the relationship between the death rate of bacterial spores, and the intensity of the temperature and the concentration of alkali was established. The equation reveals that the death rate of bacterial spores is affected in an exponential manner by temperature and in a direct relationship by alkali concentration.

RADIATION DESTRUCTION OF Vibrio parahaemolyticus. J. R. Matches & J. Liston. *J. Food Sci.* **36**, 339–340 (1971)–Japanese and Puget Sound strains of *Vibrio parahaemolyticus* suspended in peptone water and in fish homogenate and inoculated onto crab meat were found highly sensitive to gamma irradiation.

DETINNING IN CANNED TOMATOES CAUSED BY ACCUMULATIONS OF NITRATE IN THE FRUIT. R. P. FARROW, J. H. JOHNSON, W. A. GOULD & J. E. CHARBONNEAU. *J. Food Sci.* **36**, 341–345 (1971)–Experimental packs at the University of Florida in 1965 accumulated no nitrate and in 2 yr removed only 15% of the tin from their cans. The 1966 Florida pack accumulated 50–80 ppm and produced extensive detinning. The nitrate was exhausted in 6–9 mo and thereafter the detinning rate leveled off. Field studies at Ohio State University in 1965 and 1966 resulted in widely varying but relatively modest nitrate accumulation in the fruit. The extent of detinning corresponded closely with the initial nitrate concentration. Pooled results of field studies and work on nitrate fortification of non-aggressive tomatoes suggest that an initial nitrate concentration of the order of 100 ppm can constitute a rapid detinning problem in a 303 can having a tin coating weight of 1.00 lb/bb.

EVALUATION OF MONOSACCHARIDES, DISACCHARIDES AND CORN SYRUPS AS DISPERSANTS FOR HEAT-PROCESSED DRIED SOY MILK PROTEINS. H. SUGIMOTO & J. P. VAN BUREN. *J. Food Sci.* **36**, 346–348 (1971)–The presence of sugar during the drying of heated soy milk significantly improved the redispersibility of the dried soy milk proteins in water, reaching nearly 100% at sugar levels in the range of 50–60% of total solids. Monosaccharides, disaccharides and corn syrups were tested. Enzyme-converted syrups having D.E. around 48.5 seemed to be quite suitable because of high dispersant efficiency, moderate sweetness and easier drying. One possible reason for the dispersant effects of sugars is suggested to be a physical separation of soy protein molecules.

REFRIGERATED APPLE SLICES: EFFECTS OF pH, SULFITES AND CALCIUM ON TEXTURE. J. D. PONTING, R. JACKSON & G. WATTERS. *J. Food Sci.* **36**, 349–350 (1971)–Apple slices of two varieties were dipped in solutions at various pH values with and without sulfite and with and without calcium. After dipping, the apples were packed in plastic bags and stored at 34°F. At weekly intervals shear strength was measured and related to pH of the dipping bath for the following treatments: 1) unsulfited, 2) sulfited, 3) calcium treated, unsulfited and 4) calcium treated, sulfited. An alkaline sulfite dip resulted in firmer appie slices than an acidic sulfite dip. Addition of calcium to an alkaline sulfite dip resulted in a further increase in firmness, but addition of calcium to an acidic sulfite dip did not. Sulfite was necessary to preserve the light color of apple slices during storage of refrigerated apple slices for several weeks.

IMPROVED METHODS FOR DETERMINATION OF CERTAIN ORGANIC ACIDS IN PASTEURIZED AND UNPASTEURIZED LIQUID AND FROZEN WHOLE EGG. J. G. REAGAN, L. R. YORK & L. E. DAWSON. *J. Food Sci.* **36**, 351–354 (1971)–A modified procedure was developed and evaluated for quantitating short-chain organic acids in liquid whole egg. Egg products to which acetic, butyric, lactic, propionic and succinic acids were added or which had previously been incubated at room temperature to different microbial populations were pasteurized at 60.5°C for 3.5 min. Lactic and succinic acids were recovered from the liquid whole egg samples and chromatographed as their butyl ester derivatives and acetic, propionic and butyric acids were recovered and chromatographed as the acids per se. The pasteurization process did not affect the concentration of these short-chain organic acids; thus, the procedure should be valid for analyses of commercially pasteurized egg products.

ANALYSIS OF COFFEE, TEA AND ARTIFICIALLY FLAVORED DRINKS PREPARED FROM MINERALIZED WATERS. R. M. PANGBORN, I. M. TRABUE & A. C. LITTLE. *J. Food Sci.* **36**, 355–362 (1971)–Coffee, black tea and artificially flavored and artificially sweetened orange and grape soft drinks were prepared from solutions of 8 minerals each at 750 ppm. Additionally, coffee and tea were brewed, using 6 natural drinking waters which ranged from 42–1,725 ppm of total dissolved solids. Beverages made from solutions containing carbonates were the least desirable, having flat, insipid characteristics. Although distilled water resulted in an acceptable soft drink, coffee and tea prepared from it were excessively sour and astringent, respectively. The recommended formula of 53.3 g of coffee per liter of water was considered too strong and "burnt" for the panel of trained judges, so that the experiments were repeated at a lower concentration of 47 g coffee/liter water. Large differences in the direction and magnitude of the visual characteristics of the coffee and tea brewed from the various waters also were observed in the marked changes in luminous transreflectance, purity and shifts in dominant wavelengths measured by thin-layer reflectometry.

JFS BASIC SCIENCE

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STATISTICAL ASPECT OF THE CORRELATION BETWEEN OBJECTIVE AND SUBJECTIVE MEASUREMENTS OF MEAT TENDERNESS

SUMMARY—The importance of obtaining a high degree of correlation between instrumental method and sensory evaluation is apparent when we are searching for a method to replace the conventional and expensive taste panel test. Unfortunately, discouraging results have been reported in regard to the degree of their correlation. Attempts have been made to clearly define what is actually being measured in both the objective and subjective methods. However, few reports have dealt with methods of data analysis. The purpose of our study was to present a method of analysis of data for removing sources of variation influencing objective and subjective measurements. Statistical models were developed for the Warner-Bratzler and taste panel methods of tenderness evaluation. It was found that by expressing the experimental data as a deviation from their contemporary mean, extraneous sources of variation were minimized, resulting in a substantial improvement in the degree of correlation as theoretically expected. A contemporary mean is defined as an average value derived from observations collected in the same substratum which is assumed to be homogeneous by virtue of proper experimental design.

INTRODUCTION

THE IMPORTANCE of a high degree of correlation between an instrumental method and sensory evaluation is apparent if an instrumental method is to replace the conventional and expensive taste panel test. Unfortunately, conflicting and discouraging results have been reported in regard to the degree of their correlation (Deatherage and Garnatz, 1952; Hurwicz and Tischer, 1954 and Wells et al., 1962). Several attempts have been made to clearly define what is actually being measured in both the objective and subjective methods (Cover and Hostetler, 1960; Schutz, 1969; Sheppard, 1953 and Szczesniak, 1968). However, few reports have dealt with methods of data analysis for removing the sources of variation influencing the objective and subjective measurements (Kramer, 1969; Mason and Koch, 1953 and Sheppard, 1953).

The purpose of this paper is to present a method of data analysis for minimizing extraneous sources of variation in experimental data.

EXPERIMENTAL

Taste panel

Beef muscles from 24 animals were used in this study. Using a paired design, 1 side of each animal was treated and the other side served as a control. The muscles evaluated were longissimus dorsi, semimembranosus, adductor and biceps femoris. 12 judges, thoroughly acquainted with texture evaluations, were selected for their ability to determine tenderness. They scored paired samples (control vs. treated) in individual taste panel booths under controlled conditions. The judges were asked to evaluate the initial tenderness of the meat on an 8-point scale ranging from 1 = extremely tough to 8 = extremely tender, and to record the number of chews necessary for mastication. 24 taste panel evaluations were conducted for both the strip loins (l. dorsi) and the rounds (semimembranosus, adductor and biceps femoris).

Sample preparation

The paired short loins were cut from the frozen condition with a band saw. The anterior end of the loin was squared off by removing an approximately 5-cm-thick wedge-shaped piece. Then 6, 4-cm-thick steaks were cut. The tenderloin and tails were trimmed off each steak and individually numbered in the order of cut starting from the anterior end. Steaks numbered 1 through 4 were submitted for Warner-Bratzler shear test.

For the frozen paired round steaks, a section of the round was removed by making a parallel cut just behind the aitch bone. Then 2, 5-cm-thick steaks were cut from the remaining portion of the round and numbered accordingly.

All the samples were thawed overnight at 4°C. Thermometers were inserted into the center of each loin steak. Conventional electric ovens were preheated on "broil" for 5 min before using. The broiling pan was then placed on a rack positioned to broil the meat 10 cm from the unit. The steaks were broiled for 10 min on one side, then turned and broiled on the other side until the internal temperature reached 66°C. The rounds were placed on a rack in a roasting pan with the thermometer inserted into the center of the meat. Then the meat was placed in the center of a preheated 180°C conventional electric oven until it reached an internal temperature of 66°C. The steaks were immediately placed in a 4°C cooler and allowed to cool, 5 hr for the loins and 20 hr for the rounds. Cores 2 cm in diameter were cut, uniformly trimmed to 3 cm long, thus removing the exterior browned surface. The randomized coded samples were presented to the judges according to a predetermined design. The panel scores reported here are the averages of 12 judgments. For the shear values, it is the average of 6–10 shears depending upon the size of the muscle.

RESULTS & DISCUSSION

Statistical model

In a general sense, a statistical model is defined as a simplified numerical representation of a system. The definition implies that a statistical sampling of the system is involved. Why do we need a statistical-biological model in experimentation? There are at least 2 reasons: 1) The model gives the researcher a better appreciation of what he is doing and how to approach his problem, and 2) a statistical model serves as guideline for the design, analysis and interpretation of experimental results. It is necessary that the formulation of a statistical model for describing an experimental condition should simulate as closely as possible the real existing system. In many instances, failure of a research investigator to attain his objective has been attributed to the use of an inappropriate statistical-biological model.

To illustrate a simple model, consider a set of taste panel data. Ideally, it is desired that an observed score, say X_{11}, should equal the true population mean

$\hat{\mu}_1$, that is

$$X_{11} = \hat{\mu}_1$$

where $\hat{\mu}_1$ is the mean of all the panel scores in the experiment, which is assumed to be the true population mean. This equality is impossible in practice, as no measurement is entirely exact. For this reason we have to add a component of error,

$$X_{11} = \hat{\mu}_1 + e_{11}$$

where e_{11} is a random error effect peculiar to the X_{11} observation. The total observations can be represented in a series of observation equations,

$$\begin{aligned} X_{11} &= \hat{\mu}_1 + e_{11} \\ X_{12} &= \hat{\mu}_1 + e_{12} \\ &\cdots\cdots\cdots \\ X_{1i} &= \hat{\mu}_1 + e_{1i} \qquad (i = 1,2,\ldots,n) \end{aligned}$$

Similarly, the observation equations for the Warner-Bratzler measurements are

$$\begin{aligned} X_{21} &= \hat{\mu}_2 + e_{21} \\ X_{22} &= \hat{\mu}_2 + e_{22} \\ &\cdots\cdots\cdots \\ X_{2i} &= \hat{\mu}_2 + e_{2i} \qquad (i = 1,2,\ldots,n) \end{aligned}$$

where X_{2i} is an observed shear value in the i^{th} determination, $\hat{\mu}_2$ is the population mean and e_{2i} is a random error peculiar to the i^{th} measurement. The above equations represent the simplest type of a statistical model.

When simple correlation coefficients are computed between taste panel scores and Warner-Bratzler shears, we are really correlating the right-hand side of the model based on the values in the left-hand side of the observation equation. The magnitude of the association is given by the covariance (cov) between X_{1i} and X_{2i}. That is

$$\text{cov}\,(X_{1i}, X_{2i}) = \text{cov}\,(\hat{\mu}_1 + e_{1i}, \hat{\mu}_2 + e_{2i}).$$

In some cases, the correlation coefficient has been misused in experimental investigation by applying it to a regression problem. However, depending upon the underlying model assumed and the experimental design, regression or correlation analysis can be used. In regression analysis, we make a strict distinction between the independent (fixed variable) and the dependent variables (random variable); whereas, in correlation analysis there is no such clear-cut distinction, both variables are random, hence both subject to error of measurement. Therefore, in the study of linear association, one should know whether the study concerns a regression or a correlation problem. For further reference, one may consult Fisher (1958), Snedecor and Cochran (1967), Sokal and Rohlf (1969) and Wine (1964).

Correlation between Warner-Bratzler shear and taste panel

Evidently, the association between Warner-Bratzler and taste panel score is a correlation problem. We are pursuing how the 2 measurements co-vary or vary together because of a common cause. Both variables are subject to error, and neither variable could be classed as an independent or dependent variable. If, indeed, a correlation exists, we have to identify the common cause of the correlation. The observed correlation could be a result of both direct and indirect causes. Figure 1 shows the possible line of causes of the correlation between Warner-Bratzler shear and panel score.

The statistical model for describing a panel score is shown below:

$$\begin{aligned} X_{1ijkmn} = {} & \hat{\mu}_1 + a_{1i} + b_{1j} + c_{1k} + d_{1m} \\ & + (a_1 b_1)_{ij} + (a_1 c_1)_{ik} + (a_1 d_1)_{im} \\ & + (b_1 c_1)_{jk} + (b_1 d_1)_{jm} + (c_1 d_1)_{km} \\ & + 4 \text{ three-way interaction} \\ & + (a_1 b_1 c_1 d_1)_{ijkm} + e_{1ijkmn}, \end{aligned}$$

where:

X_{1ijkmn} = an observation (panel score) by the i^{th} panel in the j^{th} period on the k^{th} sample and m^{th} treatment.

$\hat{\mu}_1$ = population mean.

a_{1i} = effect due to panel differences; this source includes level and direction of motivation, sensitivity of the panelists, adaptation level, informational bias, central tendency and instability of individual panelists at different score levels; this source can be biologically and statistically controlled to a certain degree.

b_{1j} = an effect due to time period; learning process and attitudinal changes are included in this effect; other sources are incomplete repeatability of panelist's score and location differences; physical and statistical control is possible.

c_{1k} = an effect attributed to heterogeneity of experimental materials; this includes differences due to internal muscle structure, anatomical location, sex, age, biological cycle, nutrition and genetic effect; statistical local control is possible.

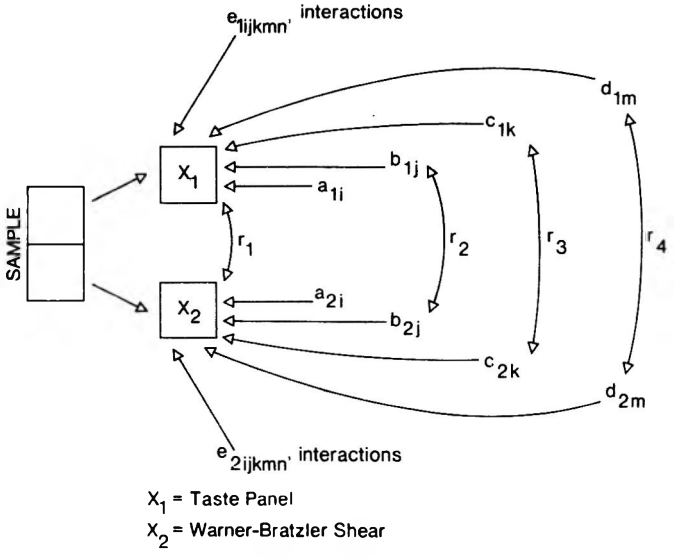

Fig. 1–Systems of correlated causes.

Table 1—Simple correlation coefficients between Warner-Bratzler measurements and taste panel scores.

Group	No. of animals	Experimental conditions	Actual data[a]	Deviation data[b]
Round	24	Treated	−.36	−.86**
		Control	−.15	−.66**
Loin	24	Treated	−.35	−.48*
		Control	−.51*	−.69**
Combined[c]	18	Treated	−.87**	−.46
		Control	−.80**	−.51**

[a]Uncorrected data (actual model).

[b]Adjusted data expressed as a deviation from their contemporary mean (reduced model).

[c]Combined data for 3 muscle structures.

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

Table 2—Correlation values between Warner-Bratzler and panel scores computed by a parametric and a nonparametric procedure using deviation data.[a]

Stratum No.	No. of animals	Experimental conditions	Loins		Rounds	
			r_1	r_s	r_1	r_s
I	6	Treated	−.51	−.44	−.96**	−.76
		Control	−.85*	−.71	−.84*	−.77
II	6	Treated	.32	.16	−.91*	−.90*
		Control	.54	.60	−.75	−.71
III	6	Treated	−.82*	−.77	−.79	−.26
		Control	−.93**	−.94**	−.08	−.09
IV	6	Treated	−.39	−.31	−.97**	−.86*
		Control	−.36	−.44	−.92**	−.59

[a]r_1 = product-moment correlation (parametric procedure); r_s = Spearman's rank correlation (nonparametric procedure).

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

d_{1m} = an effect due to treatment; this includes magnitude of treatment effect.

$(a_1 b_1)_{ij}$ = interaction effect between panelist and time.

$(a_1 c_1)_{ik}$ = interaction effect between panelist and experimental material.

$(a_1 d_1)_{im}$ = interaction effect between panelist and treatment.

$(b_1 c_1)_{jk}$ = interaction effect between time and sample.

$(b_1 d_1)_{jm}$ = interaction effect between time and treatment.

$(c_1 d_1)_{km}$ = interaction effect between sample and treatment.

e_{1ijkmn} = random errors peculiar to the n^{th} observation assumed to be normally and independently distributed.

The remaining interactions (3- and 4-way) are defined similarly. In other words, the model can be simplified as:

Score X_1 =
Mean + panelist + time + sample
+ treatment + 2–3–4-way interactions
+ random error,

that is, an observation X_1 is equal to the mean plus the sum of the effects of panelist, time, sample, treatment, all interactions and the unaccounted sources of variation. If these effects are negligibly small, the observed value X_1 will approach the true value and the observation equation reduces to

$$X_{1i} = \hat{\mu}_1 + e_{1i}.$$

The Warner-Bratzler shear measurement is assumed to follow a similar model:

$$X_{2ijkmn} = \hat{\mu}_2 + a_{2i} + b_{2j} + c_{2k} + d_{2m} + (a_2 b_2)_{ij} + (a_2 c_2)_{ik} + (a_2 d_2)_{im} + (b_2 c_2)_{jk} + (b_2 d_2)_{jm} + (c_2 d_2)_{km} + 4 \text{ three-way interaction} + (a_2 b_2 c_2 d_2)_{ijkm} + e_{2ijkmn},$$

where:

X_{2ijkmn} = a shear measurement by the i^{th} operator at the j^{th} time in the k^{th} sample and m^{th} treatment group.

$\hat{\mu}_2$ = population mean.

a_{2i} = effect due to operator differences; this source includes motivation and experience of the operator and systematic and personal biases; it can be minimized by physical and statistical control.

b_{2j} = effect due to time period; this source includes inaccurate calibration of the instrument, differences due to instrument and possibly directional bias; control by statistical means is possible.

c_{2k} = effect due to heterogeneity of meat samples; this effect is similar to c_{1k} discussed previously.

d_{2m} = effect due to treatment; this includes magnitude of treatment effect.

The interactions and the error component for this model are defined accordingly as in the taste panel model. Again, if these sources of variation are nil, the model reduces to

$$X_{2i} = \hat{\mu}_2 + e_{2i}.$$

The importance of the factors affecting the Warner-Bratzler and taste panel test measurements may be found elsewhere (Amerine et al., 1965; Filipello, 1957; Hamilton, 1968; Hurwicz and Tischer, 1954; Mitchell, 1957a; 1957b; Paul and Bratzler, 1955; Pettit, 1958; Schutz, 1969 and Tarver and Schenck, 1958).

There are 4 possible sources of correlations between X_1 and X_2 denoted by r_1, r_2, r_3 and r_4 (Fig. 1). These are shown by a double-headed arrow. A single-headed arrow indicates a directional effect by a particular source or factor. The correlation coefficient r_1 is the postulated correlation of primary interest. This is the direct correlation between panel score (X_1) and Warner-Bratzler shear (X_2). The simultaneous variation in X_1 and X_2 is also influenced by the factors defined in the models. In this paper, the association resulting from correlation between these factors (source of variation) is termed indirect correlation, and is given by their respective covariances. In Figure 1, r_2 is the correlation arising from time trend effect, cov (b_{1j}, b_{2j}); r_3 is the correlation between adjacent samples, cov (c_{1k}, c_{2k}) and r_4 is the correlation due to treatment effect, cov (d_{1m}, d_{2m}). These correlations could contribute to the direction and extent of r_1, although the contribution of r_2 and r_3 is still unsubstantiated. The likely contribution of r_4 to r_1 could be estimated by comparing r_1 obtained from treated and control samples. Studies have shown that higher correlations are found in treated groups than in controls (Bockian et al., 1958 and Deatherage and Garnatz, 1952). In the present study, we find that this is true for the rounds, but not for the loins (Tables 1 and 2).

The deviation data

It is likely that extraneous sources of variation are present in the data collected

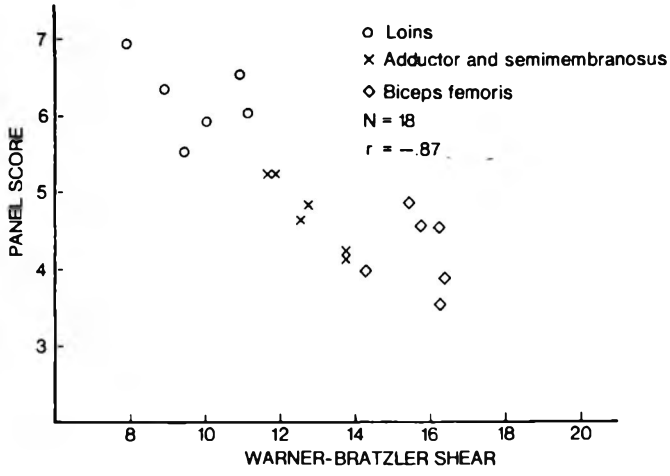

Fig. 2–Scatter diagram of actual data showing the distinct separation among the 3 muscle structures.

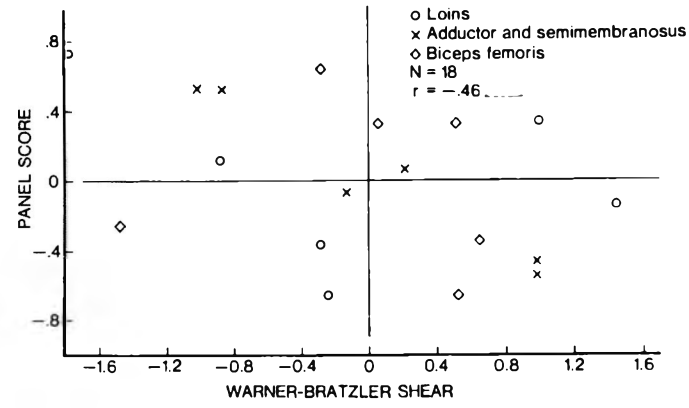

Fig. 3–Scatter diagram of the data shown in Figure 2 when expressed as a deviation from their contemporary mean.

over a period of time. To remove these variations, either of 2 things can be done: 1) express the individual observations as a function of the mean, or 2) apply an additive or multiplicative correction on the observations, using the method of fitting constants. However, with experimental data where the specific effects are not separable, an additive or multiplicative correction is not possible.

In the present case, we find it appropriate to express the individual observations as a function of their contemporary mean. A contemporary mean is defined here as a mean computed from observations collected in the same substratum, which is assumed to be homogeneous. Homogeneity can be achieved by proper blocking, grouping and balancing. For example, a complete day's run of an experiment may be considered a substratum in the whole experimental run. If the experiment is to be run for 4 days, we will have 4 substrata, each stratum having its own contemporary mean. This procedure is essentially a statistical control comparing observations on a within substratum basis. There is one caution which should be observed, that the grouping of the data is completely independent of observations and thus does not remove variation through relations among observations.

The data of the present study consist of 4 strata (subpopulation), designated as I, II, III and IV. Only the effects of strata are definable in the data. As a result, we can reformulate the Warner-Bratzler and taste panel models as

$$X_{ij} = \hat{\mu} + s_i + e_{ij}$$

where s_i is an effect due to the i^{th} strata. This effect includes the a_i, b_j, c_k, d_m and their interactions. These 4 main sources of variation are said to be confounded with the i^{th} strata.

The deviation data were generated by the equation of the form,

$$d_{ij} = X_{ij} - \bar{X}_{i.}$$

$$(i = 1,2,3,4; j = 1,2,\ldots,n)$$

where d_{ij} is the deviation data, X_{ij} is an observation within a stratum and $\bar{X}_{i.}$ is the respective stratum mean. The equation for each stratum is as follows:

$$\begin{aligned} \text{I:} \quad & d_{1j} = X_{1j} - \bar{X}_{1.} \\ \text{II:} \quad & d_{2j} = X_{2j} - \bar{X}_{2.} \\ \text{III:} \quad & d_{3j} = X_{3j} - \bar{X}_{3.} \\ \text{IV:} \quad & d_{4j} = X_{4j} - \bar{X}_{4.} \end{aligned}$$

When the observations are expressed this way, the data are free of the sources of variation we wish to remove. Finally, the reduced model for the taste panel is

$$d_{1i} = \hat{\mu}_1 + e_{1i}$$

and for the Warner-Bratzler shear,

$$d_{2i} = \hat{\mu}_2 + e_{2i}.$$

The covariance between these models is

$$\text{cov}\,(d_{1i}, d_{2i}) = \text{cov}\,(\hat{\mu}_1 + e_{1i}, \hat{\mu}_2 + e_{2i}),$$

and the product-moment correlation is

$$r_1 = \frac{\text{cov}\,(d_{1i}, d_{2i})}{\sqrt{(\hat{\sigma}^2 d_1)(\hat{\sigma}^2 d_2)}}$$

The computation of the correlation coefficients was based on the above reduced models.

Estimated correlation coefficients

Table 1 shows the simple correlation coefficients between Warner-Bratzler shear and taste panel for both the actual data and the deviation data. The correlation coefficients in the rounds and loins were larger using the deviation data. This change is the result of the removal of the extraneous sources of variation in the data. These sources, as defined previously, may have affected the estimate of the true correlation.

The combined data shown in this table came from population IV, in which we deliberately included 3 different muscle structures. The correlations obtained were higher in the actual data than in those of the deviation data. A graphical examination of the data indicated that the data belong to 3 distinct populations; 2 of the populations have values distributed in both extremes of the bivariate distribution (Fig. 2), resulting in a biased correlation. The deviation data are plotted in Figure 3 for comparative purposes. The analysis by the reduced model has obviously removed the effect of population differences.

In some cases, the experimental data do not conform with the assumption of bivariate normal distribution required in parametric statistical methods. Under this condition, we resort to the use of the nonparametric procedure where the underlying assumptions are minimal. For further reference, consult Bradley (1968). In this part of the study, we have used the Spearman's rank correlation coefficient r_s. One advantage of this procedure is the simplicity involved in the calculation of r_s. We have calculated the product-moment correlation and the rank correlation for each of the substrata. The result is given in Table 2. In most of the cases, the difference between the 2 procedures for estimating correlation is negligible. A low correlation of $r_s = -.26$ was obtained in the round of substratum III. This was attributed to the many tied ranks in the panel data. In cases where simplicity and speed are desired, the

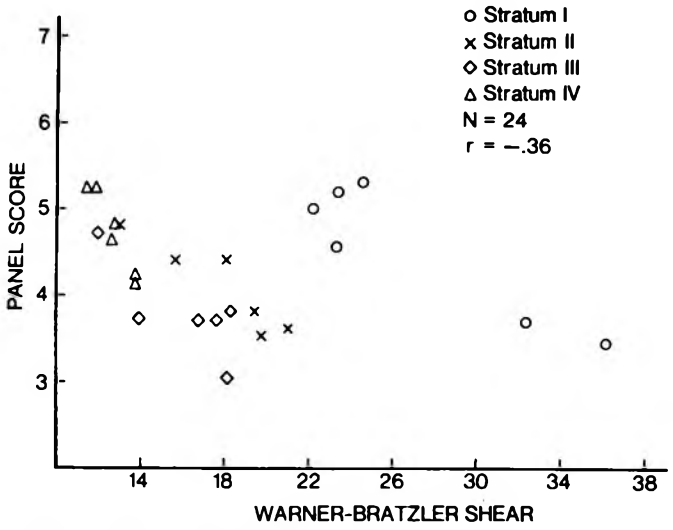

Fig. 4—Scatter diagram of actual data from 4 strata using the rounds.

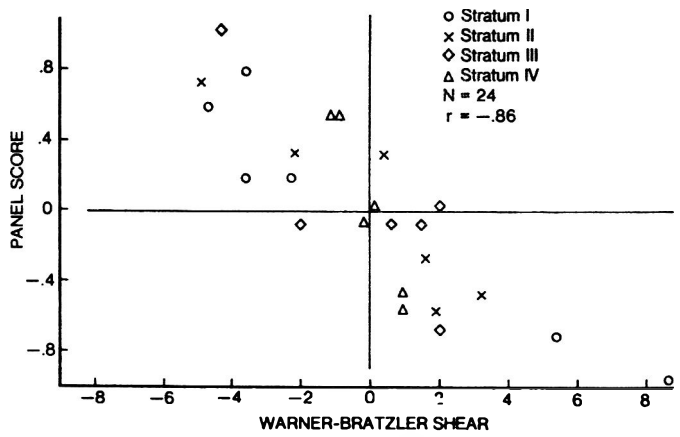

Fig. 5—Scatter diagram of the data in Figure 4 when expressed as a deviation from their contemporary mean.

method of rank correlation may be of value.

A comparison of the correlation values given in Tables 1 and 2 indicated that the pooling of the 4 subpopulations in the analysis of the data resulted in lowered correlation coefficients. This was because of the contribution of substratum II (loins) to the over-all correlation, where a direct association was obtained instead of the expected inverse relationship. It is therefore suggested that the analysis of data collected over time and space should be evaluated on a "within substratum basis" when statistical or other means of error control is not possible.

Figure 4 shows a scatter diagram of the 4 substrata studied using the round data. The values plotted are the actual data. The plot visually indicates a low linear correlation of −.36. The low correlation can be traced to external influences affecting the subpopulation in a different manner, and the instability of the population means over a period of time, especially in the taste panel data. As a result, the real correlation is masked by these factors. Note that the "within stratum" correlation obtained for these particular data ranged from −.79 to −.97 (Table 2). The scatter diagram for the deviation data is shown in Figure 5. The distribution of the data points is mostly found in the upper left and lower right of the 4 quadrants, indicating an inverse association. The correlation coefficient obtained was −.86, a substantial improvement over that obtained in the actual data. In general, the results of the study suggest that the analysis and evaluation of experimental data derived from several runs or experiments without proper control of internal and external influences will likely result in erroneous conclusions.

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BOVINE MUSCLE TENDERNESS AS RELATED TO PROTEIN SOLUBILITY

SUMMARY *The longissimus (modest degree of marbling) from forty beef ribs selected 48–56 hr post-mortem was used in two trials. Trial I involved A, C and E maturity ribs (10 each classification). Each rib was subjectively scored for texture (fresh) and adjacent longissimus samples were removed for the determination of protein solubility (fresh) and tenderness. Tenderness (cooked muscle) was measured with a Warner-Bratzler shear and taste panel. Protein solubilities were determined using 0.154M Krebs-Ringer-Bicarbonate buffer, 0.2M KCl + 0.01M K phosphate buffer, 1.1M KI + 0.1M K phosphate buffer, and 0.03M K phosphate buffer. Trial II involved 10 A maturity ribs. The 0.2M KCl, 1.1M KI and 0.03M K phosphate buffers as described for trial I were used for protein extraction. Additionally, sarcomere length was measured in formalin. Multiple regression equations were developed to predict tenderness in trial II. Protein solubilities were not significantly different between the carcass maturity groups although there were trends toward increased solubility as maturity increased. Tenderness tended to decrease from A to E maturity indicating a negative relationship between protein solubility and tenderness. Several significant negative correlations between protein solubility and tenderness were found in trial I (A maturity group) and trial II. Additionally, several significant negative correlations between texture and solubility were calculated. Correlations within the C and E maturity groups were variable and showed no definite trends. Multiple regression analyses showed that a combination of protein solubilities, texture score and sarcomere length accounted for 88% of the variation in shear force and 72% of the variation in taste panel tenderness.*

INTRODUCTION

TENDERNESS is the most desirable eating characteristic of meat. Early reports have shown that rigor mortis is associated with large differences in meat tenderness and significant increases occur in tenderness of excised beef muscles after 24 hr post mortem. Davey and Gilbert (1966), Locker (1960) and Sharp (1963) suggest that post mortem tenderization may be due in part to temperature-dependent nonproteolytic alterations in the contractile apparatus.

Wierbicki et al. (1954) first suggested that post-mortem tenderization may originate from a dissociation of the actin-myosin complex, but these same authors subsequently reported that they could not find any evidence for this hypothesis (Wierbicki et al., 1956). Weinburg and Rose (1960) found that an increase in tenderness post-mortem was paralleled by an increase in extractability of contractile proteins. Partman (1963) stated there was little doubt that the post-mortem tenderization of meat was directly related to the dissociation or an inhibition of the actin-myosin interaction. Hegarty et al. (1963) found that fibrillar proteins were least extractable at 24 hr post-mortem but solubility significantly increased with aging and paralleled an increase in tenderness. Goll et al. (1964) showed that significantly greater amounts of protein were extracted from muscles which had been excised immediately post-mortem than from muscles left attached to the carcass. Since the excised muscles were less tender a negative relationship was found between myofibrillar solubility and tenderness.

Marsh and Leet (1966) described "background-toughness" of meat as that toughness due to connective tissue. It is generally agreed by most workers that connective tissue affects meat tenderness, but to what extent has not been agreed upon. That total collagen had a small relationship with meat tenderness was shown by Hershberger et al. (1951), Wierbicki et al. (1954), Ritchey et al. (1963), and more recently by McClain et al. (1965). However, some workers (Husaini et al., 1950; Adams et al., 1960; Loyd and Hiner, 1959; Parrish et al., 1962) indicated a significant negative assocation between collagen content and meat tenderness. Hill (1966) suggested collagen solubility should be considered when attempting to biochemically explain the toughness of meat. Hill (1966) and Herring et al. (1967) found collagen solubility to decrease with advancing maturity. Herring et al. (1967) found that collagen solubility was higher ($P < .05$) in the longissimus than in the semimembranosus. Solubility was also related to panel tenderness in both muscles but within maturity group correlations of solubility with tenderness were low. Field et al. (1970) reported that collagen solubility was higher for tender than for tough muscles.

This study was undertaken in an attempt to evaluate the solubility of bovine muscle proteins as related to meat tenderness. The primary objective of evaluating this relationship was to develop a biochemical method for determining meat tenderness. Such a method could be quite useful in beef carcass evaluation and, more important, it could be very valuable in predicting tenderness from a muscle biopsy.

Two buffered salt solutions were used which, according to the literature, have not been used to extract bovine muscle proteins, while the two others used are commonly reported in the literature. Trial I was a preliminary study in which ribs of wide maturity and tenderness differences were selected to determine if protein solubility was related to tenderness. Trial II was conducted using A maturity ribs based on results of Trial I and the fact that more mature carcasses are of much less retail economic importance.

EXPERIMENTAL

Source of material

The longissimus (modest degree of marbling) was used from forty beef ribs selected 48–56 hr post-mortem by the senior author in a commercial packing plant. The cut surface (fresh) of the longissimus was subjectively scored for texture using a scale of 1 through 6 with (1) very coarse, (2) coarse, (3) slightly coarse, (4) slightly fine, (5) fine and (6) very fine. Two adjacent steaks (2.6 cm thick) were cut from the posterior end of each rib. The most anterior steak was frozen and stored (2 wk) at −29°C and later used for Warner-Bratzler shear measurement and taste panel evaluation. Ramsbottom (1947), Smith et al. (1969) and Smith (1970) have shown that freezing for this length of time has no effect on tenderness. A sample from the adjacent steak was frozen in liquid nitrogen, pulverized in a stainless steel Waring Blendor and stored at −29°C (less than 24 hr) for the determination of protein solubility. Helander (1957) showed that storage of samples more than 48 hr results in decreased protein extraction.

Protein extraction

Approximately 1g of each pulverized sample was transferred to a 50 ml polyethylene centrifuge tube. Duplicate extractions were run for each of the following buffers:

1. 0.154M Krebs-Ringer-Bicarbonate buffer, pH 7.4;
2. 0.2M KCl + 0.01M K phosphate buffer, pH 7.4;
3. 1.1M KI + 0.1M K phosphate buffer, pH 7.4;
4. 0.03M K phosphate buffer, pH 7.4.

10 vol of extracting buffer were added to the tubes containing the samples and the tubes were slowly agitated with a wrist action shake-

(1–4°C). All samples were extracted for 3 hr and then centrifuged for 20 min at approximately 4500 × g. The supernatant was saved and the muscle residue re-extracted for 2 hr with 10 vol of the same buffer and recentrifuged. The supernatant from the second extraction was then added to the first supernatant.

Nitrogen determination

1 ml of supernatant containing extracted protein was digested with 5 ml concentrated H_2SO_4 for 70 min. The digest was cooled 5–10 min, several drops of 30% H_2O_2 added and then heated 30 min. The clear digest was neutralized and diluted to 2–5 mcg of nitrogen per ml. Nitrogen was determined according to Weatherburn (1967) as modified by Beecher et al. (1970).

Hydroxyproline determination

2 ml of supernatant containing 0.154M Krebs protein was hydrolyzed with 10 ml of 6N HCl at 120–125°C for 3 hr. neutralized (pH 6.5–7.0) with KOH, and hydroxyproline content determined by the procedure of Woessner (1961) modified by Goll et al. (1963). Since no detectable hydroxyproline was found in the hydrolysates from muscle extracts using this procedure, a two-dimensional qualitative descending paper chromatography method was employed using phenol saturated with water, and n-butanol. Hydroxyproline was identified from standard chromatograms after spraying with ninhydrin.

Tenderness evaluation

The steaks were thawed overnight in a cooler at 2–4°C. Steaks were cooked to an internal temperature of 66°C (five at one time) in a pre-heated (163°C) rotary oven. The steaks were allowed to cool approximately 30 min, then a 1.27 cm core from each of the medial, central and lateral areas was removed for Warner Bratzler shear measurement and five additional cores were removed from the central area for taste panel evaluation. The taste panel (five members) scored the steaks for tenderness and overall acceptability on a 7-point hedonic scale. In trial II, thermometers were placed in two of the five steaks cooked at one time and when the temperature of these two steaks averaged 66°C, all steaks were removed from the oven.

Sarcomere length

Sarcomere length was determined by the procedure of Locker (1960) in trial II.

Statistical analysis

A one-way analysis of variance was used to study differences in the three maturity groups. Simple correlation coefficients were calculated on an individual maturity group basis. Multiple regression analyses were used to develop prediction equations.

RESULTS & DISCUSSION

Trial I

Effect of carcass maturity on protein solubility, texture score and tenderness. The means for extractable nitrogen for the individual maturity groups are presented in Table 1. Protein solubility was not significantly ($P < .05$) affected by carcass maturity; however, solubility tended to increase from A to E maturity when using the 1.1M KI and 0.2M KCl buffers. Also, solubility tended to be lower in the A than in the C and E groups when using 0.154M Krebs and 0.03M K phosphate buffers.

Table 1–Longissimus extractable nitrogen[a] means for three maturity groups.

Buffer	No. of replicates	Maturity A	C	E
0.03M K phosphate	(5)	0.416	0.458	0.432
0.154M Krebs-Ringer Bicarbonate	(5)	0.420	0.436	0.408
0.2M KCl + 0.01M K phosphate	(10)	0.371	0.376	0.382
1.1M KI + 0.1M K phosphate	(10)	0.656	0.685	0.713

[a]Expressed as a proportion of total muscle nitrogen.

Table 2–Longissimus shear force values and subjective scores for three maturity groups.

	Maturity[a] A	C	E
Warner-Bratzler shear, kg.	2.65[a]	3.43[ab]	4.06[b]
Taste panel tenderness[b]	5.51[a]	4.52[b]	4.50[b]
Taste panel acceptability[b]	5.19[a]	4.51[b]	4.37[b]
Texture score[c]	4.2[a]	3.9[a]	3.5[a]

[a]Means in the same line having different superscripts are significantly ($P < .05$) different due to carcass maturity.
[b]Score of 1 = Extremely undesirable, 7 = Extremely desirable.
[c]Score of 1 = Very coarse, 6 = Very fine.

Table 3–Simple correlation coefficients between several muscle quality and protein solubility variables.[a]

Buffer	No. of replicates	Warner-Bratzler shear	Taste panel tenderness[b]	Taste panel acceptability[b]	Texture score[c]
0.03M K phosphate	(5)	0.54	– .77	– .40	– .05
0.154M Krebs-Ringer-Bicarbonate	(5)	0.54	– .16	– .50	– .62
0.2M KCl + 0.01M K phosphate	(10)	0.43	– .25	– .28	– .63
1.1M Kl + 0.1M K phosphate	(10)	0.06	0.19	0.05	– .18
Texture score	(10)	.02	– .03	– .02	1.00

[a]Longissimus from A maturity ribs only; correlations > 0.63 are significant ($P < .05$) and correlations > 0.77 are significant ($P < .01$).
[b]Score of 1 = Extremely undesirable, 7 = Extremely desirable.
[c]Score of 1 = Very coarse, 6 = Very fine.

The 0.03M K phosphate and 1.1M KI buffers have commonly been used to extract bovine muscle proteins and extract sarcoplasmic and myofibrillar proteins, respectively. The 0.154M Krebs buffer is a physiological buffer and protein solubility in it could be related to physiological characteristics which influence tenderness. The 0.2M KCl buffer principally extracts sarcoplasmic proteins and myosin. However, since protein solubility was lower than in the phosphate and Krebs buffer (Table 1), some unidentified protein was less extractable in this extracting solution.

Panel tenderness decreased significantly ($P < .05$) from A to C maturity with little difference occurring between the C and E groups (Table 2). Warner-Bratzler shear force increased from A to C maturity and there was also an increase from C to E, although nonsignificant. Texture score tended to decrease from A to E maturity which paralleled the decrease in tenderness as evaluated by the Warner Bratzler shear.

Relationships of protein solubility with texture score and tenderness. Simple correlation coefficients between several muscle quality and protein solubility variables are presented in Table 3. Muscle proteins solubilized (A maturity) in 0.2M

KCl, 0.154M Krebs or 0.03M K phosphate were positively, but not significantly, related to shear values (r = 0.43, 0.54 and 0.54, respectively). Muscle proteins solubilized in 0.03M K phosphate were significantly negatively related to taste panel tenderness (r = −.77) and those correlations with 0.2M KCl and 0.154M Krebs were negative, but low. Protein solubility in the 1.1M KI buffer appeared to have no relationship to tenderness in this trial (r = 0.06 and 0.19), which does not support results presented by Hegarty et al. (1963), and Aberle and Merkel (1966).

Coarse textured muscle was associated with a higher proportion of 0.154M Krebs (r = −.62) and 0.2M KCl (r = −.63) extractable proteins. However, texture score had no relationship with tenderness (r = −.02 and −.03).

Correlations of protein solubility with texture score and tenderness in the C and E maturity groups were variable and showed no definite trends. For example, the correlations of 0.2M KCl extractable protein with taste panel tenderness was −.33 and with overall acceptability was 0.42. The variability is probably due to marked physiological maturity changes.

Collagen solubility related to tenderness. Hydroxyproline, as a measure of collagen, was not detected in any of the protein extracts by either the colorimetric procedure or paper chromatography method and it was therefore assumed that collagen was insoluble under the experimental conditions of this study. It was the intent of the present study to determine if collagen was soluble at 2–4°C in the 0.154M Krebs buffer used in this study and to relate its solubility to tenderness. Goll (1965) reported 11–20 mcg of hydroxyproline/g in a K phosphate buffer of considerably greater ionic strength than that used in the present study. Perry (1955), Barber and Canning (1966), Mihalyi and Rowe (1966) and Chaudhry et al. (1969) have suggested that K phosphate may have important effects on the solution's extracting properties in addition to making a sizable contribution to the ionic strength.

Trial II

Only A maturity ribs (10) were used in Trial II since the results within C and E maturity ribs and pooled within maturity correlations were quite variable and showed no definite trends. Also, it was decided that studying C and E maturity carcasses was insignificant to the main objective of the study. The 0.154M Krebs buffer was eliminated since it extracted essentially the same proportion of nitrogen as the 0.03M K phosphate buffer.

Relationships of protein solubility, texture score and sarcomere length with tenderness. Simple correlation coefficients between several variables indicated that definite trends exist between protein solubility and muscle tenderness (Table 4). Those muscles having high extractable protein with 0.03M K phosphate and 0.2M KCl buffers tended to have higher shear force values (r = 0.22 and 0.21, respectively), lower taste panel tenderness scores (r = −.71 and −.56, respectively) and lower taste panel acceptability scores (r = −.54 and −.46, respectively). Some of these correlations were significant and those of 0.03M K phosphate and 0.2M KCl fractions tend to support results in Trial I while the correlations of 1.1M KI extractable protein with tenderness disagree with those found in Trial I. The relationship of the latter fraction to tenderness supports results of other workers (Hegarty et al., 1963; Aberle et al., 1966).

Fine textured muscle was negatively correlated (r = −.57) with shear force and positively related (r = 0.64) to taste panel tenderness and acceptability (r = 0.56). These results disagree with those found in Trial I but confirm the accepted idea that fine textured muscle is more tender. Fine textured muscle was also negatively correlated with the amount of muscle protein soluble in 0.03M K phosphate (r = −.75) and 0.2M KCl (r = −.68). It is also interesting to note that fine textured muscle was positively associated with longer sarcomeres (r = 0.77). In two of the three buffers used, longer sarcomeres appeared to be negatively related to protein solubility, although only 13% of the variation was accounted for. Sarcomere length had a slight positive correlation with taste panel tenderness (r = 0.26) and a small negative correlation (r = −.30) with shear force.

Multiple regression analyses. A summary of the variables studied and the regression coefficients for Warner-Bratzler shear force, taste panel tenderness and taste panel acceptability are presented in Table 5. To predict taste panel tenderness for example, the following equation would be used: $\hat{Y} = 14.33 - 28.98$ (0.03M K phosphate soluble fraction) + 3.27 (1.1M KI soluble fraction).

Multiple regression analyses (Table 5) show that 88% of the variation in shear force was accounted for by using 0.03M K phosphate, 0.2M KCl and 1.1M KI soluble fractions plus texture score and sarcomere length.

62% of the variation in taste panel tenderness was accounted for using the 0.03M K phosphate and 1.1M KI soluble protein fractions. Although texture score, sarcomere length and the 0.2M KCl soluble fraction did not contribute significantly (P < .05) to R-square in predicting taste panel tenderness, when they were added to the regression equation, 72% of

Table 4—Simple correlation coefficients between several muscle quality and protein solubility variables.[a]

	Warner-Bratzler shear	Taste panel tenderness[b]	Taste panel acceptability[b]	Texture score[c]	Sarcomere length
0.03M K phosphate	0.22	− .71	− .54	− .75	− .36
0.2M KCl + 0.01M K phosphate	0.21	− .56	− .46	− .68	− .28
1.1M KI + 0.1M K phosphate	− .47	0.42	0.66	0.06	− .05
Texture score	− .57	0.64	0.56	1.00	0.77
Sarcomere length	− .30	0.26	0.38	0.77	1.00

[a]Longissimus from 10A maturity ribs from Trial II; correlations > .63 are significant (P < .05) and correlations > 0.77 are significant (P < .01).
[b]Score of 1 = Extremely undesirable, 7 = Extremely desirable.
[c]Score of 1 = Very coarse, 6 = Very fine.

Table 5—Multiple regression analyses summary.[a]

	Dependent variables		
Independent variables	Warner-Bratzler shear	Taste panel tenderness	Taste panel acceptability
0.03M K phosphate	−37.29	−28.98	
0.2M KCl + 0.01M K phosphate	−30.19		+0.32
1.1M KI + 0.1M K phosphate	− 6.08	+ 3.27	+4.02
Texture score	− 2.52		
Sarcomere length	+ 7.71		
Intercept	30.87	14.33	0.65
R-square	0.88	0.62	0.74
S.D. of Y	0.67	0.66	0.47

[a]Coefficients are presented for only those independent variables which contributed significantly to R-square for the dependent variables.

panel tenderness variation was accounted for by the equation. 71% of the variation in taste panel acceptability was accounted for by using the 1.1M KI soluble protein fraction and texture score.

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RELATIONSHIPS OF SERUM, MUSCLE AND SUBCUTANEOUS LIPIDS TO BEEF CARCASS TRAITS AND FLAVOR

SUMMARY—An examination of the means and range coefficients of the total serum, intramuscular (longissimus) and subcutaneous lipids, the major lipid classes and fatty acid compositions of the 3 tissue sites from 30 heifers and 31 bulls of the Hereford breed revealed some interesting aspects of lipid metabolism. When placed on a concentration (percent of tissue) basis, significant differences due to sex, environment and sires were shown by an analysis of variance for many of the lipid components. Phenotypic correlations, also computed on a concentration basis, gave indications of the growth and development of the animals, in addition to showing the associations of various lipid components of the 3 tissue sites to various carcass cutout traits and flavor. With a few exceptions, low positive correlations were shown for the serum lipid components with flavor, whereas, most of the marbling and subcutaneous fat components exhibited low negative correlations with flavor.

INTRODUCTION

THE VALUE of marbling or other depot fats with regard to meat quality and flavor has remained largely unresolved. Consumer resistance to meat surrounded by large amounts of waste fat and the encouragement of the American Heart Association for the public to consume less animal fat has resulted in a certain amount of meat being offered by some meat processors on a "trimmed of waste fat" basis. However, cattle yielding carcasses which are graded USDA Choice and Prime, therefore bringing the feeder top price for his product, usually carry a considerable amount of high-cost finish.

Using odor as a flavor characteristic, Hornstein and Crowe (1960) and Hornstein et al. (1961) examined the effects of free fatty acids, carbonyls, neutral fats, cephalin, sphingomyelin and lecithin on raw and cooked beef flavor. Volatile fatty acids and various carbonyls were the variables considered by Yueh and Strong (1960) as possible odor-flavor contributors. Neither group of investigators was able to arrive at definite conclusions. Batzer et al. (1960) were also unable to isolate or identify specific lipid components as odor-flavor contributors. Much of the more recent work has been concerned with the differences in fatty acid composition of raw and cooked meats and specific muscles (Siedler et al., 1964; Campbell and Turkki, 1967; Hornstein et al., 1967 and Yang et al., 1966). Terrell et al. (1968) presented simple correlations of the fatty acids of neutral and phospholipid fractions of bovine longissimus with carcass and growth characteristics, and also considered the effects of broiling and sex. They reported low nonsignificant correlations between neutral and phospholipid fatty acids with tenderness and juiciness.

Richardson (1968) obtained the carcass cutout measurements and directed the organoleptic studies used in this report. His approach to the analysis of the data was to hold various carcass traits constant and then to determine simple and partial correlation coefficients, by sex, between cutability traits and taste panel evaluations. Richardson concluded that marbling score may be receiving more attention than it deserves in relationship to beef palatability.

The purpose of the study reported in this paper was to determine phenotypic relationships of serum lipids of beef cattle to the amount, distribution and composition of body fats. In addition, various lipid components were correlated with carcass cutout and taste panel evaluations.

EXPERIMENTAL

Sample description

The cattle used for determining the cutability and quality of meat were Hereford heifers (30) and bulls (31) of known genetic and environmental background from the San Juan Basin Experiment Station at Hesperus, Colorado. The heifers were slaughtered at 18.3 ± 1 month of age and the bulls were 17.4 ± 2 months of age. The average left side weight of the heifers was 120.6 kg, the bulls averaging 122.2 kg. The heifers were on full feed an average of 215 days as compared to 174 days for the bulls. At the time of slaughter, blood samples were collected from which the serum was separated and stored at −20°C. The cutting procedure and management of the taste panel were described in detail by Richardson (1968). The left sides of the carcasses were aged at 2°C for 7–8 days before cutting. 12th-rib steaks were wrapped in cellophane and polyethylene-coated freezer wrap and stored at −34°C approximately 60 days at which time organoleptic evaluations were conducted. A 6-member, experienced taste panel was selected to evaluate the steaks cooked in an electric oven to an internal temperature of 71.1°C. A 15-point hedonic scale was used to rank flavor, juiciness, tenderness and over-all acceptability.

Extraction of lipids

The method of Bligh and Dyer (1959) was used for extractions of intramuscular and serum lipids with the following modifications: utilization of separatory funnels, an extra water purification step, removal of aqueous wash layers by aspiration and an additional extraction of the chloroform:water interface. A 0.5-in. slice of the longissimus, trimmed of surrounding fat and fascia, was used for the gravimetric determination of percent intramuscular fat and dry matter. 2 ml of serum in duplicate were used for gravimetric quantification of serum lipids and for separation of the lipid classes for subsequent fatty acid determinations. The maximum allowable difference between duplicate serum samples was 50 mg per 100 ml of serum. Fat was extracted from the subcutaneous adipose tissue, trimmed from the outer edge of the ribeye, with petroleum ether (30–40°C b.p.) on Bailey-Walker apparatus.

Qualitative thin-layer chromatography (TLC)

Glass plates, 20 by 20 cm were prepared as follows: 100 g of silica gel without binder (Adsorbosil-2, Applied Science Laboratories, Inc.) was mixed for 30 sec in a Waring Blendor with 150 ml of a solution consisting of 20 ml of 0.1% ethanolic 2′,7′-dichlorofluorescein indicator in 130 ml water. The slurry was applied to the plates with an adjustable CAMAG applicator (Arthur H. Thomas Co.) within 1.5 min in layers 0.30 mm thick. After preliminary air drying, the plates were activated for 1 hr at 110°C, then stored in a desiccator until ready for use.

Table 1—Means, ranges and range coefficients (H/L) of the % dry-matter and % fat in the longissimus muscle and total serum lipid of the 1965 San Juan Hereford heifers and bulls.

	N	Mean ± std. dev.	Range	H/L
		% Dry matter		
Heifers	26	30.1 ± 2.3	24.7 – 35.4	1.4
Bulls	23	24.7 ± 2.6	15.9 – 28.1	1.8
		% IM fat (on d.m. basis)		
Heifers	26	23.0 ± 6.1	15.7 – 31.8	2.0
Bulls	23	9.9 ± 3.5	3.6 – 18.7	5.4
		mg % Serum lipid		
Heifers	26	344 ± 55.4	175 – 460	2.6
Bulls	23	300 ± 65.7	190 – 470	2.5

3 V-shaped channels were marked into each plate and approximately 50 mg of fat applied to each channel by repeated streaking with capillary pipettes. Lipid classes were separated in ascending chromatographic tanks using a solvent system of petroleum ether (40–60°C. b.p.), diethyl ether (peroxide-free) and glacial acetic acid, 85:15:1. After development, the bands were visualized by ultraviolet light, identified with the aid of Hormel Institute standards and outlined. The bands were scraped off the plates into sublimation tubes for subsequent direct esterification.

Esterification of fatty acids

The esterification procedure employed was a modification of Marchello's method (1965). The fat was put into solution with chloroform:methanol, 2:1, prior to refluxing for 0.5 hr in 5% dimethyl sulfate in superdry methanol. The methyl esters were extracted from the reaction mixture with petroleum ether (30–40°C. b.p.) in separatory funnels and washed 3 times with water. By controlling the volume of petroleum ether, the esters could be transferred to glass vials, evaporated to dryness under a stream of air and refrigerated until ready for analysis.

Gas-liquid chromatography

A Perkin-Elmer, Model 154D, vapor fractometer, equipped with a hydrogen ionization detector and connected to a Leeds-Northrop strip-chart recorder fitted with a Perkin-Elmer, Model 194, printing integrator was used for determination of fatty acid compositions. Separation of the acids was effected by 20% diethyleneglycol succinate on Chromosorb P, 80–100 mesh (Applied Science) packed in .0.25-in. by 7-ft columns. The operating parameters were approximately: injector port temperature, 300°C ; isothermal oven temperature, 208°C ; detector block temperature, 265°C. Nitrogen carrier gas pressure was 30–40 ml per minute; hydrogen and air pressures, about 80 and 220 ml per minute, respectively. Reference standards (Hormel Institute) were used to assist in peak identification and to check the linearity of the detector, recorder and integrator.

Quantitative TLC

Serum lipids were prepared for TLC quantitative analysis of lipid classes by adding 0.8 ml of serum to 4 ml of cold chloroform:methanol:water (2:1:0.1) in centrifuge tubes, with vortex mixing. The tubes were refrigerated for 1 hr, then centrifuged for 5 min at 2000 rpm. The top methanolic phase was removed by aspiration. The remainder (protein mat and lipid in chloroform) was again mixed, filtered through filter paper into vials and evaporated to dryness under air. This procedure was rapid and automethanolysis was avoided.

Quantitative analysis of lipid classes was carried out on 20 by 20 cm precoated silica gel TLC plates (Brinkman Instruments Inc.). A template of stainless steel was constructed to facilitate rapid, uniform marking of the plates into 7 strips. Lipids were applied in narrow bands using a No. 1 camel's-hair brush. The amount applied was controlled by dilution of the lipids with heptane.

Separation of the lipid classes was effected by using 2 solvent systems. The first system, previously described, was allowed to ascend 12–13 cm. The second system, ascending 18 cm, was petroleum ether (40–60°C. b.p.) and diethyl ether (peroxide-free), 94:6. After development the plates were dipped into a solution of 20% ammonium sulfate and 20% sulfuric acid, drained and dipped into clean, cold water, drained and charred in a cross-air convection oven at about 190°C for 6–10 min. The plates were cut into strips. Relative areas and densities of the bands were determined by densitometry (Photovolt, Densicord, Model 542, complete with a strip-chart recorder fitted with a disc integrator).

Analysis of the data

All of the carcass cutout measurements were placed on a basis of 100 kg of side weight. All of the lipid characteristics were placed on a concentration or percent of tissue basis for the statistical analyses. Phenotypic, environmental and genetic correlations and paternal half-sib estimates of heritability were computed according to the procedures set forth by Harvey (1960). Because of the findings of Terrell et al. (1968), mentioned previously, and the extensiveness of this study, flavor was the only taste panel evaluation used for the computer-programmed analyses.

Table 2–Means, ranges and range coefficients (H/L) of classes of serum, muscle and subcutaneous lipids of the 1965 San Juan heifers and bulls.

	Heifers (N = 26)			Bulls (N = 23)		
	Mean	Range	H/L	Mean	Range	H/L
Lipid class[a]	(% of Fat)			(% of Fat)		
Serum P–L	24.0	17.4–31.8	1.8	21.6	17.6–24.7	1.4
Chol	15.1	10.8–20.0	1.8	17.4	12.2–21.5	1.8
FFA	9.0	5.9–15.0	2.5	7.0	3.8–14.7	3.9
T–G	5.9	2.3–11.3	4.9	4.5	1.3–8.3	6.4
C–E	45.9	37.5–52.2	1.4	49.0	39.8–58.8	1.5
Muscle P–L	4.5	2.5–10.1	4.0	8.9	3.2–21.3	6.6
M–G	2.2	0.0–5.1	51.0[b]	2.8	0.6–6.0	10.0
D–G	3.1	0.0–7.1	77.0[b]	3.3	0.0–9.2	92.0[b]
Chol	4.8	1.5–8.2	5.5	13.8	8.2–19.6	2.4
FFA	4.8	0.6–18.8	31.3	7.5	3.6–13.9	3.9
T–G	79.4	65.5–89.9	1.4	60.4	48.4–72.2	1.5
C–E	1.2	0.0–3.0	30.0[b]	3.1	0.6–5.6	9.3
Sub-Q P–L	0.6	0.4–1.7	4.2	0.8	0.5–2.8	5.6
M–G	0.9	0.4–2.6	6.5	0.8	0.2–1.7	8.5
D–G	1.7	0.3–3.9	13.0	1.8	0.4–3.9	9.8
Chol	2.6	0.6–4.1	6.8	1.9	0.5–6.1	12.2
FFA	3.8	2.0–6.1	3.0	4.6	2.1–7.0	3.3
T–G	90.0	84.5–95.5	1.1	89.8	84.9–95.8	1.1
C–E	0.0	0.0–0.0	----	0.1	0.0–1.0	10.0[b]

[a]P–L, phospholipid: Chol, cholesterol; FFA, free fatty acids; M–G, D–G, T–G, mono-, di-, triglycerides; C–E, cholesterol-esters; Sub-Q, subcutaneous adipose tissue.
[b]For the purpose of illustration, 0.1% was used for the division.

RESULTS & DISCUSSION

CONCOMITANT with the establishment of interrelationships and genetic aspects of bovine lipids, it was possible to examine the means and normal biological variations of serum (s), intramuscular (IM) and subcutaneous (SQ) lipid components. Values presented in Tables 1, 2 and 3 are those of 49 of the 61 animals. These animals were slaughtered in the same year; therefore, only their data are presented to avoid introduction of potential

Table 3–Means of the major fatty acids (as a percent of fat) of serum, intramuscular and subcutaneous lipids of 26 heifers and 23 bulls from the San Juan Station.

Fatty acid[a]	14:0	16:0	16:1	17:0	18:0	18:1	18:2
	Total serum fatty acids (% of fat)						
Heifers	1.8	23.5	4.8	3.8	26.5	15.8	18.5
Bulls	1.6	20.6	5.1	2.5	28.5	15.7	19.9
	Total intramuscular fatty acids (% of fat)						
Heifers	8.8	32.7	8.3	1.3	8.1	30.1	3.3
Bulls	6.4	32.2	6.2	1.4	11.4	28.9	7.4
	Total subcutaneous fatty acids (% of fat)						
Heifers	4.7	26.9	7.2	0.8	12.3	42.5	1.6
Bulls	3.7	26.7	5.0	1.0	17.4	40.4	3.1

[a]Carbon number and unsaturation indicated.

Table 4—Least-squares analysis of variance of cutout, flavor and lipid analysis of 61 San Juan Herefords.

	Sex	Year	Sires/Year			Sex	Year	Sires/Year	
d.f.	1	1	8	6		1	1	8	6
Side weight	5.3		3.5*		% D–M	14.4			
Thickness	10.7*		2.7*		IM fat	30.7		2.1	
IMF: Thickness		4.9	2.9*		S, Lip				
Kidney fat	29.0								
SRM	65.0	4.0			S– 14:0				3.4*
Trim	98.2		3.8		16:0			2.4	
IMF: trim					16:1	4.5		2.8	4.5*
Flavor	7.8*	4.3			17:0				
S, P–L					18:0		5.8*	2.6*	
S, Chol			2.0	4.4*	18:1		2.6	2.5*	5.6*
S, FFA				2.2	18:2	4.7			
S, T–G			2.3						
S, C–E					IM– 14:0				
IM, P–L					16:0	11.0*			
IM, M–G					16:1	28.6*			
IM, D–G					17:0	9.8*			
IM, Chol					18:0				
IM, FFA					18:1	35.7*		2.4	4.7*
IM, T–G	42.2				18:2	5.5			
IM, C–E		4.1	2.0						
SQ, P–L					SQ– 14:0	25.6*			
SQ, M–G			2.5		16:0				
SQ, D–G					16:1	24.9*		2.0	
SQ, Chol				2.2	17:0				3.2*
SQ, FFA			3.5*	2.2	18:0	18.9*			
SQ, T–G					18:1				
SQ, C–E					18:2	14.6			4.3*

d.f. = degrees of freedom. Only significant, 5 and 1%, F values are listed; 1% = *.

year effect. Although nutritional regimens and other environmental factors reportedly produce minimal changes in bovine lipids, as compared to other animals (Hilditch, 1956), the environmental effects were shown to be significant for a number of lipid components. The analysis of variance (Table 4) shows the effects of year, sex and sire. For the sake of clarity, only those "F" values significant at the 5 and 1% level of confidence were tabulated.

Table 5—Phenotypic correlations between various carcass measurements[1] of the San Juan cattle killed in 1965. (N = 61.)

	Side weight	IMF: Thickness	Thickness	Kidney	SRM	Trim	IMF: Trim	Flavor	IMF
Side weight	1.00								
Thickness[a]	.06	1.00							
IMF: thickness	–.07	–.66	1.00						
Kidney fat[b]	.41	.07	–.05	1.00					
SRM[c]	–.25	–.16	–.01	–.48	1.00				
Total fat trim[d]	.60	.33	–.33	.68	–.48	1.00			
IMF: trim	–.23	–.10	.57	–.22	–.03	–.43	1.00		
Flavor	–.00	–.04	–.14	.00	–.04	–.00	–.17	1.00	
% DM[e]	.18	–.02	.32	.12	–.17	.08	.28	–.03	
% IMF[f]	.45	.20	.32	.31	–.36	.36	.49	–.22	
% S, Lip[g]	.53	.25	–.18	.24	–.07	.37	–.25	.11	.27

Level of significance: 5% = .25, 1% = .32.
[1] All carcass characteristics were placed on a basis of 100 kg, of side weight for computation.
[a] Fat thickness as measured on the distal edge of the twelfth rib area.
[b] Perinephric fat.
[c] Steak and roast meat.
[d] Total trimmable fat, including perinephric fat.
[e] Percent dry matter of longissimus dorsi.
[f] Percent intramuscular fat.
[g] Percent total serum lipid.

Heifers had considerably more intramuscular fat than bulls, but the amount of serum lipid between the 2 sexes was not appreciably different (Table 1). As in other species, females had lower serum and intramuscular cholesterol than did males (Table 2). Although the heifers obviously had lower cholesterol levels when calculated as percent of fat, the values did not appear statistically significant between sexes when computed on a concentration basis (Table 4).

An examination of the range coefficients (H/L) in Table 2 revealed some interesting aspects of the data. Some of the values for these 49 animals appear somewhat extreme due to the occasional 0.0 percentage values observed for some components. For the purpose of illustration, however, they were considered as 0.1% for the division. When calculated on another group of more uniform animals, the values were not as extreme, but the trends in both instances were the same. The range coefficients of serum phospholipids (P–L), cholesterol (Chol) and cholesterol-esters (C–E) were rather small, indicating that the supply into and out of the blood stream was quite constant, reflecting a primarily endogenous source. In contrast, the variations of the serum triglycerides (T–G) and free fatty acids (FFA) were considerably larger, reflecting an exogenous or dietary source. In cattle, a considerable amount of FFA is synthesized from ruminally produced acetate and other short-chain lipid precursors (Ballard et al., 1969). This, too, was reflected by the somewhat larger FFA variations. Projecting the same considerations to the muscle and subcutaneous lipid classes revealed which classes were the most stable. Triglycerides are known to be the major storage form in muscle and subcutaneous fat depots, and the T–G were shown to be considerably more stable than the more transient types of lipids, as indicated by the relatively low variation in the range coefficients in muscle and subcutaneous tissue. These observations are in agreement with those reported in other species by Masoro (1968).

The fatty acid composition of the 3 tissues did not appear remarkably different between sexes when observed as a percent of fat (Table 3). However, when the fatty acids were placed on a concentration basis, the differences again became statistically significant, as shown in Table 4, column 1. Tissues of the heifers contained significantly more unsaturated fatty acids than those of the bulls.

The phenotypic correlations of the various lipid components with the cutout data and flavor evaluations are presented in Tables 5, 6 and 7. Only those correlations statistically significant at the 5 and 1% level or those of special interest were tabulated. The approximate stage of de-

Table 6–Some phenotypic correlations of cutout data, flavor and lipids of 61 San Juan Herefords.

	Side wt	SRM	IMF	Kidney	Thickness	IMF: Thickness	Trim	IMF: Trim	Flavor
S– P–L	.54	–.14	.41	.41	.23	–.14	.46	–.10	.06
Chol	.50	–.11	.16	.12	.21	–.27	.38	–.34	.14
FFA	.01	–.12	.20	–.01	.11	.15	.02	.01	–.22
T–G	–.14	.17	–.25	–.12	.24	–.24	–.07	–.21	.17
C–E	.52	.01	.21	.22	.16	–.13	.29	–.22	.16
IM– P–L	–.31	.16	.08			.38	–.26	.45	–.11
M–G	.43	–.12	.33	.29					–.08
D–G	.44	–.18	.45	.34	.35		.34		.00
Chol	.33	–.20	.44				.34	.25	–.02
FFA	.47	–.19	.41	.38					.02
T–G	.32	–.36	.93	.25		.37	.29	.49	–.24
C–E	.42	–.01	.28						.08
SQ– P–L		.18	–.14		–.15				.13
M–G		.26	–.25	–.35	.00		–.32		–.05
D–G		.25	–.01	–.25	.06				–.03
Chol		.26	–.06	–.32	–.07				.12
FFA		–.00	.17		.28			.33	.02
T–G		–.24	.06	.35	–.06				–.07
C–E		–.06	–.01		–.06			.35	–.00

5% = .250, 1% = .325.

velopment of these animals was verified by the phenotypic correlations of Table 5. The correlations of the side weight with steak and roast meat (SRM) was –.25 and with the total fat trim, .60, which indicated that the animals were well past the stage of maximum muscle development and that any further increase in side weight would be due primarily to increased deposition of fat.

Correlations between kidney fat, fat thickness and intramuscular fat were also interpreted as an indication of the growth, development and relative rates of change of the fat depots, which would be in agreement with early work done by Haecker (1920) and Hammond (1932). That is, the perinephric fat is one of the first adipose tissues to be developed and it is physically limited in the amount of fat that can accumulate. Subcutaneous fat depots develop later and are virtually unrestricted in the amount that can accumulate. The kidney fat deposits of these animals had nearly reached a maximum and the rate of increase was very slow; whereas, the subcutaneous adipose tissue was continuing to develop at a comparatively rapid rate. If a plot were made of the amount of fat in these 2 depots with respect to age or weight, both lines would be positive but far from parallel; thus, the low correlation (.07) between kidney fat and subcutaneous fat thickness.

Intramuscular fat is the last adipose tissue to be developed and is also limited in the amount that can accumulate. A plot of the intramuscular fat vs. age or weight would, therefore, fall between those plotted for subcutaneous fat thickness (.20) and kidney fat, being considerably closer to the kidney fat line (.31).

Probably the most controversial correlation derived from all the various analyses was the nearly significant negative correlation (–.22) between the intramuscular or marbling fat and flavor (Table 5). Essentially none of the intramuscular lipid components were positively correlated with flavor (Tables 6 and 7). Of the subcutaneous lipid classes, only the cell structural components (P–L and Chol) were positively correlated with flavor. One way of considering this, is that if triglycerides were decreased there would be relatively higher percentages of P–L and Chol, thereby effecting a favorable influence on flavor. By contrast, the serum lipid classes and the serum fatty acids by and large correlate positively with flavor (Tables 6 and 7). However, correlations with flavor should be interpreted with caution as organoleptic flavor is difficult to measure unless there are rather large differences or off-flavors.

Other interesting series of correlations were those of the subcutaneous lipid classes with fat thickness. One would expect very high correlations; instead, they were low and negative (except for FFA). These results were explained on the basis of a report by Ballard et al. (1969) and the correlations obtained from analysis of data from 297 animals. These data suggested that the major source of triglycerides in subcutaneous fat depots was by de novo synthesis of FFA from ruminally produced acetate. This would result in a positive, phenotypically expressed association between the FFA and fat thickness.

Although none of the correlations of the lipid components with flavor was statistically significant at the 5% level of confidence, the number of animals used for the study should lend considerable confidence to the positive and negative trends indicated. The somewhat low correlations observed could be one explanation for the inability of other investiga-

Table 7–Some phenotypic correlations of cutout data, flavor and fatty acids of 61 San Juan Herefords.

	Side wt	SRM	IMF	Kidney	Thickness	IMF: Thickness	Trim	IMF: Trim	Flavor
S– 14:0	.35	–.02	.17						.03
16:0	.28	–.04	.09		.31	–.32		–.30	.12
16:1		.10	.25						–.16
17:0		.17	–.14	–.26					.03
18:0	.62	–.08	.21	.31		–.26	.50	–.36	.16
18:1		.17	–.04					–.26	.05
18:2		–.11	.21	.30		.26			.10
IM– 14:0		–.05	.49					.29	–.17
16:0	.44	–.31	.88	.30		.30	.28	.40	–.15
16:1	.30	–.23	.65	.26				.32	–.09
17:0		–.22	.27	.28			.38		–.01
18:0		–.25	.55		.37			.34	–.07
18:1		–.15	.70			.33		.34	–.17
18:2		–.31	.22				.25		–.14
SQ– 14:0	–.27	–.16	–.04						–.07
16:0		.03	–.12						.07
16:1		–.46	.38	.26		.28	.25		–.03
17:0		.29	–.31			–.31			.23
18:0	–.46	.32	–.22	–.28			–.27		–.06
18:1	.53	–.16	.26				.27		–.09
18:2		.37	–.23	–.25		–.25			.16

5% = .250, 1% = .325.

tors to positively identify lipid flavor components. Considering components from the standpoint of the total amount present, rather than as a "part-of-a-part," may also offer advantages worth considering.

That more economical beef, higher percent cutability and better flavor can be obtained by slaughtering at a younger age or with less "finish," or both, is not a new concept. However, the suggestion that excessive marbling does not contribute appreciably to better flavor is a challenge to current thinking and practices which should stimulate more extensive research in this area. Perhaps there is a point of diminishing returns with regard to the amount of fat deposited and the enhancement of flavor. Further research might lead to the establishment of lower (optimum) levels of marbling (without penalizing the producer), thereby decreasing the human intake of animal fats.

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EPIMYSIAL CONNECTIVE TISSUE POLYSACCHARIDES OF BOVINE SEMIMEMBRANOSUS MUSCLE AND ALTERATIONS IN THEIR TYPE WITH AGE AND SEX DIFFERENCES

SUMMARY—Newborn veal, 11.5-month-old steers, 14.5-month-old heifers and 9 to 10.5-year-old cows were used to identify the polysaccharide types present in the epimysium and to determine the relationship between the amount of connective tissue polysaccharides and the amount of collagen in the epimysium. Trimmed muscle was used for tenderness evaluation by shear force. Average amounts of 332, 247, 230 and 202 mg dry polysaccharides per 100g dry, defatted epimysium were isolated from the veal, steer, heifer and cow groups, respectively. A negative correlation was obtained between age of animals and amount of isolated polysaccharides. Only 20% of the hexosamine in the dried defatted epimysium was extracted as soluble connective tissue polysaccharides. It is possible that some selective solubilization of certain polysaccharides occurred during extraction and, consequently, the extracted polysaccharides may not reflect the actual polysaccharide composition of the intact tissue. Considering this, it was found by using Dowex 1 X-2 chromatography that an average of 42% of the total uronic acids of the veal and heifer groups was eluted with 0.5M NaCl. 17 and 19% of the cow and steer uronic acids, respectively, were eluted with 0.5M NaCl. In the veal and heifer groups, 37 and 38% of the total uronic acids were eluted with 1.5M NaCl, whereas in the cow and steer groups the amount represented 71 and 70%. The percentages of uronic acids eluted with 2.0M NaCl were 20, 11, 19 and 12% in the veal, steer, heifer and cow groups, respectively. Dermatan sulfate was found to be the main polysaccharide eluted with 1.5M NaCl for the veal, heifers and cows. It also represented an important type in the steers, although other sulfate polysaccharides seemed to be present. Cellulose polyacetate electrophoresis confirmed that hyaluronic acid and dermatan sulfate were present in the epimysium. The ratio of hexosamine to insoluble collagen in the epimysium was positively associated with muscle tenderness.

INTRODUCTION

IT IS generally accepted that collagen affects the eating qualities of meat. In muscle and in other tissues, collagen occurs in close association with connective tissue polysaccharides (Clayson et al., 1962; Meyer, 1965). Specific connective tissue polysaccharide types tend to occur along tightly packed collagen fibers; others are associated with loosely organized collagen fibers (Meyer, 1965). So far, eight different types of connective tissue polysaccharides have been isolated from a variety of tissues such as cartilage, bone, tendon, skin and vascular tissue (Meyer, 1957; Walker, 1961). These are hyaluronic acid, chondroitin, chondroitin sulfate A, chondroitan sulfate C, dermatan sulfate, heparin, heparitin sulfate and keratosulfate.

It has been observed that transformations take place in the connective tissue polysaccharides of several organs or structures during aging (Milch, 1966; Jackson and Bentley, 1968). Some researchers found that total connective tissue polysaccharides, as measured by hexosamine, decreased during aging (Houck and Jacob, 1958; Sobel et al., 1954; Shetlar and Masters, 1955). Other researchers pointed out that there were some alterations in polysaccharide types during aging (Loewi and Meyer, 1958). McIntosh (1967) suggested that during the postmortem aging of meat, degradation of connective tissue polysaccharides occurs, resembling the type of breakdown caused by papain. Fox (1968) found that in three muscles of bulls, steers and cows, the hexosamine content of the polysaccharides eluted with 0.5, 1.25, 1.5 and 2.0M NaCl did not vary significantly between sexes or time post-mortem. No significant association was found between hexosamine content of muscle and tenderness as measured by shear force. No significant relationships were found between mucopolysaccharide fractions and tough or tender meat. Wipf et al. (1970) observed that porcine muscle classified as pale, soft and exudative had a higher hexosamine content, and higher dermatan sulfate, residual acid mucopolysaccharide and chondroitin content than normal muscle. Tenderness of porcine muscle was positively correlated with dermatan sulfate content.

Additional research is needed to establish more definitely the type of connective tissue polysaccharides in bovine muscle. The present study was undertaken to isolate the polysaccharides of bovine epimysium, to fractionate and further characterize them and to determine if alterations in polysaccharide types occurred with age and sex differences.

MATERIALS & METHODS

Experimental animals

3 female veal (2 to 3 days of age), 3 steers (11.5 months), 3 heifers (14.5 months) and 3 cows (9 to 10.5 years) were used to study the age and sex differences of muscle connective tissue polysaccharides. The study was designed mainly to study intensively the connective tissue polysaccharides of animals varying widely in age. Thus, the number of experimental animals per age group was kept small. Steers were included in the experimental group, since they constitute a large proportion of the animals slaughtered for meat. All experimental animals were of the Holstein breed. The veal, steers and heifers were obtained from sources where actual birth records were kept, while the age of the last group was estimated by a veterinarian. Carcasses averaged 27 kg for the veal, 275 kg for the steers, 125 kg for the heifers and 273 kg for the cows. All animals were slaughtered at the Cornell Department of Animal Science Meat Abattoir according to practices normally employed at the laboratory.

Processing of muscle

Within 30 min after death, the right and left semimembranosus muscles were removed and frozen in large polyethylene bags at −29°C. The frozen storage period varied from 4–6 months. At the convenient time, the muscles were allowed to thaw and age in a 2°C cooler for 8 days. At the end of the aging period, the muscles were removed from the polyethylene bags, the right muscle utilized for the chemical determinations and the left muscle immediately cooked and used for tenderness evaluation.

Characterization of connective tissue polysaccharides

This study was conducted on the epimysial sheath of the semimembranosus muscle because it represented a concentrated source of connective tissue. The epimysium of the right semimembranosus muscle was carefully dissected with a scalpel, cut into pieces and extracted with a 0.6M KCl buffer at 2°C during 3 hr according to the method of McIntosh (1961). Solvent-to-tissue ratios of 20:1 (v/w) were used. Following this extraction, the epimysium was defatted in 10 vol of acetone at 2°C for 12 hr with occasional stirring. The acetone was changed and the same procedure was repeated. The defatted epimysium was dried to constant weight in a vacuum desiccator, powdered in a Wiley mill and stored in a desiccator at −29°C. Triplicate 30-mg samples of dry defatted epimysium were assayed for hexosamine as described subsequently, to calculate recoveries after the isolation procedure.

To isolate the polysaccharides of the epimysium, 10g of dried defatted epimysium was digested with papain according to a modification of the original procedure of Schiller et al. (1961). Major changes in the Schiller procedure consisted of increasing the amount of papain from 2 to 25 mg per g of dried defatted material, extending the 0.5N NaOH treatment from 4 to 24 hr and omitting the trypsin digestion. Type II crude papain from Sigma Chemical Company, St. Louis, Missouri, was purified according to the method of Kimmel and Smith

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Table 1—Dry weight, hexosamine and hexuronic acid content of polysaccharides isolated from bovine epimysium.

Animal group	Animal no.	Dry weight of polysaccharide (mg/100g dry epimysium)	Hexosamine (μg/mg dry polysaccharide)	Hexuronic[a] acid (μg/mg dry polysaccharide)	Hexosamine: hexuronic acid ratio[b] Dry epimysium	Hexosamine: hexuronic acid ratio[b] Extracted polysaccharide
Veal	1	290	206	213	2.41	1.05
	2	385	234	239	2.59	1.11
	3	289	206	197	2.50	1.13
Heifer	1	233	228	210	3.13	1.18
	2	218	223	214	3.37	1.13
	3	240	199	201	3.10	1.07
Cow	1	195	197	183	2.77	1.17
	2	217	199	173	3.25	1.25
	3	193	212	197	4.28	1.15
Steer	1	236	219	197	3.69	1.20
	2	275	225	227	3.57	1.07
	3	231	203	186	3.68	1.18

[a]Hexuronic acid by carbazole procedure (Dische, 1947).
[b]Molar ratio.

Table 2—Elution pattern of polysaccharides rechromatographed on cellulose columns, expressed in terms of percent hexosamine eluted at each ethanol concentration.

Percent ethanol in elution solvent[a]	Veal 1	Veal 3	Heifer 1	Heifer 2	Heifer 3	Cow 3	Steer 1
	(%)						
80	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0
30	0	7.8	0	5.0	0	0	7.1
25	0	0	0	0	0	0	4.0
20	0	5.6	0	0	5.3	0	5.5
15	6.5	0	21.6	0	0	0	3.9
10	13.3	8.4	3.7	8.9	0	9.1	0
5	13.6	17.7	14.1	27.9	35.2	5.5	20.8
0	66.6	60.5	60.6	58.2	59.5	85.4	58.7
0[b]	0	0	0	0	0	0	0

[a]8-ml fractions were collected at each ethanol concentration.
[b]An additional tube was collected with 0% ethanol to ensure complete elution of the polysaccharides.

(1954) and utilized for the digestion. The polysaccharides were isolated as a cetylpyridinium chloride complex, redissolved and precipitated with ethanol. The purified polysaccharides were washed with ethanol and ether, dried in vacuo over P_2O_5 and the dry weight of the isolated polysaccharides recorded. They were analyzed for hexosamine or hexuronic acid, and utilized for anionic exchange chromatography and electrophoresis.

The colorimetric analysis for hexosamine was carried out as described by Boas (1953). The hydrolysis conditions were modified according to the procedure of Anastassiadis and Common (1958) to ensure maximum recovery of the hexosamine. Balazs et al. (1965) reported that when the color intensity produced by the Boas procedure was measured at 530 mμ, the amount of color produced by glucosamine was different from the amount of color produced by galactosamine. To determine the relative amounts of glucosamine and galactosamine in the isolated polysaccharides, therefore, their absorption spectra were recorded between 580 and 510 mμ and compared to a standard curve prepared with known ratios of glucosamine to galactosamine. A Beckman Model DU 2 recording spectrophotometer was used for these measurements. The colorimetric carbazole method of Dische (1947) was used for the determination of hexuronic acids. In this method, iduronic acid, which is the uronic acid moiety of dermatan sulfate, gives less color than does glucuronic acid. However, there is another method of uronic acid determination based on an orcinol reaction (Brown, 1946), in which both iduronic acid and glucuronic acid give the same color yield. This method was used when an attempt was made to establish the type of polysaccharide present in the epimysium.

For the purpose of fractionating the epimysial polysaccharides, chromatography on Dowex 1 x-2 chloride columns was conducted according to the method of Schiller et al. (1961). The polysaccharides were theoretically expected to be fractionated in the following manner: hyaluronic acid is eluted with 0.5M NaCl; heparitin sulfate, chondroitin, chondroitin sulfates A and C and dermatan sulfate are eluted with 1.5M NaCl; heparin and part of keratosulfate are eluted with 2.0M NaCl (it requires 3.0M NaCl to obtain a satisfactory elution of keratosulfate). The effluent was collected in 8-ml fractions with an automatic fraction collector and analyzed for hexuronic acid (Dische, 1947). To obtain the resolution of the polysaccharide mixture eluted with 1.5M NaCl, the effluent was pooled, dialyzed until free of chloride ions, concentrated and refractionated on a cellulose column by means of ethanol gradient solution. The method used in this fractionation was that of Gardell (1957), which elutes the polysaccharides as follows: chondroitin sulfate C at 40–50% ethanol (Meyer et al., 1956); chondroitin sulfate A at 30–40% ethanol (Meyer et al., 1956); heparin sulfate at 10% ethanol (Gardell, 1957). In preliminary work for this research, pure chondroitin sulfate A was nearly completely eluted with 30% ethanol and dermatan sulfate was eluted between 15 and 0% ethanol (Cormier, 1969). The effluent was collected in 8-ml fractions and analyzed for hexosamine.

Cellulose polyacetate electrophoresis was used to separate the polysaccharides isolated from the epimysium of the semimembranosus muscle of the four groups of animals. The separation was conducted according to the method of Mathews (1961). Cellulose polyacetate strips—Seraphore III—were soaked in pyridine formic acid buffer at pH 3, the samples were spotted along with known purified standards (generously furnished by Dr. J. A. Cifonelli, University of Chicago). A voltage of 170 was applied for 45 min. The strips were dried, sprayed with 1% acridine orange, washed and dried in a fume hood.

Analysis of epimysial collagen

Since it has been shown that collagen and polysaccharides interact under physiological conditions (Mathews, 1965), it was felt that a better understanding of meat connective tissue as a whole would be obtained if the solubility characteristics of epimysial collagen were studied concomitantly with the characterization of the connective tissue polysaccharides. Duplicate 200-mg samples of dry defatted epimysium were analyzed for soluble and insoluble collagen. The alkali-soluble fraction was obtained following the procedure of Kao and McGavack (1959). The insoluble collagen fraction was extracted by the method of Fitch et al. (1955). The two collagen fractions were analyzed for hydroxyproline content using the method of Prockop and Udenfriend (1960). Collagen content was calculated by multiplying the amount of hydroxyproline by 7.52 (Goll et al., 1963).

Tenderness evaluation

Clayson et al. (1962) found there was some degree of uniformity in the proportion of collagen and polysaccharides present in the epimysium compared to the intramuscular connective tissue. This uniformity was deemed to justify a comparison between the amount of connective

Table 3–Optical densities at 570 and 530 mμ of glucosamine and galactosamine standards and hexosamines from the epimysial polysaccharides.

Origin of hexosamine		Optical density (OD) 570 mμ	530 mμ	Glucosamine: galactosamine ratio
Hexosamine standards				
50 μg glu: 0 μg gal		.111	.414	1.00:0
40 μg glu:10 μg gal		.116	.406	0.80:0.20
30 μg glu:20 μg gal		.134	.420	0.60:0.40
20 μg glu:30 μg gal		.143	.425	0.40:0.60
10 μg glu:40 μg gal		.145	.408	0.20:0.80
0 μg glu:50 μg gal		.149	.392	0:1.00
Isolated epimysial polysaccharides				
Animal no.	μg[a]			
Veal 1	20.3	.102	.305	0.41:0.59[b]
Veal 2	20.4	.122	.379	0.52:0.48
Veal 3	8.3	.068	.205	0.43:0.57
Heifer 1	19.2	.113	.347	0.48:0.52
Heifer 2	20.0	.119	.366	0.49:0.51
Heifer 3	19.6	.094	.297	0.57:0.43
Cow 1	19.9	.111	.305	0.14:0.86
Cow 2	19.5	.114	.319	0.20:0.80
Cow 3	No sample left			
Steer 1	5.5	.055	.167	0.45:0.55
Steer 2	20.0	.131	.398	0.45:0.55
Steer 3	No sample left			

[a]Weight of dry isolated polysaccharides giving the intensity of absorption obtained during the scanning procedure.

[b]Values obtained from a standard curve constructed of the ratio $\frac{\text{OD at 570 m}\mu}{\text{OD at 530 m}\mu}$ for the pure hexosamine standards.

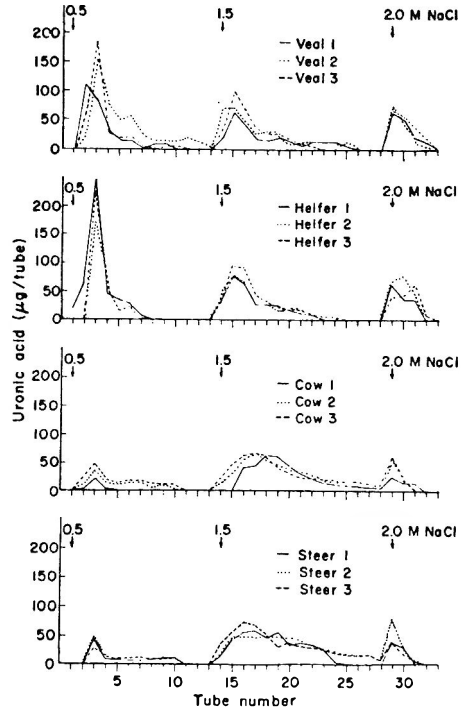

Fig. 1–Dowex 1 x-2 chloride column chromatography of 5 mg of polysaccharides from veal, heifer, cow and steer using NaCl of increasing molarity as the eluant. 8-ml fractions were collected in an automatic fraction collector.

tissue polysaccharides present in the epimysium and the tenderness attributes of the trimmed semimembranosus muscle. To conduct the tenderness evaluation, four slices, each 3.8 cm thick, were cut from the middle section of the left semimembranosus muscle, perpendicular to the length of the muscle. The slices were placed into deep beef fat previously heated to 150°C and cooked to an internal temperature of 63°C. The meat was allowed to cool at 25°C and 8 cores, each having a diameter of 1.27 cm, were mechanically removed from each slice. Each core was sheared in half on a Warner-Bratzler shearing apparatus. An average of 32 shear readings was recorded as the shear value for that muscle.

Data analysis

The data were analyzed statistically by analysis of variance according to Steel and Torrie (1960). Non-orthogonal single degree of freedom contrasts were used to determine significance of mean differences. Simple correlations as outlined by Steel and Torrie were used to determine relationships between the variables under study.

RESULTS & DISCUSSION

Isolation of the polysaccharides

Table 1 presents a summary of the quantity of polysaccharides isolated from the epimysium along with an analysis of their hexosamine and hexuronic acid content. There were fewer polysaccharides isolated with increasing animal age. A correlation of -0.63, $P < .05$, was obtained between quantity of polysaccharides and animal age. This relationship must be interpreted with caution, however, since the isolated polysaccharides contained only approximately 20% of the total hexosamine originally present in the dry defatted epimysium. This low percentage recovery can be attributed to the difficulty of working quantitatively during the numerous steps of the isolation procedure and to the interference of hexosamine containing mucoproteins in the pre-isolation hexosamine measurement. Table 1 presents the molar ratios of hexosamine to hexuronic acid in the pre-isolation and post-isolation material. The pre-isolation hexosamine:hexuronic acid ratios ranged from 2.41 to 4.28, with an average ratio of 3.19. The average post-isolation ratio came down to 1.14 and there were no statistically significant differences between the animal groups. These results can probably be explained by the incomplete removal of hexosamine containing mucoproteins from the epimysium during the pre-isolation treatment with a 0.6M KCl buffer. Boas (1955) found that almost 50% of the hexosamine present in rat connective tissue was from plasma mucoprotein, whereas the other 50% was from the connective tissue polysaccharides.

In the present research, the pieces that the epimysium was cut into for the KCl buffer extraction were not very small. A more effective removal of mucoproteins would have been accomplished if the epimysium had been pulverized before the extraction procedure. When pre- and post-isolation hexuronic acid measurements were used to calculate percent recovery during the polysaccharide isolation procedure, an average recovery of 49% was obtained. There were no significant differences between animal groups for uronic acid recovery. It cannot be ruled out that selective losses of polysaccharides did not occur during the isolation procedure. Further experimentation is needed to clarify this point. The hexosamine content of the isolated polysaccharides ranged from 197–234 μg per mg of isolated polysaccharides; the hexuronic acid content as measured by the carbazole method ranged from 173–228 μg per mg of isolated polysaccharides. An analysis of variance revealed no statistically significant differences between the bovine groups for the hexosamine or the hexuronic acid content of the isolated polysaccharides.

Chromatography of the isolated polysaccharides on Dowex 1 x-2 chloride columns

Results obtained with Dowex 1 x-2 chloride chromatography are summarized in Figure 1. The data for the four groups–veal, heifer, cow and steer–are graphed separately to emphasize the similarities and differences observed between these groups. The veal and heifer groups had similar polysaccharide peak intensities at 0.5, 1.5 and 2.0M NaCl; the cow

Table 4—Uronic acid values obtained by two different colorimetric procedures for polysaccharide standards, polysaccharides eluted with 0.5M NaCl and polysaccharides rechromatographed on cellulose column.

Origin of material	Carbazole procedure (μg/ml)	Orcinol procedure (μg/ml)	Carbazole:orcinol ratio
A. Polysaccharide standards[a]			
Chondroitin sulfate A	59.8	29.4	2.03
Chondroitin sulfate C	–	–	2.0–2.5[b]
Dermatan sulfate	21.2	67.7	0.31
Heparin	85.6	22.8	3.75
Hyaluronic Acid	60.9	40.0	1.52
B. Epimysial polysaccharides eluted with 0.5M NaCl (hyaluronic acid fraction)[c]			
Veal 1	6.2	3.5	1.77
Veal 2	10.2	6.4	1.61
Veal 3	8.8	5.8	1.51
Heifer 1	11.8	8.5	1.39
Heifer 2	11.1	7.2	1.54
Heifer 3	15.7	9.9	1.59
Cow 1	4.6	2.5	1.79
Cow 2	5.7	4.1	1.37
Cow 3	3.6	3.1	1.18
Steer 1	6.6	4.1	1.61
Steer 2	8.2	5.0	1.65
Steer 3	5.2	3.4	1.55
C. Epimysial rechromatographed polysaccharides on a cellulose column[d]			
Veal 1	1.9	4.5	0.42
Veal 3	1.9	5.6	0.34
Heifer 1	1.9	3.8	0.51
Heifer 2	1.1	3.5	0.30
Heifer 3	1.7	6.6	0.26
Cow 3	2.2	6.0	0.37
Steer	2.2	6.1	0.36

[a]Purified chondroitin sulfate A, dermatan sulfate, hyaluronic acid and heparin were gifts of Dr. J. A. Cifonelli from the University of Chicago. They were rechromatographed on cellulose columns and the material assayed came from the peak of the elution curve at the proper ethanol concentration. The heparin was used without further purification.

[b]Personal communication with Dr. J. A. Cifonelli.

[c]The material assayed came from the peak of the elution curve with 0.5M NaCl during Dowex 1 x-2 chromatography.

[d]The material assayed came from the peak of the elution curve, which occurred at 0% ethanol. The five missing values were rejected because of imperfections in the cellulose columns.

and steer groups also had similar peak intensities at the three NaCl concentration levels but had a pattern strikingly different from the veal and heifer groups. An analysis of variance of the percentage of uronic acid eluted at the three levels of NaCl concentration indicated that at the 0.5M NaCl level (hyaluronic acid) there was a statistically significant difference, $P < .01$, between the veal and the heifer groups compared to the cow and the steer groups. These same comparisons were also significantly different with the 1.5M NaCl eluant, $P < .001$, and with the 2.0M NaCl eluant, $P < .05$. An average of 42% of the total uronic acids was eluted as hyaluronic acid in the veal group compared to 17% in the aged cow group. This observation was in general agreement with previous findings made with pig skin (Loewi and Meyer, 1958) and bovine vitreous humor (Chvapil, 1967), and indicated that the level of hyaluronic acid tended to decrease with aging. The relative proportion of hyaluronic acid, 42% of the total uronic acids, was exactly the same in the veal and heifer groups. This observation was unexpected in light of the references just cited. The percentage of uronic acids eluted as hyaluronic acid in the steer group was 19, a value close to the 17 obtained for the aged cow group. Asboe-Hansen (1963) reported that the female hormone estrogen increased the polysaccharide content of connective tissue and, more specifically, the hyaluronic acid content. The reduced level of estrogens in the aged cows and the absence of estrogen and testosterone in the castrated steers conceivably could explain the low hyaluronic acid content observed in these two groups of animals.

Refraction of polysaccharides eluted with 1.5M NaCl

The polysaccharides eluted with 1.5M NaCl were dialyzed, concentrated, precipitated on top of cellulose columns and then gradually redissolved by means of a decreasing ethanol gradient. Table 2 presents the results obtained. The data from five columns had to be rejected either because of an overflow of the eluting solvent on top of the column or because of air pockets developing in the column. The average recovery for the seven samples appearing in Table 2 was 89%.

Of the seven samples rechromatographed, most of the polysaccharides were eluted with an ethanol concentration of 15% or less. The elution pattern of the epimysial polysaccharides closely followed the pattern of a pure dermatan standard run along with the isolated polysaccharides. Traces of chondroitin sulfate A seemed to be present in veal No. 3, heifer No. 2 and steer No. 1. According to these limited observations, no change in sulfated polysaccharide type could be detected among bovine epimysia of different ages.

Electrophoresis of isolated epimysial polysaccharides

The isolated polysaccharides were resolved into two or three components by electrophoresis on cellulose polyacetate at pH 3; typical results of one member of each group are presented in Figure 2. The veal contained two spots, one running parallel to hyaluronic acid and the other parallel to dermatan sulfate. The heifer contained two similar spots plus traces of an additional one running parallel to chondroitin sulfate A. The cow had only traces of hyaluronic acid and a major spot running parallel to dermatan sulfate. The steer had one spot running parallel to hyaluronic acid and a major component running parallel to dermatan sulfate. The information obtained from the fractionation of epimysial polysaccharides with electrophoresis confirmed results previously reported for the Dowex 1 x-2 chloride chromatography. In both procedures, the veal and heifer were found to have a sizable amount of hyaluronic acid whereas the cow and steer had a smaller amount. The electrophoretic result also supported the findings obtained with the cellulose column fractionations, which indicated that dermatan sulfate was the major sulfated polysaccharide in bovine epimysium.

Absorption spectrum of hexosamines in isolated polysaccharides

Table 3 presents the optical density at 570 and 530 mμ of pure hexosamine standards and of the hexosamine obtained from the isolated polysaccharides. The veal, heifer and steer groups had glucosamine:galactosamine ratios ranging

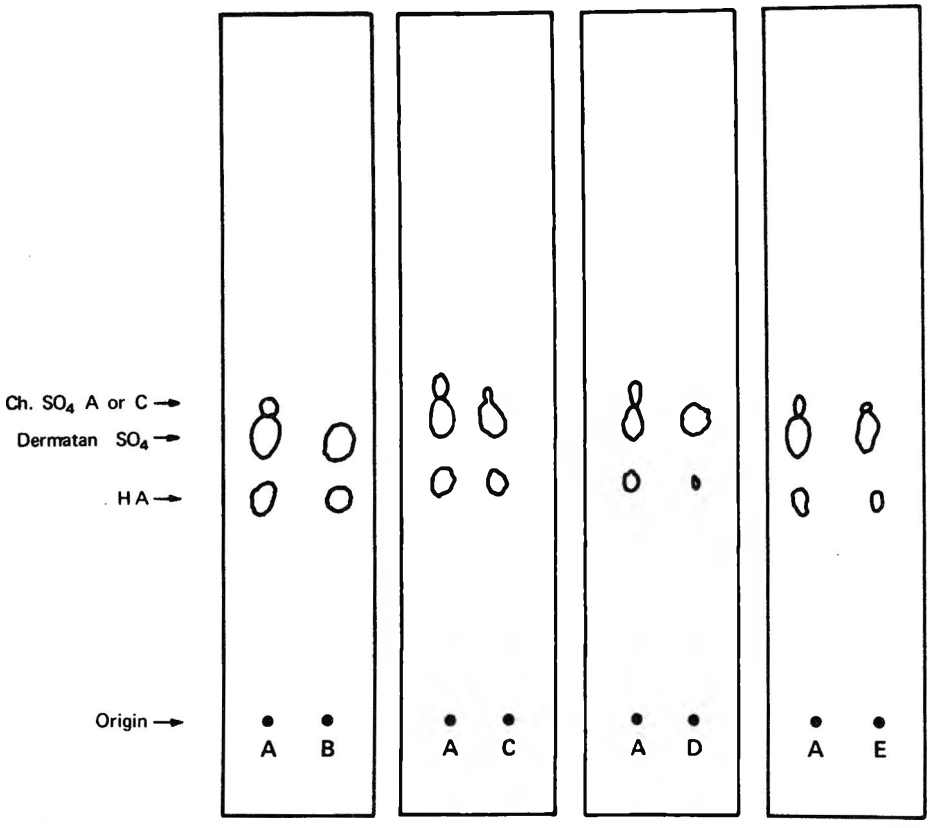

Fig. 2–Cellulose polyacetate electrophoresis in pyridine formic acid buffer pH 3 of the polysaccharides isolated from bovine epimysium and of polysaccharide standards. At point A, three polysaccharide standards were spotted: hyaluronic acid (HA), dermatan sulfate and chondroitin sulfate A (Ch SO_4 A). The veal polysaccharides were spotted at point B, the heifer at point C, the cow at point D and the steer at point E.

Table 5–Collagen fractions of semimembranosus epimysium and hexosamine:collagen interrelationship.

Animal group	Animal no.	Alkali-soluble collagen	Insoluble collagen	Hexosamine	Hexosamine:insoluble collagen ratio
		(g/100g dry epimysium)			
Veal	1	0.99	40.59	60	1.47
	2	1.03	39.68	90	2.27
	3	1.01	42.36	67	1.57
Heifer	1	0.27	32.71	53	1.62
	2	0.40	37.94	49	1.28
	3	0.43	31.82	48	1.50
Cow	1	0.40	35.31	38	1.09
	2	0.26	37.88	43	1.14
	3	0.50	34.90	41	1.17
Steer	1	0.90	39.60	52	1.31
	2	0.62	42.94	62	1.44
	3	1.23	43.55	50	1.13

from 0.41:0.59 to 0.57:0.43. These ratios indicated that 41 to 57% of the hexosamines in these three groups were glucosamine. The cow group had a glucosamine:galactosamine ratio, indicating that 80–86% of the hexosamines were galactosamine. Assuming that the glucosamine in the veal and heifer groups comes from hyaluronic acid, the ratios appearing in Table 3 show that 40% or more of the total polysaccharides of these groups is accounted for by hyaluronic acid; the cow group shows 14–20% glucosamine and thus 14–20% hyaluronic acid. In the steer group, approximately 45% of the hexosamines was glucosamine. In the Dowex chromatography, 20% of the uronic acids was eluted as hyaluronic acid. The remaining 25% of the hexosamine could arise from heparitin sulfate, keratosulfate and heparin, which also have glucosamine as their hexosamine moiety.

Identity of the hexuronic acids

In another attempt to identify the polysaccharide types, advantage was taken of the different behavior of uronic acid in the carbazole and orcinol procedures. Table 4 presents the results obtained by these two colorimetric methods for a) polysaccharide standards, b) the 0.5M NaCl fraction from Dowex chromatography (hyaluronic acid), and c) the polysaccharides rechromatographed on cellulose columns.

Results obtained from analysis of the 0.5M NaCl fraction of the Dowex columns confirmed that hyaluronic acid was definitely the polysaccharide eluted at this NaCl concentration, since the carbazole:orcinol ratios for all animals were close to that of the hyaluronic acid standard. Results obtained with the rechromatographed polysaccharides on cellulose columns confirmed that dermatan sulfate was the polysaccharide eluted near 0% during the ethanol fractionation.

Epimysial collagen

Table 5 presents the values obtained for the proportion of collagen which was alkali-soluble and alkali-insoluble. The quantity of collagen which could be solubilized in 0.1N NaOH varied among the animals: the highest amount was found in the veal group, the second highest in the steer group, the cow group came next and, finally, the heifer group had the least alkali-soluble collagen. An analysis of variance revealed that the veal group had significantly more alkali-soluble collagen than the steer group, $P < .10$ and the cow and heifer groups, $P < .01$; the steer group had significantly more alkali-soluble collagen than the heifer and the cow groups, $P < .01$. There were statistically significant differences in total collagen between the veal group and the heifer group, $P < .01$; between the veal group and the cow group, $P < .01$; between the steer group and the heifer group, $P < .001$ and between the steer and the cow group, $P < .01$. The highest amount of collagen observed in the veal compared to the cow group was in agreement with Goll et al. (1963) and Carmichael and Lawrie (1967). The lower collagen values observed in heifers No. 1 and No. 3 cannot be fully explained. Polysaccharides and collagen are thought to be intimately related in connective tissue (Meyer, 1965). In the present

study, a relationship was found between the hexosamine content of the isolated polysaccharides and the amount of insoluble collagen in the epimysium. These data appear in Table 5, presenting the amount of hexosamines in the isolated polysaccharides along with the hexosamine:insoluble collagen ratio. There was a significant negative correlation, −.45 and $P < .10$, between the amount of isolated epimysial polysaccharides as measured by hexosamine content and the amount of insoluble epimysial collagen.

Meat tenderness

Fields and Pearson (1969) reported that the collagen solubility pattern in the epimysium was closely related to the pattern found in intramuscular connective tissue. This observation justifies the comparison of findings made on the epimysium and the tenderness attributes of the whole semimembranosus muscle. In the present study, meat tenderness as measured by the Warner-Bratzler Shear varied significantly with the age of the animals. The tenderness data are summarized in Table 6. An analysis of variance indicated that the veal muscle was more tender than the heifer muscle, $P < .05$, and the cow muscle, $P < .001$. The steer muscle was more tender than the cow muscle, $P < .001$. There was no significant difference in tenderness between the veal and the steer muscle. A correlation of 0.85, $P < .001$, was obtained between the age of the animals and the shear force values. The more epimysial alkali-soluble collagen, the lower were the shear force values of the muscle. A negative correlation of −.64, $P < .05$, was obtained between tenderness as measured by shear force and percent alkali-soluble collagen in the epimysium. This finding suggests that the degree of solubility of the collagen should be considered when biochemical explanations for toughness are sought. There was also a significant association between the hexosamine:collagen ratio and the tenderness of the muscle as measured by shear force. The higher the hexosamine:collagen ratio, the lower the shear force value of the muscle. The correlation coefficient between these two measurements was 0.54, $P < .05$. No significant correlations were found between shear value and the polysaccharide fractions obtained with Dowex chromatography.

It would appear that the decrease of polysaccharides with advancing age may be a predominant factor affecting the increased insolubility of collagen with aging. When there are fewer polysaccharides forming a network around collagen fibers, there may be more chances for the formation of intramolecular cross linkages in collagen, which would decrease the solubility of collagen. The polysaccharides may play an important role in plasticizing the collagen fibers, and this may be how they contribute to meat tenderness, as has been suggested by Milch (1966).

Table 6—Tenderness of semimembranosus muscles of veal, heifer, cow and steer as determined by shear force.

Animal group	Animal no.	Number of replications	Mean shear force (lb)	Standard error
Veal	1	30	4.2	0.7
	2	30	4.2	0.5
	3	30	4.3	0.6
Heifer	1	32	11.3	1.0.
	2	32	7.4	1.3
	3	32	8.9	1.8
Cow	1	32	14.1	2.3
	2	32	14.5	2.1
	3	32	21.2	4.4
Steer	1	32	7.5	1.3
	2	32	7.5	1.2
	3	32	7.5	1.3

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EFFECTS OF DRY AND MOIST HEAT ON SELECTED HISTOLOGICAL CHARACTERISTICS OF BEEF SEMIMEMBRANOSUS MUSCLE

SUMMARY—Samples of raw and heat-treated beef semimembranosus muscle were examined histologically to determine how deep-fat frying, oven roasting, oven braising and pressure braising to 70°C affected histological characteristics of the muscle. A panel of three persons used an ocular micrometer to measure muscle fiber width, observed color and type of connective tissue and distribution of fat and estimated quantity of connective tissue of 475 histological sections. Differences among heat treatments in muscle fiber width and relative proportion of straight and wavy connective tissue were not significant. However, there was a significantly ($P < 0.05$) larger quantity of granular tissue in deep-fat fried, pressure braised, and oven braised samples than in oven roasted samples. Intact fat cell walls were observed in both raw and heated samples.

INTRODUCTION

VARIABLE and contradictory results obtained by different researchers studying the effects of similar treatments on cooked beef are found in the literature. Paul (1963) suggested that variation in type of animal, pre- and post-slaughter treatments, cuts or muscles studied, cooking methods and method of evaluating tenderness contribute to the varied results obtained by different investigators.

To compare results of studies with cooked meat it is important that meat scientists know whether variations from one laboratory to another are attributable to method of cooking. Schock et al. (1970) studied the effects of deep-fat frying, oven roasting and oven and pressure braising on pieces of beef semimembranosus muscle (SM) cooked to 70°C. Shear, pH, color, flavor, tenderness, and over-all acceptability were similar for all treatments. Oven roasted pieces had the slowest rate of heat penetration, longest cooking time, and highest moisture content, press fluid, waterholding capacity and juiciness, and appeared less well done than pieces given the other treatments.

To study the effects of cooking on muscle tissue, measurements made on raw tissue are used as a reference point for data related to the cooked tissue. The method of measurement may affect the data obtained. Various methods of determining histological characteristics of muscle have been reported, most of them having been used to study raw tissue.

The primary objective of this study was to investigate the effects of dry and moist heat on selected histological characteristics of beef SM muscle. A secondary objective was to review some of the available data on muscle fiber diameter measured by several methods.

EXPERIMENTAL

Samples

Twelve U.S. Good beef top rounds were purchased from a local wholesale meat company. The SM muscle was removed and cut in four pieces each measuring approximately 11 × 11.5 × 6.5 cm and weighing approximately 820g. Each piece of muscle was wrapped in aluminum foil (gauge 0.0015), frozen and stored at −17.8°C for 2–14 weeks. Each wrapped piece was thawed to an internal temperature of 5° ± 2°C and cooked according to an incomplete block design by one of four methods: deep-fat fried (DF), oven braised (OB), pressure braised (PB) or oven roasted (OR) to an internal temperature of 70°C (Schock et al., 1970).

For histological study, adjacent samples (2.5 × 1.3 × 1.3 cm) of raw and cooked muscle were removed from the surface of each piece of SM muscle, wrapped in aluminum foil (gauge 0.0015) and stored at −17.8°C. Samples were thawed approximately 1 hr before sectioning 8–10μ thick with a CTD International Harris Cryostat microtome. A small amount of Cryoform, an imbedding matrix, was used to secure the tissue to the tissue holder. Commercially prepared freon gas, Cryo-Quik, was used to freeze the tissue. Each section was transferred to a slide by lightly touching the slide to the section while it was on the knife blade. Sections were stained with a saturated solution of picric acid and picro-ponceau stain, cleared in xylene and mounted with permount.

Evaluation

Three persons evaluated five sections of muscle tissue per sample for muscle fiber width; color, type and quantity of connective tissue; and distribution of fat. The width of one fiber, selected at random from each of three microscopic fields per section, was measured using an ocular micrometer in the eyepiece of the microscope; magnification was 430×.

A magnification of 35× was used to observe the characteristics of connective tissue and distribution of fat in each section of muscle tissue. Scores for color of connective tissue were given on a 3-point intensity scale, 3 representing the most intense color. For heated samples, hue of stained connective tissue was described in terms of changes from the bright pink-red of connective tissue in raw muscle.

Type (wavy, straight, granular) and quantity of connective tissue were estimated using the system of Ramsbottom et al. (1945). Scores of 7, 5, 3, and 1 represented a large, medium and small proportion or quantity and none, respectively. Distribution of fat was recorded as clumped or scattered.

Statistical analysis

For each histological measurement, differences between values for heated muscle and

Table 1—Mean data for histological measurements on raw and heat treated muscle.

	Treatment[a]					Significance	
Measurement	Raw	PB	OR	DF	OB	of F	LSD*
Muscle fiber width, u	48.2	43.9	43.7	43.3	42.8		
Difference, u[b]		− 4.3	− 4.5	− 4.9	− 5.4	ns	---
Difference, %[b]		− 8.9	− 9.3	−10.2	−11.2		
Type of connective tissue[c]							
Wavy	6.4	3.7	4.0	3.6	3.8		
Difference[b]		− 2.7	− 2.4	− 2.8	− 2.6	ns	---
Straight	3.6	4.2	4.5	4.3	4.3		
Difference[b]		+ 0.6	+ 0.9	+ 0.9	+ 0.7	ns	---
Granular	1.1	6.3	5.7	6.4	6.4		
Difference[b]		+ 5.2	+ 4.6	+ 5.3	+ 5.3	*	0.586[d] 0.599
Quantity of connective tissue[c]	4.6	5.8	4.8	5.5	5.5		
Difference[b]		+ 1.2	+ 0.2	+ 0.9	+ 0.9	ns	---

[a]PB, Braised – 10 p.s.i.g.; OR, Oven roasted; DF, Deep-fat fried; and OB, Braised, atmospheric pressure.
[b]Difference between raw and cooked tissue.
[c]Panel score: 7, Large proportion or quantity; 5, Medium proportion or quantity; 3, Small proportion or quantity; and 1, None.
[d]Use LSD 0.586 for comparing values among DF, OB, and OR heat treatments; use LSD 0.599 for comparing values of PB with DF, OB, and OR heat treatments.
*$P < 0.05$.

Table 2–Variation in muscle fiber width, in microns, for raw and heat treated samples.

Treatment[a]	Mean	Low	High	Difference
Raw	48.2	43.7	57.0	13.3
DF	43.3	39.8	46.4	6.6
OB	42.8	39.5	47.0	7.5
PB	43.9	40.6	47.7	7.1
OR	43.7	40.5	47.7	7.2
All heat treatments	43.4	40.1	47.2	7.1

[a]DF, Deep-fat fried; OB, Braised, atmospheric pressure; PB, Braised, 10 p.s.i.g.; and OR, Oven roasted.

corresponding samples of raw muscle were analyzed by analysis of variance. When the F-value was significant, the least significant difference ($P < 0.05$) was calculated.

RESULTS & DISCUSSION

Muscle fiber width

Mean muscle fiber width for samples representing each heat treatment was smaller than mean fiber width for raw muscle. However, the change from raw to cooked tissue did not vary significantly among the four heat treatments. The mean decrease for all heat treatments ranged from 8.9% for PB to 11.2% for OB, a difference of 2.3% between the two moist heat treatments. The decrease for OR and DF was 9.3 and 10.2%, respectively, or a difference of 0.9% between the two dry heat treatments. Irrespective of treatment, heating decreased fiber width approximately 10%; the difference between dry and moist heat was minute (0.25%), Table 1. Satorius and Child (1938) reported decreases in fiber diameter of 12–16% for beef muscles heated to 58°C by oven roasting, and further decreases during heating to 67°C. Hostetler and Landmann (1968) heated longissimus dorsi fibers on slides on a microscope stage to 53°–77°C. They observed decreases of approximately 20 to 25% in muscle fiber width.

The variation in width of muscle fibers from raw and heated samples is indicated by data in Table 2. Fiber width for raw samples averaged 48.2μ and ranged from 43.7–57.0μ; fiber width for all heated samples averaged 43.7μ and ranged from 39.5–47.7μ. Differences between low and high values for heated samples were approximately one-half of the difference between low and high values for raw samples.

Values for fiber width and diameter have been obtained by measuring fibers in longitudinal and cross sections of muscle tissue. There are problems associated with making measurements on both types of sections. Width of fibers in longitudinal sections may not truly indicate the size of muscle fibers and the quantity of sarcoplasm present. Fibers occur in a wide variety of shapes and measuring fiber width in a longitidinal plane could affect the accuracy of the data.

Swanson et al. (1965) noted that care must be taken to cut true cross sections, because any variation from the true cross section will increase the area of fiber exposed for measurement. They also pointed out that muscle fibers seldom are perfectly round, which makes it difficult to select a representative area for measurement. However, M. R. Feede (personal communication, 1970) found a high correlation between the diameter calculated from the cross-sectional area of fibers measured with a polar planimeter and the diameter of fibers obtained by averaging the largest and smallest diameters of a muscle fiber. His measurements were made on photomicrographs.

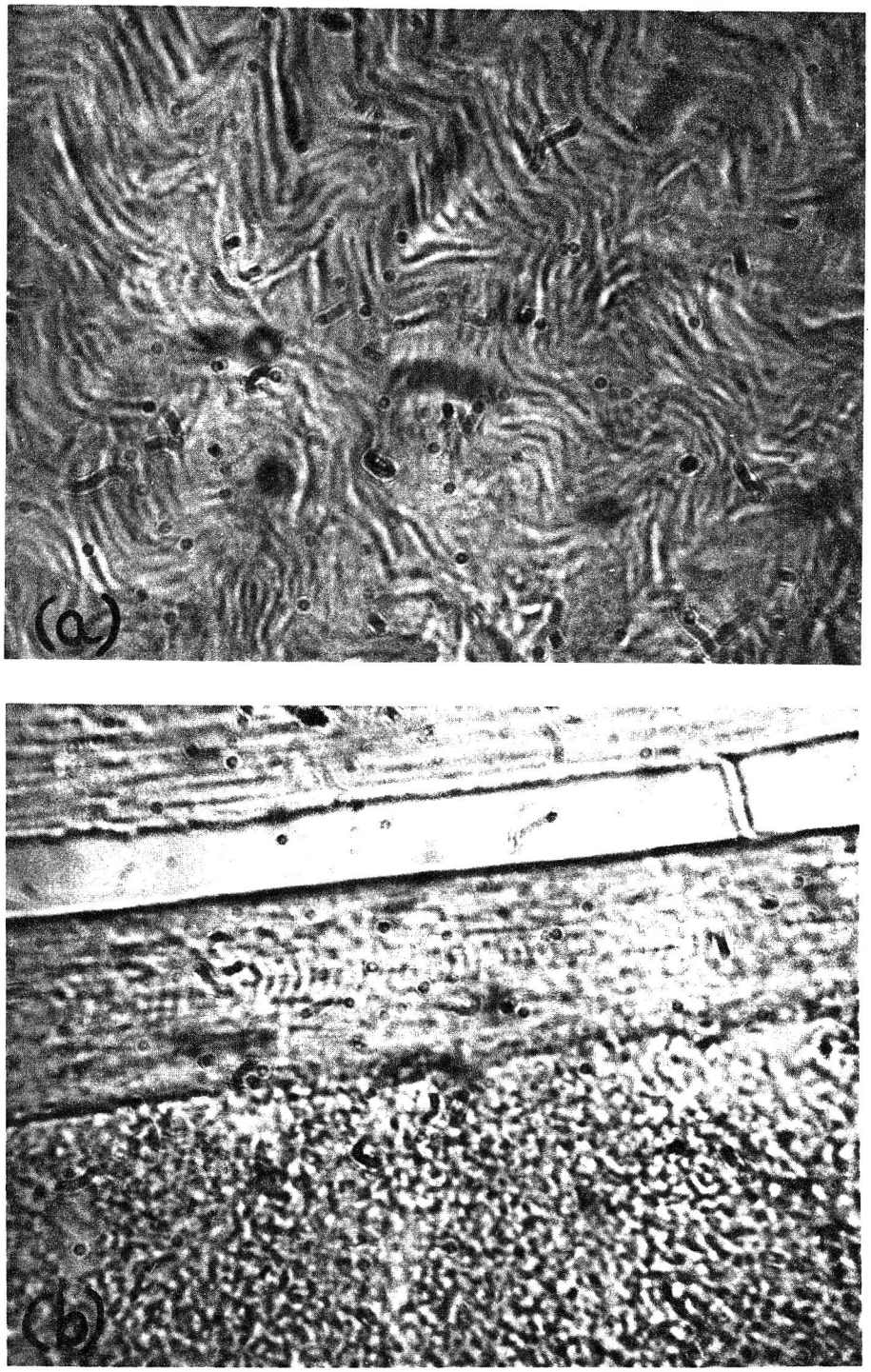

Fig. 1–Examples of histological characteristics: (a) A large area of wavy collagenous connective tissue fibers. Raw tissue, 645×. (b) A mixture of straight collagenous tissue fibers and granular tissue. Oven braised, 430×.

Several researchers have used the method described by Tuma et al. (1962) in which a microblender with the blades reversed was used to separate fibers and break them into pieces for measurement of fiber diameter. Hiner et al. (1953) used a dissecting microscope and needles to "tease" fibers from muscle before measuring fiber diameter. Values for fiber diameter or width reported in the literature may depend partly on the method of measurement.

Herring et al. (1965) reported a mean fiber diameter of 50μ for cross sections of raw SM muscle from 325–350 kg animals, which was similar to the mean value found in this study for width of fibers in raw SM muscle (48.2μ). Hiner et al. (1953) reported a slightly larger mean fiber diameter (57.1μ) for muscles of the round (semimembranosus, semitendinosus, and biceps femoris) from 900 lb, 14 mo old U.S. Good grade steers. Ely (1967), employing the same method used in this study, found slightly smaller average widths (30.0–45.3μ) for raw SM muscle from 16–25 mo old steers.

Connective tissue

Collagenous connective tissue in raw samples stained bright pink to red. In cooked samples some of the collagenous tissue had a granular appearance and was stained yellow by the picric acid, but did not stain red with the picro-ponceau stain. Paul et al. (1944), Lowe and Kastelic (1961) and Skelton et al. (1963) reported similar findings. It was postulated that changes in staining affinity might be evaluated by estimating the color intensity of each stained section. However, when preparing slides it was noted that freshness of the stain affected the length of staining time required. As it was not practical to prepare a new batch of stain for each sample, the staining period was increased as necessary to obtain a good contrast. Sections from heated muscle stained more rapidly than did sections from raw muscle.

Estimates were made of the relative proportion of wavy, straight and granular types of collagenous connective tissue present in each section and the quantity of total collagenous tissue (Table 1). Wavy fibers were predominant in sections of raw muscle, and made up a small to medium proportion of collagenous fibers in heated tissue. The proportion did not vary significantly among heat treatments. They occurred as narrow strands between muscle fibers, but were more common in large areas (Fig. 1a).

Straight fibers made up a small to medium proportion of the total collagenous tissue in raw sections. They comprised a slightly larger proportion of the connective tissue in cooked than in raw sections, but differences among heat treatments were not significant. Straight fibers appeared as small strands between muscle fibers and in large areas.

In most sections of raw tissue no granular connective tissue was observed, whereas in sections of heated tissue a medium to large proportion of the total collagenous tissue was noted (Table 1). The difference in the amount of granular tissue between raw and heated samples was significantly ($P \leqslant 0.05$) larger in sections from samples given PB, DF, and OB treatments than in those from OR samples. In sections from heated samples, generally, granular tissue was mingled with straight connective tissue fibers (Fig. 1b). Narrow strands of connective tissue tended to be completely granular, whereas larger areas tended to contain both granular and straight fibers.

The panel observed a greater quantity of total connective tissue in sections from cooked samples than in sections from adjacent raw samples (Table 1). Skelton et al. (1963) also observed more connective tissue in cooked than in raw muscle and attributed the apparent increase in connective tissue to the swelling and redistribution of the connective tissue during heating.

Distribution of fat

Although the solvent used in the staining technique dissolved the fat droplets from the cells, fat cell walls were distinguishable in both raw and heated samples. In the majority of sections fat was present in both clumped and scattered forms, the clumped form being predominant. Wang et al. (1954) explained that during heating some fat disperses from the fat cells to the degraded collagen without causing structural damage.

Conclusion

Generally the effects of heat on selected histological characteristics of beef SM muscle did not vary significantly among four heat treatments (two dry heat and two moist heat treatments). Variations among similar data in the literature probably resulted from procedural variations such as method of measurement; age, maturity or grade of the animal; or other pre- and post-slaughter treatments rather than heat treatment.

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POST-MORTEM GLYCOLYSIS IN OX SKELETAL MUSCLE: EFFECTS OF MINCING AND OF DILUTION WITH OR WITHOUT ADDITION OF ORTHOPHOSPHATE

SUMMARY—The rates of pH fall and of changes in glycolytic intermediates and cofactors in ox sternomandibularis muscle minced soon after slaughter were compared with the rates in unminced muscle. Mincing caused about a three-fold increase in the rates of most of the post-mortem changes but a much greater increase in the rate of loss of nicotinamide-adenine dinucleotide (NAD). Glycolysing muscle minces were diluted with 1 vol of a solution containing potassium chloride, with or without added potassium phosphate. Increasing the potassium chloride concentration resulted in a higher ultimate pH of the diluted preparation, whereas inclusion of inorganic phosphate (P_i) in 0.16M potassium chloride solutions resulted in lower ultimate pH values, the maximum effect being obtained usually with 50 mM P_i in the diluent. The ultimate pH was then about the same as in the undiluted mince, provided that glycogen was not exhausted. Comparisons were made of the glycolytic metabolism in undiluted minces and in minces diluted with 0.16M potassium chloride containing 0, 50 or 100 mM potassium phosphate (pH 6.7). Dilution without addition of P_i decreased the glycolytic resynthesis of adenosine triphosphate (ATP) without affecting the initial adenosine triphosphatase (ATPase) activity. Consequently ATP was lost faster, metabolism caused sooner and the ultimate pH was higher than in the undiluted mince. Inclusion of P_i in the diluent stimulated the glycolytic resynthesis of ATP without affecting the initial ATPase activity. Consequently loss of ATP was delayed until, due to loss of NAD, the glyceraldehyde-3-phosphate dehydrogenase step became rate-limiting for lactate production and fructose-1,6-diphosphate and the triose phosphates accumulated. Phosphorylase, phosphofructokinase and ATPase activities were calculated from the changes in glycolytic products and intermediates, and 'high-energy' phosphates. Reasons are discussed for the differences in the activities of these enzymes among the different types of preparation.

INTRODUCTION

PRECEDING PAPERS in this series pertained to post-mortem glycolysis in intact mucle at above zero temperatures (Newbold and Scopes, 1967) and during prerigor freezing and subsequent thawing (Scopes and Newbold, 1968). Investigations into the factors determining the extent of post-mortem glycolysis, using diluted mince preparations, have also been described (Newbold and Lee, 1965). In these investigations glycolysing muscle was minced and diluted with an equal volume of approximately isotonic (0.16M) potassium chloride, thus providing a preparation throughout which cofactors and glycolysable substrates could be readily dispersed.

The present paper compares the metabolism of intact and minced muscle, and the effect of diluting muscle minces with various concentrations of potassium chloride or potassium phosphate on the ultimate pH. It also compares the metabolism of undiluted minces and minces diluted with an equal volume of 0.16M potassium chloride with or without added P_i. It was of interest to study the effect of including P_i in the diluent since P_i is not only a substrate of phosphorylase and glyceraldehyde-3-phosphate dehydrogenase but also an activator of phosphofructokinase.

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MATERIALS & METHODS

Muscle preparations

The source of ox sternomandibularis muscle, the methods of mincing and preparing diluted minces, and the general conditions under which the experiments were conducted were as described previously (Newbold and Lee, 1965). Minces were prepared about 75 min post-mortem and diluted 10–15 min later. The diluted minces all contained 1 ml of diluent/g of mince. Mince diluted with 0.16M KCl is referred to as 'KCl mince,' with 0.16M KCl containing 50 mM potassium phosphate, pH 6.7, as '50 mM P_i mince,' and with 0.16M KCl containing 100 mM potassium phosphate, pH 6.7, as '100 mM P_i mince.'

All muscle preparations were kept at room temperature (20–25°C) unless otherwise noted, the diluted minces in air and the undiluted minces and intact muscle samples in N_2.

Measurement of pH

Unminced muscle and undiluted minces were homogenized in 5 mM sodium iodoacetate and the pH of the homogenate was measured with a Radiometer (Copenhagen, Denmark) model 22 pH-meter, scale expander and combined electrode (GK 2021C). The pH of the diluted preparations was measured directly by immersing the combined electrode in the preparation.

Analytical methods

Glycogen was measured as described previously (Newbold and Lee, 1965).

Methods of preparing perchloric acid extracts and analyzing them were as described by Newbold and Scopes (1967) except as noted below. The extracts were adjusted with 1N KOH to a pH close to 7.0 using a mixture of bromothymol blue and phenolphthalein as indicator. ATP was determined using phosphoglycerate kinase as described by Adam (1963); after this reaction was complete, creatine phosphate was measured using creatine kinase. In addition, glucose was determined using yeast hexokinase and measuring either the ADP produced (as described by Pfleiderer, 1963) or the glucose-6-phosphate produced (as described by Slein, 1963). The method for P_i (Allen, 1940) and all enzymic methods were automated using the Technicon Auto-analyser.

Expression of results

Analytical values obtained for each extract were multiplied by the ratio of the total acid-soluble phosphorus concentration of the muscle preparation from which the extract was made, to that of the extract. The total acid-soluble phosphorus content of each of the four muscles used to study the effect of mincing was assumed to be 53 μg-atoms/g. Determinations were made of the total acid-soluble phosphorus contents of all of the diluted preparations and six of the corresponding undiluted minces. Values obtained for the undiluted minces ranged from 49.0–57.0 μg-atoms/g (mean 53.0 μg-atoms/g).

'Hexose monophosphate' refers to the sum of glucose-1-phosphate, glucose-6-phosphate and fructose-6-phosphate; 'fructose diphosphate' to the sum of fructose-1,6-diphosphate and half of the triose phosphates. At all times fructose-1,6-diphosphate and the triose phosphates were present in roughly equilibrium proportions, as also were the individual hexose monophosphates.

RESULTS

Effect of mincing on subsequent glycolytic changes

The ultimate pH of a mince stored at temperatures ranging from 1°C–37°C

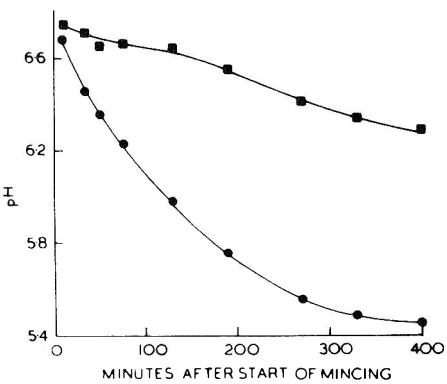

Fig. 1—Effect of mincing on the time-course of the change in pH. ■, intact (unminced) muscle; ●, minced muscle. Points are mean values for the same 4 muscles. Experimental details are given in the text.

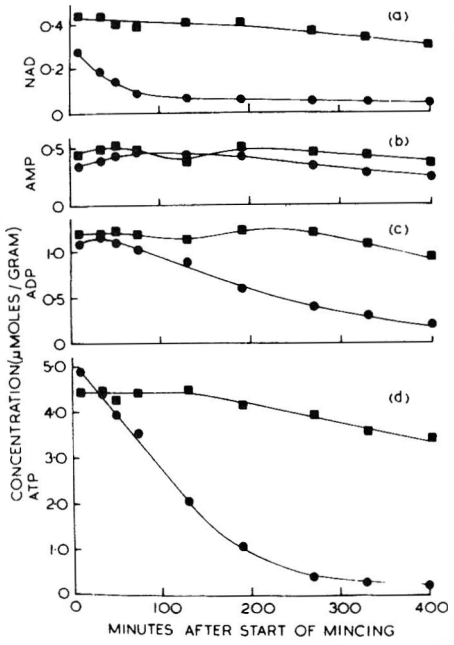

Fig. 2—Effect of mincing on the time-course of changes in the concentrations of (a) NAD, (b) AMP, (c) ADP and (d) ATP. ■, intact muscle; ●, minced muscle. Points are the mean values for the same 4 muscles as in Fig. 1. Experimental details are given in the text.

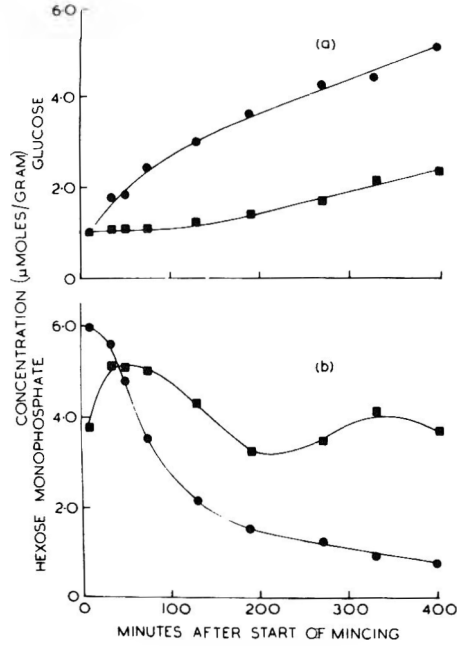

Fig. 3—Effect of mincing on the time-course of changes in the concentrations of (a) glucose and (b) hexose monophosphate. ■, intact muscle; ●, minced muscle. Points are mean values for the same 4 muscles as in Fig. 1. Experimental details are given in the text.

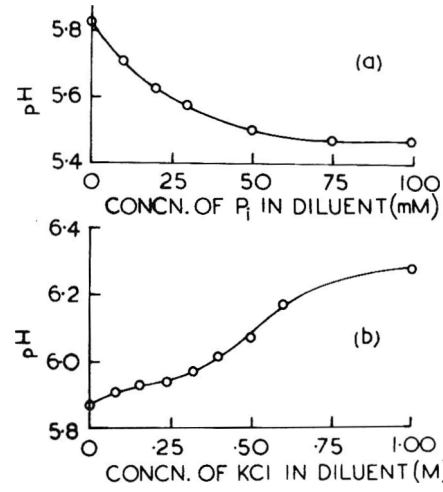

Fig. 4 Effect of composition of the diluent on the ultimate pH of diluted minces containing 1 ml of diluent/g of mince. (a) Concentrations of orthophosphate. Points are mean values for the same 9 minces. All diluents contained added glycogen (50—100 mM as glucose equivalents) in addition to orthophosphate and 0.16M potassium chloride; (b) Concentration of potassium chloride. Points are mean values for the same 4 minces. The diluents for one of these minces also contained added glycogen (55 mM as glucose equivalents).

was close to that of the corresponding unminced sample, but was reached in about one third the time. For example, at 20°C it was reached by 6—7 hr in mince compared with 20—24 hr in intact muscle.

Figures 1—3 show the changes, in the first 400 min after the start of mincing, in minced and intact samples kept at 20°C. The values are the means for four muscles. Changes in pH are shown in Figure 1. Lactate production closely paralleled the fall in pH in both intact and minced samples. Changes in the concentrations of adenine nucleotides are shown in Figure 2, and of hexose monophosphate and glucose in Figure 3.

The overall changes in ATP, adenosine diphosphate (ADP), glucose, glycogen and creatine phosphate concentrations were of the same magnitude in the mince as in the intact muscle, but took place several times faster in the mince. Changes in adenosine monophosphate (AMP) concentration (Fig. 2b) were small. The NAD content of the mince fell very rapidly (Fig. 2a) and by the time the first sample was taken it had fallen further than it did in the intact muscle in 400 min. It continued to fall only very slowly in the mince after reaching a concentration of about 0.06 μmoles/g.

At 10 min after the start of mincing the ATP concentration was always slightly higher and the ADP and AMP concentrations slightly lower in the mince than in the intact muscle. However, the sum of the ATP, ADP, AMP and NAD concentrations was at this time the same in both, and this sum plus the inosine nucleotide remained virtually constant throughout the 400 min period (mean value, 6.77 μmoles/g).

As noted previously (Newbold and Scopes, 1967) only very small amounts (less than 0.05 μmoles/g) of fructose diphosphate occur in intact ox sternomandibularis muscle. However, in the minced muscle fructose diphosphate built up to a peak of about 1 μmole/g (see Fig. 8c). The hexose monophosphate content of the mince was initially higher than that of the intact muscle (Fig. 3b), but subsequently declined steadily. In the 23 intact muscles examined to date the mean (± S.E.M.) hexose monophosphate content at about 75 min post-mortem, has been 4.15 ± 0.20 μmoles/g, and in minces freshly prepared from 12 muscles 75 min post-mortem 6.35 ± 0.48 μmoles/g.

The α-glycerophosphate content changed little in either intact or minced muscle.

At 400 min the glycogen contents (expressed as glucose equivalents) of the four minces were in the range 9—31 μmoles/g, indicating that the extent of glycolysis in these minces was not limited by lack of glycogen.

Effect of diluting with solutions of potassium salts on the ultimate pH of muscle minces

The effects of diluting with 0.16M potassium chloride with or without addition of glycogen have been described previously (Newbold and Lee, 1965). For 47 different muscles, the mean ultimate pH (± S.E.M.) of the undiluted minces was 5.59 ± 0.02 while that of the minces diluted with 0.16M potassium chloride containing 55 mM glycogen (as glucose equivalents) was 5.87 ± 0.01. When glycogen was omitted from the diluent the ultimate pH was only 0.038 ± 0.005 unit higher (22 comparisons).

The effect of diluting with different concentrations of potassium chloride is shown in Figure 4b. The ultimate pH of the four undiluted minces used in these experiments ranged from 5.54—5.71. As the concentration of potassium chloride in the diluent increased the pH fell more slowly and the ultimate pH was higher.

The effect of including potassium phosphate, pH 6.7, and glycogen, as well as 0.16M potassium chloride, in the diluent is shown in Figure 4a. Increasing the P_i concentration resulted in a lower ultimate pH, the maximum effect usually being obtained when the diluent contained 50 mM P_i. The ultimate pH was then similar to that of the undiluted mince.

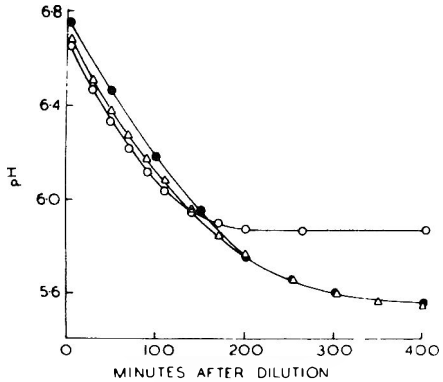

Fig. 5–Time-course of changes in the pH of undiluted minces (●), KCl minces (○) and 50 mM P_i minces (△). Points are mean values for corresponding preparations made from the same 17 muscles. Experimental details are given in the text.

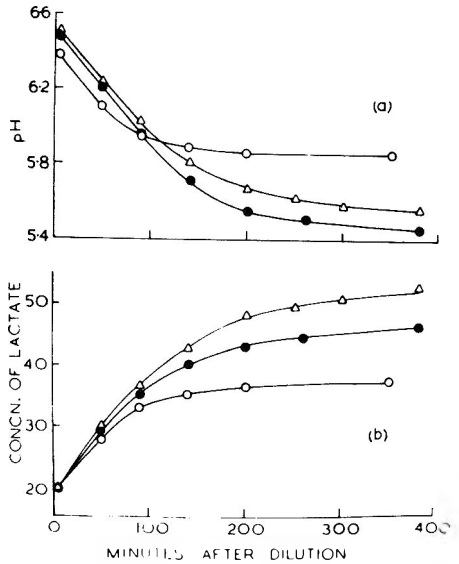

Fig. 6–Time-course of changes in (a) pH and (b) lactate concentration in undiluted minces (●), KCl minces (○) and 50 mM P_i minces (△). Concentrations in the diluted minces are expressed as μmoles/g of diluted preparation; those in the undiluted minces as μmoles/0.5g of mince. Points are mean values for corresponding preparations made from the same 3 muscles. Experimental details are given in the text.

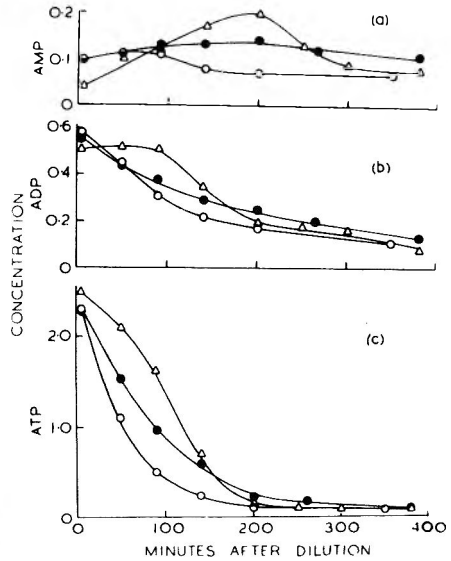

Fig. 7–Time-course of changes in the concentrations of (a) AMP, (b) ADP, and (c) ATP in undiluted minces (●), KCl minces (○) and 50 mM P_i minces (△). Concentrations in the diluted minces are expressed as μmoles/g of diluted preparation; those in the undiluted minces as μmoles/0.5g of mince. Points are mean values for the same preparations as in Fig. 6. Experimental details are given in the text.

When the diluent contained 50 mM glycogen (as glucose equivalents), the mean ultimate pH (± S.E.M.) of the 50 mM P_i minces prepared from the 47 muscles described above was 5.54 ± 0.02. When glycogen was omitted from the diluent the ultimate pH was 0.19 ± 0.02 unit higher (19 comparisons), and determinations on some of these preparations showed that the glycogen had been exhausted.

In the experiments described below, glycogen (55 – 100 mM, as glucose equivalents) was included in all the diluents to ensure that glycolysis did not cease because of lack of this substrate.

Effect of dilution on the rate of pH fall

The rates of pH fall in undiluted minces, KCl minces, and 50 mM P_i minces are shown in Figure 5. Each point in this figure is the mean value for the same 17 muscles.

The initial pH of the 50 mM P_i mince was partly determined by the pH of the diluent. The fact that the initial pH recorded for the KCl mince was lower than that recorded for the undiluted mince is attributable to the different methods used in measuring these values. The pH of the KCl mince was measured directly, whereas that of the undiluted mince was measured after homogenization in 5 mM sodium iodoacetate. It was found that, with freshly prepared mince, the pH of a homogenate in iodoacetate was consistently higher by about 0.1 unit than that of a homogenate in iodoacetate containing 0.16M potassium chloride.

Until the pH had fallen to 6.1–6.2 the rates of fall in the undiluted, KCl, and 50 mM P_i minces were very similar despite the higher buffering capacity (per gram of mince) of the 50 mM P_i minces. Subsequently, as has been noted above, the pH of the KCl minces fell only to about 5.9, reached in about 200 min; whereas in the 50 mM P_i minces, as in the undiluted minces, it fell to an average value below 5.6, reached in about 400 min.

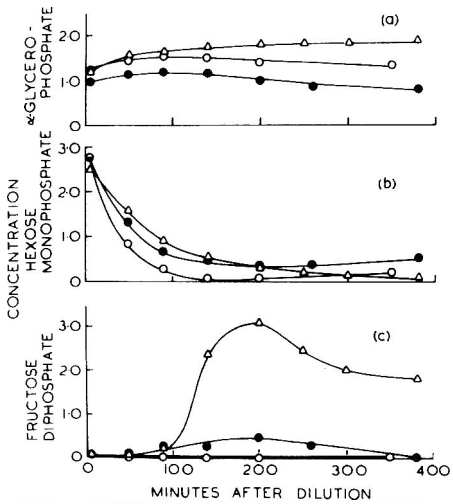

Fig. 8–Time-course of changes in the concentrations of (a) α-glycerophosphate, (b) hexose monophosphate and (c) fructose diphosphate in undiluted minces (●), KCl minces (○) and 50 mM P_i minces (△). Concentrations in the diluted minces are expressed as μmoles/g of diluted preparation; those in the undiluted minces as μmoles/0.5g of mince. Points are mean values for the same preparations as in Fig. 6. Experimental details are given in the text.

Effect of dilution on intermediary metabolism

Progressive changes in the concentrations of glycolytic intermediates and associated compounds were followed in nine KCl minces, seven 50 mM P_i minces and three 100 mM P_i minces. (In none of these was the glycogen concentration, expressed as glucose equivalents, less than 22 μmoles/g at 380 min.) Corresponding preparations from different muscles always showed the same general pattern of change but not necessarily the same time scale. The results presented in Figures 6–9 are the mean values for three muscles, the corresponding preparations from which metabolized at similar rates. In these figures, concentrations in the diluted minces are expressed as μmoles/g of diluted preparation, while those in the undiluted minces are expressed as μmoles/0.5g of mince unless noted otherwise. Similarly, comparisons made in the text are between equivalent amounts of minced muscle.

Lactate. In the interval 5–50 min after dilution more lactate was produced in the undiluted mince than in the KCl mince (Fig. 6b). The fact that, in this interval,

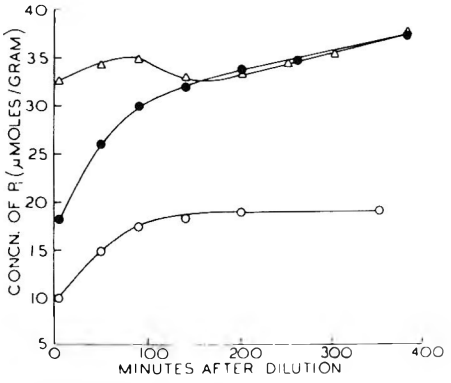

Fig. 9—Time-course of changes in the concentration of P_i in undiluted minces (●), KCl minces (○) and 50 mM P_i minces (△). Concentrations in all preparations, diluted or undiluted, are expressed as μmoles/g of preparation. Points are mean values for the same preparations as in Fig. 6. Experimental details are given in the text.

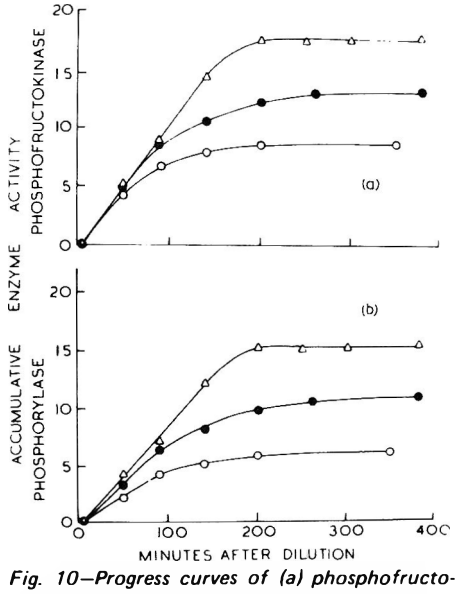

Fig. 10—Progress curves of (a) phosphofructokinase activity and (b) phosphorylase activity in undiluted minces (●), KCl minces (○) and 50 mM P_i minces (△). Accumulative phosphofructokinase and phosphorylase activities are expressed, respectively, as μmoles of substrate and μmoles of glucose equivalents transformed in 1g of diluted preparation or 0.5g of undiluted mince. Points are values derived as described in the text for the preparations described in Figs. 6–8.

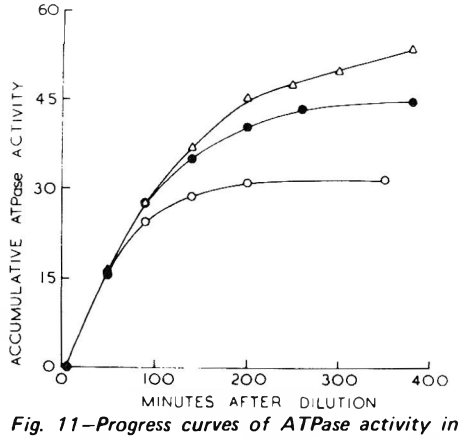

Fig. 11—Progress curves of ATPase activity in undiluted minces (●), KCl minces (○), and 50 mM P_i minces (△). Accumulative ATPase activity is expressed as μmoles of ATP dephosphorylated in 1g of diluted preparation or 0.5g of undiluted mince. Points are values derived as described in the text from mean values obtained with the preparations described in Figs. 6–8.

the change in pH was apparently the same in both (Fig. 6a) may be a consequence of the different methods used in determining pH, as described above. Lactate accumulated fastest in the 50 mM P_i mince.

Adenine nucleotides. Changes in the concentrations of the adenine mononucleotides are shown in Figure 7. Loss of ATP and ADP was faster in the KCl mince than in the undiluted mince but was delayed in the 50 mM P_i mince. However, by 200 min both the ATP and ADP contents had fallen by similar amounts in each of these preparations, the most rapid rate of loss in the 50 mM P_i mince occurring between 100–200 min. The greatest changes in AMP content occurred in the 50 mM P_i mince in which there was a steady increase to a peak at about 200 min followed by a rapid decrease. By the time of dilution the NAD content of the undiluted mince had fallen to 0.08–0.10 μmoles/0.5g, and the subsequent rate of loss was unaffected by dilution with either KCl or 50 mM P_i.

Glycolytic intermediates. Changes in the concentrations of those glycolytic intermediates which occurred in more than trace amounts are shown in Figure 8. The hexose monophosphate content fell faster in the KCl mince and slower in the 50 mM P_i mince than in the undiluted mince (Fig. 8b). In both the undiluted mince and the 50 mM P_i mince the fructose diphosphate content increased, reaching a maximum at about 200 min (Fig. 8c). The increase was much greater in the 50 mM P_i mince than in the undiluted mince and occurred during the period of rapid ATP loss. In the three KCl minces for which results are given here no more than a trace of fructose diphosphate was present at any time. However, in some others, a small temporary increase was observed. The amounts of 3- and 2-phosphoglycerate, phosphoenolpyruvate, and pyruvate in the minced muscle preparations were very small and did not change except for slow increases, in all but pyruvate, at times when substantial amounts of fructose diphosphate were present. After about 90 min the α-glycerophosphate concentration decreased slowly in the KCl minces but not in the 50 mM P_i minces (Fig. 8a).

Other compounds. The initial creatine phosphate contents were low, 1–2 μmoles/g of diluted preparation, and virtually none remained after 150 min. In the minced muscle preparations, as in intact muscle (Newbold and Scopes, 1967), changes in P_i concentration (Fig. 9) corresponded with the changes in other phosphates, the total amount of phosphorus in P_i, creatine phosphate, ATP, ADP, AMP, NAD, hexose monophosphate, fructose diphosphate, α-glycerophosphate, and inosine nucleotide (assumed to be IMP) being essentially the same at all times. Similarly the sum of the glycogen, glucose, lactate and glycolytic intermediates (all expressed as glucose equivalents) was virtually constant throughout the period of examination. Free glucose accumulated at about the same rate in the KCl mince as in the undiluted mince but more rapidly in the 50 mM P_i mince.

Metabolism of 100 mM P_i minces

Glycolytic changes in 100 mM P_i minces were similar to, but of greater magnitude than those in 50 mM P_i minces. Lactate accumulated at a faster rate but, as a consequence of the higher buffering capacity, the pH fell rather more slowly. The final lactate concentration was considerably higher, but the ultimate pH was not significantly different. Loss of ATP was further delayed but took place more rapidly during the period when fructose diphosphate was accumulating. Loss of ADP was also further delayed. The AMP peak was reached at about the same time as in the 50 mM P_i minces but was somewhat higher. Loss of NAD was not affected. Fructose diphosphate reached a peak concentration of about twice that in 50 mM P_i minces and subsequently declined only a little. Hexose monophosphate concentrations were generally higher in the 100 mM P_i minces, and some remained when the ultimate pH was reached. Changes in α-glycerophosphate were much the same in the 100 mM

P_i as in the 50 mM P_i minces. Glycogen utilization and glucose accumulation were both greater in the 100 mM P_i minces.

Effects of mincing and dilution on enzyme activities

In all preparations, since changes in the total concentration of monophosphoglycerates, phosphoenolpyruvate and pyruvate (1,3-diphosphoglycerate was not measured) were very small compared with those in lactate concentration, changes in lactate concentration reflect the activities of glyceraldehyde-3-phosphate dehydrogenase and the subsequent enzymes of glycolysis.

Accumulative phosphofructokinase (Fig. 10a), phosphorylase (Fig. 10b), and ATPase (Fig. 11) activities were calculated from changes in the concentrations of lactate, glycolytic intermediates, and 'high-energy' phosphates. (ATPases were assumed to be responsible for all the utilization of ATP not attributable to the action of phosphofructokinase or creatine kinase.) In plotting these activities, values of the following at 5 min after dilution were subtracted from those at later times:

Phosphofructokinase: $-0.5 \times$ lactate + $0.5 \times$ α-glycerophosphate + fructose diphosphate

Phosphorylase: $-0.5 \times$ lactate + $0.5 \times$ α-glycerophosphate + fructose diphosphate + hexose monophosphate

ATPase: $-1.5 \times$ lactate $-$ ($0.5 \times$ α-glycerophosphate + fructose diphosphate + $2 \times$ ATP + ADP + creatine phosphate)

As has been noted previously (Newbold and Scopes, 1967), in calculating phosphofructokinase activity it was not possible to allow for the utilization, if any, of fructose-1,6-diphosphate by fructose-1,6-diphosphatase. Similarly, in calculating ATPase activity it was not possible to allow for the breakdown, if any, of ATP to ADP and P_i by the combined action of phosphofructokinase and fructose-1,6-diphosphatase.

It is clear, without presenting curves, that mincing increased the ATPase activity and stimulated all of the glycolytic enzymes. Figures 10 and 11 each include curves derived from the data obtained for the undiluted minces, KCl minces and 50 mM P_i minces described in Figures 6–8. The corresponding curves for 100 mM P_i minces are given in the following paper (Newbold and Scopes, 1971). A notable feature shown by these curves is the almost complete cessation of phosphorylase and phosphofructokinase activities in the 50 mM P_i and 100 mM P_i minces after 200 – 250 min.

During the period 5–50 min the ATPase activity was essentially the same in the undiluted, KCl, 50 mM P_i and 100 mM P_i minces, but both the phosphorylase and phosphofructokinase activities increased with phosphate concentration, the former much more than the latter. This is illustrated more clearly in Table 1.

DISCUSSION

Effects of mincing

ATPase activity and glycolytic metabolism were stimulated about three-fold by mincing. The present results do not give any indication as to whether myofibrillar or other ATPases were involved. During mincing phosphorylase was stimulated more than phosphofructokinase, since the samples taken immediately after mincing had a higher hexose monophosphate content than the intact muscle. Subsequently, however, phosphorylase was relatively less active than phosphofructokinase, since the hexose monophosphate concentration decreased. NAD was lost rapidly after mincing, and it appears that lack of this coenzyme was responsible for the glyceraldehyde-3-phosphate dehydrogenase step becoming rate-limiting for lactate production, and the consequent accumulation of fructose-1,6-diphosphate and triose phosphates. In the following paper (Newbold and Scopes, 1971) it is shown that addition of NAD prevents this occurrence.

Effects of dilution

Dilution of the mince with 0.16M potassium chloride slowed the rate of glycolytic resynthesis of ATP but had little immediate effect on the ATPase activity. As a result there was more rapid loss of ATP, glycolysis ceased earlier and the ultimate pH was higher than in the undiluted mince. On the other hand, inclusion of 50 mM or 100 mM phosphate in the diluent accelerated the rate of glycolytic resynthesis of ATP, and the ATP concentration remained high until glyceraldehyde-3-phosphate dehydrogenase became rate-limiting and fructose diphosphate accumulated. In the KCl minces, the glyceraldehyde-3-phosphate dehydrogenase step usually did not become rate-limiting before phosphofructokinase activity ceased.

It should be noted that the rate of ATP loss is not directly a reflection of the ATPase activity, but of the AMP deaminase activity. At the relatively slow rates of metabolism in these minced preparations it can be assumed that myokinase is able to maintain equilibrium among the three adenine mononucleotides, and until the ATP content approaches its minimum most of the available adenine nucleotide is in the form of ATP. The activity of the deaminase depends partly on pH being maximal around pH 6 (Lee, 1957), partly on P_i concentration (P_i being an inhibitor–Nikiforuk and Colowick, 1956), but mainly on the AMP concentration at the enzyme site. Thus faster loss of ATP implies greater AMP deaminase activity which, in turn, implies a higher available AMP concentration.

Among the several types of minced muscle preparation, differences in both phosphorylase and phosphofructokinase activities were apparent from the time of dilution. On the other hand, ATPase activity was virtually unaffected for about 50 min and subsequent differences can be ascribed to differences in pH and

Table 1—Amounts of substrates used by various enzymes in undiluted and diluted minces during the period 5–50 min after preparation of the diluted minces[a]

Group	Preparation	P_i Conc.[b]	Hexose used at the phosphorylase step	Hexose used at the phosphofructokinase step	ATP used by ATPases
A[c]	KCl mince	12.5	2.2	4.1	15.4
	Undiluted mince	22.1[d]	3.2	4.7	15.9
	50 mM P_i mince	33.4	4.2	5.2	16.4
B[c]	50 mM P_i mince	32.9	4.2	5.1	15.0
	100 mM P_i mince	53.7	5.2	5.2	15.4

[a]Methods used in assessing these values are described in the text.

[b]P_i concentrations are the mean values for each of the different preparations during the period under consideration. Concentrations of P_i and the amounts of substrates used in the diluted minces are expressed as μmoles/g of diluted preparation, and in the undiluted mince as μmoles/0.5 g of mince, unless noted otherwise.

[c]Values in Group A are means for the same three muscles. Those in Group B are means for the same two muscles.

[d]μmoles/g of mince.

ATP concentration. Initially, the differences in phosphorylase activity may have been due to small changes in the amount of phosphorylase present in the *a* form, but they can also be explained in terms of phosphorylase *b* activity alone. The available AMP concentration, calculated from the equilibrium constants of the creatine kinase and myokinase reactions (Scopes and Newbold, 1968) must have been very small initially, much less than the K_m of phosphorylase *b* for AMP. However this K_m value is greatly affected by P_i, being smaller the higher the P_i concentration (Helmreich and Cori, 1964a), so that higher P_i concentrations would result in greater activity of phosphorylase *b* at a given AMP concentration. Also, at high P_i concentrations, because AMP deaminase is largely inhibited, the available AMP concentration can be higher without deamination occurring faster. This would further stimulate phosphorylase *b*. Differences in P_i and available AMP concentrations would also help to explain the differences in phosphofructokinase activity since both of these compounds activate this enzyme. Phosphofructokinase activity is also dependent on the rate of fructose-6-phosphate production and hence on phosphorylase activity.

Phosphorylase and phosphofructokinase ceased acting at about pH 5.9 in the KCl minces and 5.7 in both the 50 mM P_i and 100 mM P_i minces, but they continued to act down to pH 5.5, or lower, in the undiluted minces (as in the intact muscle). At these pH values phosphorylase *a* is still very active (Helmreich and Cori, 1964b). Hence it is concluded that, when phosphorylase activity ceased, the phosphorylase was completely in the *b* form and that none of the measured AMP was available to it. The most likely reason for the cessation of phosphofructokinase activity at the higher pH values in the diluted preparations is that one of its substrates was exhausted. At the time phosphofructokinase stopped acting only small amounts of fructose-6-phosphate were present in the KCl minces and 50 mM P_i minces, but substantial amounts remained in the 100 mM P_i minces. On the other hand the ATP concentration had fallen to about the same low level in all these preparations (and also in the undiluted minces). These findings suggest that the small amount of ATP still present in all the mince preparations when phosphofructokinase stopped acting was not available to this enzyme.

In the diluted minces where fructose diphosphate accumulated, lactate production from fructose-1,6-diphosphate continued after phosphofructokinase activity had ceased. Hence there must have been sufficient ADP present for the phosphoglycerate kinase and pyruvate kinase reactions to proceed. The ATP produced by these reactions was apparently available to the ATPases but not to phosphofructokinase. Eventually, in these preparations, metabolism ceased despite there still being some fructose diphosphate present, possibly through lack of NAD.

Glucose production in minced muscle preparations

In all the minced muscle preparations (as in intact muscle), the glucose concentration increased continuously, but at a slower rate after phosphorylase activity stopped. The enzymes responsible for this are amylo 1,6-glucosidase, which splits glucose from phosphorylase-limit glycogen chains, and α-amylase plus maltase. In the diluted minces (which all contained added glycogen) the more glucose produced the greater the phosphorylase activity. However, although the phosphorylase activities differed, the rates of glucose production in the undiluted minces (which contained no added glycogen) and KCl minces were much the same (per gram of mince). This was probably due to the outer chain length of the added glycogen being different from that of the endogenous glycogen.

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POST-MORTEM GLYCOLYSIS IN OX SKELETAL MUSCLE: EFFECT OF ADDING NICOTINAMIDE-ADENINE DINUCLEOTIDE TO DILUTED MINCE PREPARATIONS

SUMMARY—Samples of mince, prepared from ox sternomandibularis muscle about 75 min post-mortem, were diluted with one volume of 0.16M potassium chloride containing 0, 50 or 100 mM potassium phosphate with or without 1 mg of nicotinamide-adenine dinucleotide (NAD)/ml. Changes in pH and in the concentrations of glycolytic intermediates and the principal nucleotides were followed for about 6 hr after dilution. NAD was lost rapidly, its adenine moiety being converted to inosine nucleotide by the enzymes present. When the diluent did not contain inorganic phosphate (P_i) inclusion of NAD resulted in a somewhat faster loss of adenosine triphosphate (ATP), earlier cessation of glycolysis and higher ultimate pH. Inclusion of NAD in the preparations containing added P_i led to a reduction in both phosphorylase and phosphofructokinase activity. It also prevented the accumulation of fructose diphosphate. This was the result of lowering the phosphofructokinase activity rather than of increasing the glyceraldehyde-3-phosphate dehydrogenase activity. Adenosine triphosphatase (ATPase) activity, after an initial period during which it was unaffected by inclusion of NAD, was greater in the preparations containing added P_i and NAD than in the corresponding preparations without added NAD.

INTRODUCTION

IN THE PRECEDING paper (Newbold and Scopes, 1971) it was concluded that accumulation of fructose diphosphate in undiluted and diluted mince preparations was a consequence of the very rapid loss of NAD from these preparations. The present paper describes the effects of adding NAD to diluted mince preparations at the time of dilution.

MATERIALS & METHODS

MATERIALS and methods were as described previously (Newbold and Lee, 1965; Newbold and Scopes, 1971). All diluents contained 0.16M potassium chloride and, unless otherwise stated, 55 mM glycogen (expressed as glucose equivalent). Some contained, in addition, 50 mM or 100 mM potassium phosphate (pH 6.7). When NAD (Sigma, Grade III) was included it was at a concentration of 1 mg/ml. The pH of the diluent containing NAD but no other phosphate was adjusted to about 6.7 with 0.1N KOH.

Preparations from the same muscle, which differed only in that one of them contained added NAD and the other did not, are referred to as pairs. The diluted preparations are referred to as KCl, 50 mM P_i, and 100 mM P_i minces, with or without added NAD, according to the composition of the diluent.

Progressive changes in glycolytic intermediates and related compounds were measured in 3 pairs of KCl minces, 5 pairs of 50 mM P_i minces and 3 pairs of 100 mM P_i minces. Corresponding preparations made from different muscle minces always showed the same general pattern of change but not necessarily the same time-scale. The figures summarize the results obtained with those muscles from which the corresponding preparations had similar time-scales. The first samples were taken 5 min after dilution.

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RESULTS

IN EACH OF the different types of preparation (1) the sum of the ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), NAD, and inosine nucleotide concentrations, (2) the total amount of phosphorus present as P_i and in the other phosphates measured (assuming the inosine nucleotide to be IMP) and (3) the sum of the concentrations of glycogen, glucose, lactate and glycolytic intermediates (all expressed as glucose equivalents) remained virtually constant throughout the duration of the experiments.

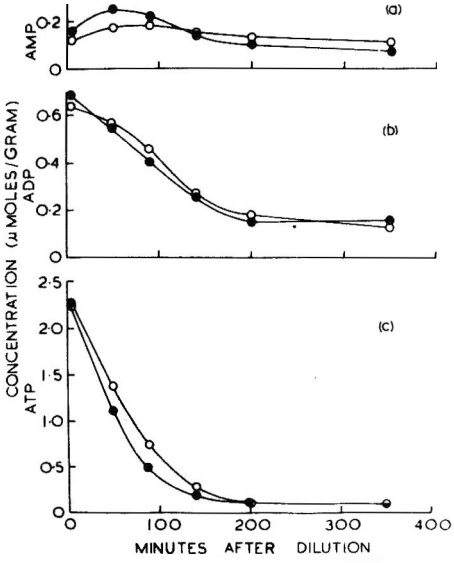

Fig. 1—Time-course of changes in the concentrations of (a) AMP, (b) ADP and (c) ATP in KCl minces without added NAD (○) and with added NAD (●). Points are mean values for corresponding preparations made from the same 3 muscles. Experimental details are given in the text.

Effect of adding NAD to KCl minces

Inclusion of NAD in the diluent had very little effect on the metabolism of KCl minces. In the preparations with added NAD, the pH fell at about the same rate as in those without added NAD but stopped falling sooner, with the result that the ultimate pH was 0.05 ± 0.01 (S.E.M.) unit higher (mean of 9 comparisons). Accumulation of lactate was correspondingly less in the preparations with added NAD.

Inclusion of NAD always resulted in faster loss of ATP but had little effect on the ADP or AMP concentration (Fig. 1). In the preparations with added NAD, the concentration of NAD, initially about eight times that in the preparations without added NAD, fell rapidly and at 250 min was about the same as the initial concentration in the preparations without added NAD (Fig. 2). The inosine nucleotide concentration increased at a faster rate and reached a higher final level in the preparations with added NAD (Fig. 3). Some of the extra amount of inosine nucleotide formed in a given time was due to the somewhat faster fall in the total concentration of ATP + ADP + AMP, and the remainder coincided closely with the amount of NAD broken down in that time. Hence it is clear that one of the products of NAD breakdown was

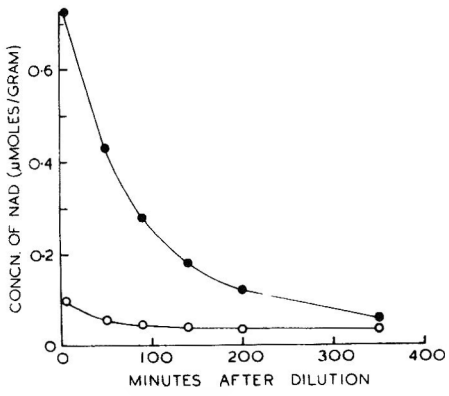

Fig. 2—Time-course of changes in the concentration of NAD in KCl minces without added NAD (○) and with added NAD (●). Points are mean values for the same preparations as in Fig. 1. Experimental details are given in the text.

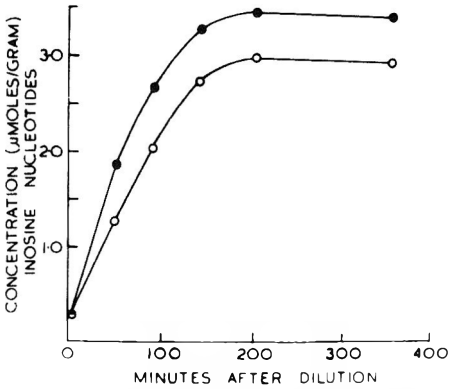

Fig. 3—Time-course of changes in the concentration of inosine nucleotides in KCl minces without added NAD (○) and with added NAD (●). Points are mean values for the same preparations as in Fig. 1. Experimental details are given in the text.

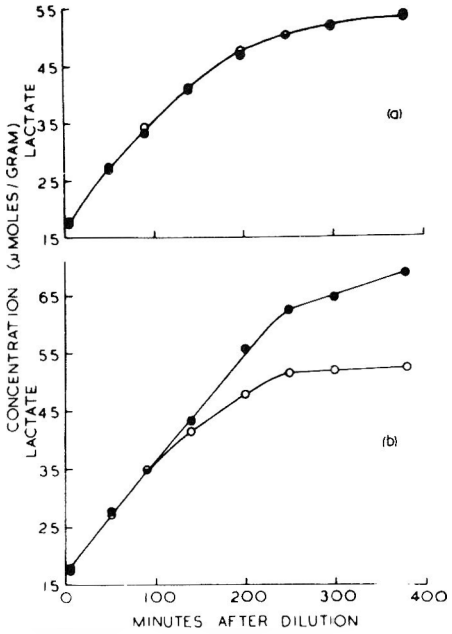

Fig. 4—Time-course of changes in the concentration of lactate in (a) 50 mM P_i minces and (b) 100 mM P_i minces prepared with (●) and without (○) addition of NAD. Points in (a) are mean values for preparations from the same 3 muscles and in (b) for preparations from the same 2 muscles. Experimental details are given in the text.

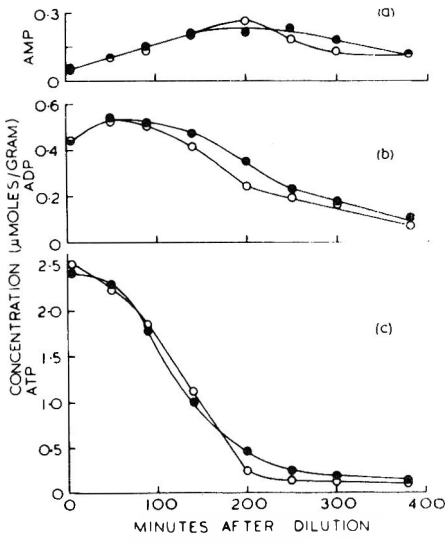

Fig. 5—Time-course of changes in the concentrations of (a) AMP, (b) ADP and (c) ATP in 50 mM P_i minces prepared with (●) and without (○) addition of NAD. Points are mean values for the same preparations as in Fig. 4a. Experimental details are given in the text.

readily converted to inosine nucleotide.

Changes in hexose monophosphate, glucose, α-glycerophosphate, P_i, and creatine phosphate concentrations were virtually unaffected by addition of NAD at the time of dilution. Fructose diphosphate did not accumulate in any of the preparations with added NAD but a small amount did accumulate temporarily in one of the preparations without added NAD.

Effect of adding NAD to P_i minces

pH. When the diluent contained 50 mM P_i, inclusion of NAD did not affect the rate of pH fall. The ultimate pH, although not affected on the average, was less variable in the preparations with added NAD, being 5.58 ± 0.01 (S.E.M.) in these and 5.56 ± 0.04 (S.E.M.) in the corresponding preparations without added NAD (10 comparisons).

With 100 mM P_i and 83 mM glycogen (expressed as glucose equivalent) in the diluent, inclusion of NAD had no effect on the rate of pH fall or the ultimate pH (5.43) in one experiment. In the other two experiments, the diluted preparations metabolized more rapidly and the rate of pH fall, though not affected over the first 100 min or so, became slower in the preparations without added NAD. The ultimate pH values of these two pairs were, with added NAD, 5.53 and 5.36, and without added NAD, both close to 5.7. The result obtained for these two pairs are summarised in the appropriate Figures.

Lactate. At both P_i concentrations, the effect of adding NAD on the rate and extent of lactate production (Fig. 4) corresponded to its above-noted effect on the pH changes.

Nucleotides. Inclusion of NAD in the 50 mM P_i minces had little effect on the changes in ATP, ADP and AMP (Fig. 5), other than to slow the loss of ATP after about 150 min and delay the loss of ADP a little. It did, however, delay the changes in all three of these compounds in the 100 mM P_i minces (Fig. 6). The rate of loss of NAD was much the same in the P_i minces with added NAD as in the corresponding KCl minces.

Glycolytic intermediates. At both P_i concentrations, hexose monophosphate was maintained at a higher concentration in the preparations with added NAD (Fig. 7a and 8a). In marked contrast to its accumulation in 50 mM P_i minces without added NAD, fructose diphosphate never amounted to more than about 0.05 μmoles/g in the corresponding preparations with added NAD (Fig. 7b). Similarly, inclusion of NAD in the diluent prevented the very large accumulation of fructose diphosphate in 100 mM P_i minces, although some accumulation did occur at a late stage (Fig. 8b) when the NAD concentration had been greatly reduced. The concentrations of 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate and pyruvate were always very small in the P_i minces with added NAD. Changes in α-glycerophosphate concentration were not significantly affected by inclusion of NAD in the diluent.

Other compounds. At both P_i concentrations, inclusion of NAD in the diluent had no clear-cut effect on the loss of creatine phosphate, and influenced the changes in P_i concentration in accordance with its above-noted effects on the changes in organic phosphates, the total amount of phosphorus in the P_i and other phosphates measured being, as noted earlier, essentially constant at all times in each type of preparation.

Effect of adding NAD on enzyme activities

In all the diluted preparations, whether they contained added NAD or not, concentrations of the glycolytic intermediates between triose phosphate and lactate were always very small. Hence the lactate production curves reflect the accumulative activities of glyceraldehyde-3-phosphate dehydrogenase and the subsequent enzymes of glycolysis. Curves for accumulative phosphofructokinase (Fig. 9a, 10a), phosphorylase (Fig. 9b, 10b) and ATPase activities (Fig. 11) were derived as described previously (Newbold and Scopes, 1971).

For about the first 100 min the activity of each of the enzymes was essentially the same in the KCl minces with added NAD as in those without, but slowed down and stopped sooner in the preparations with added NAD.

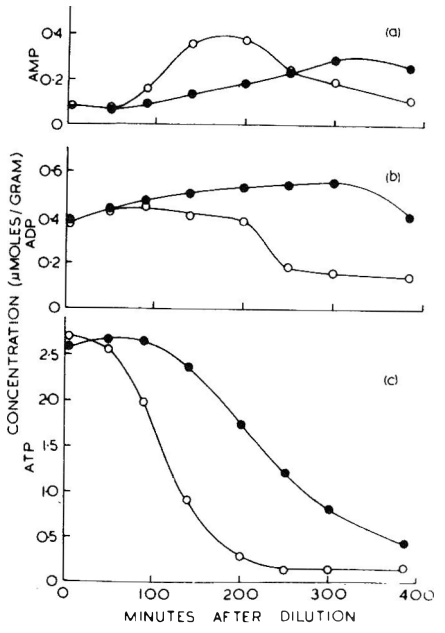

Fig. 6—Time-course of changes in the concentrations of (a) AMP, (b) ADP and (c) ATP in 100 mM P_i minces prepared with (●) and without (○) addition of NAD. Points are mean values for the same preparations as in Fig. 4b. Experimental details are given in the text.

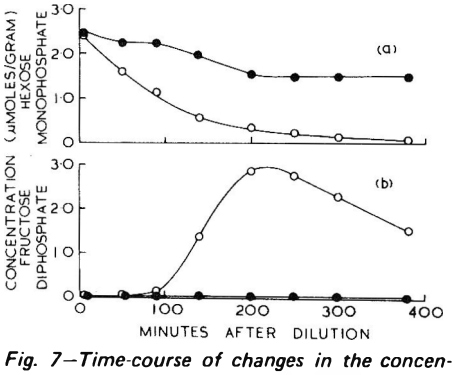

Fig. 7—Time-course of changes in the concentrations of (a) hexose monophosphate and (b) fructose diphosphate in 50 mM P_i minces prepared with (●) and without (○) addition of NAD. Points are mean values for the same preparations as in Fig. 4a. Experimental details are given in the text.

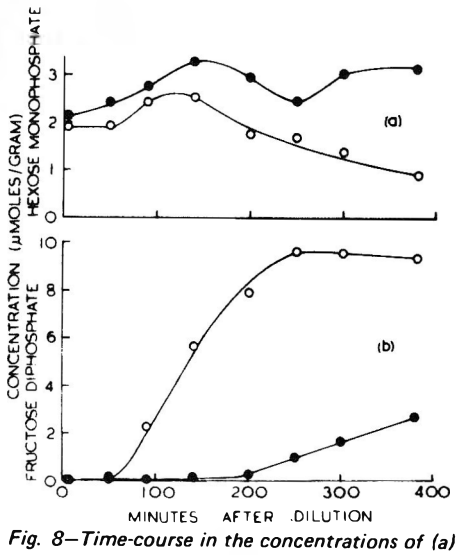

Fig. 8—Time-course in the concentrations of (a) hexose monophosphate and (b) fructose diphosphate in 100 mM P_i minces prepared with (●) and without (○) addition of NAD. Points are mean values for the same preparations as in Fig. 4b. Experimental details are given in the text.

Figures 9a and 10a show that, after an initial period during which added NAD did not affect the phosphofructokinase activity, the activity of this enzyme was greater in the P_i minces without added NAD than in the corresponding preparations with added NAD but persisted for longer in the latter.

Inclusion of NAD affected phosphorylase activity in the case of the 100 mM P_i minces in a similar manner to phosphofructokinase activity (Fig. 10b). However, although it affected the phosphofructokinase activity of the 50 mM P_i minces after about 90 min, it had no clear-cut effect on the phosphorylase activity until after about 140 min (Fig. 9b).

The ATPase activity was unaffected for about the first 90 min by inclusion of NAD in the P_i minces. For a period thereafter, however, it was slightly greater in the 50 mM P_i minces (Fig. 11a) and much greater in the 100 mM P_i minces with added NAD (Fig. 11b) than in the corresponding preparations without added NAD.

DISCUSSION

ADDITION OF NAD to the diluted mince preparations at the time of dilution had the expected result of preventing (or delaying in the case of the 100 mM P_i minces) the glyceraldehyde-3-phosphate dehydrogenase step from becoming rate limiting. It also had other effects which were not entirely anticipated.

Inclusion of NAD in the KCl minces always resulted in a somewhat faster loss of ATP, with consequent earlier cessation of glycolysis and a higher ultimate pH. As was pointed out in the previous paper (Newbold and Scopes, 1971) faster loss of ATP implies faster deamination of AMP and hence a higher concentration of available AMP. However, there was no increase in either phosphorylase or phosphofructokinase activity, apparently because stimulation by the extra AMP was counterbalanced by inhibition by the added NAD. We have recently found that NAD at these concentrations inhibits both phosphorylase *b* and phosphofructokinase, at least at pH values around 6.5 (Scopes, unpublished results).

Inclusion of NAD in the 50 mM P_i minces did not affect the glyceraldehyde-3-phosphate dehydrogenase activity even though it did prevent this enzyme from becoming rate limiting. Apparently in the preparations without added NAD the increase in glyceraldehyde-3-phosphate concentration (indicated by the increase in fructose diphosphate) was sufficient to compensate for the lack of NAD, with the result that the rate of ATP formation by glycolysis was unimpaired. With the 100 mM P_i minces, however, inclusion of NAD did lead to greater dehydrogenase activity and so to faster ATP formation by glycolysis after about 90 min.

During about the first 90 min for the 50 mM P_i minces and 50 min for the 100 mM P_i minces added NAD had little effect apart from maintaining hexose monophosphate at a somewhat higher concentration. Presumably inhibition of phosphofructokinase by the added NAD was counterbalanced by the higher hexose monophosphate concentration. Inclusion of NAD could be expected to reduce the phosphorylase *b* activity, since this enzyme is inhibited by both glucose-6-phosphate (Morgan and Parmeggiani, 1964) and NAD. The fact that the total phosphorylase activity was not reduced suggests that inclusion of NAD in the P_i minces resulted in the production of a small amount of phosphorylase *a*. In the rest of the discussion it is assumed that phosphorylase *a* activity was at no time less in the preparations with added NAD than in those without.

In the case of the 100 mM P_i minces the greater phosphorylase and phosphofructokinase activities, after about 50 min, in the preparations without NAD can be attributed largely to stimulation of phosphorylase *b* and phosphofructokinase by AMP, since the faster loss of ATP indicates a higher concentration of available AMP in these preparations. Even the measured AMP concentration was higher, but the significance of this is not clear. However, in the case of the 50 mM P_i minces inclusion of NAD had little effect on the rate of loss of ATP for the first 150 min. Thus the greater phosphofructokinase activity between about 90 and 150 min in the preparations without added NAD cannot be attributed to a higher concentration of available AMP. The

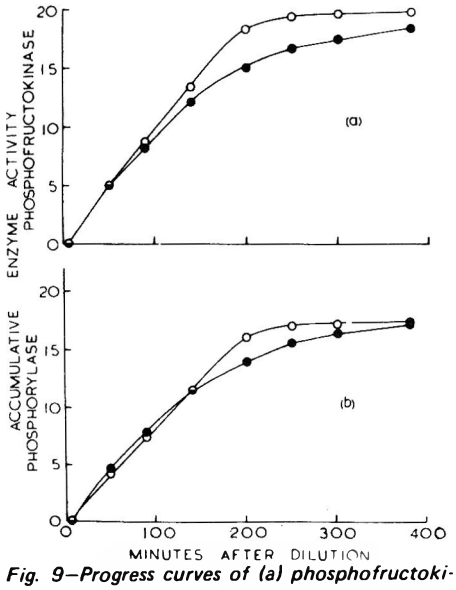

Fig. 9—Progress curves of (a) phosphofructokinase and (b) phosphorylase activity in 50 mM P_i minces prepared with (●) and without (○) addition of NAD. Accumulative phosphofructokinase and phosphorylase activities are expressed, respectively, as μmoles of substrate and μmoles of glucose equivalents transformed in 1g of diluted preparation or 0.5g of undiluted mince. Points are values derived as described in the text for the preparations described in Fig. 4a, 5 and 7.

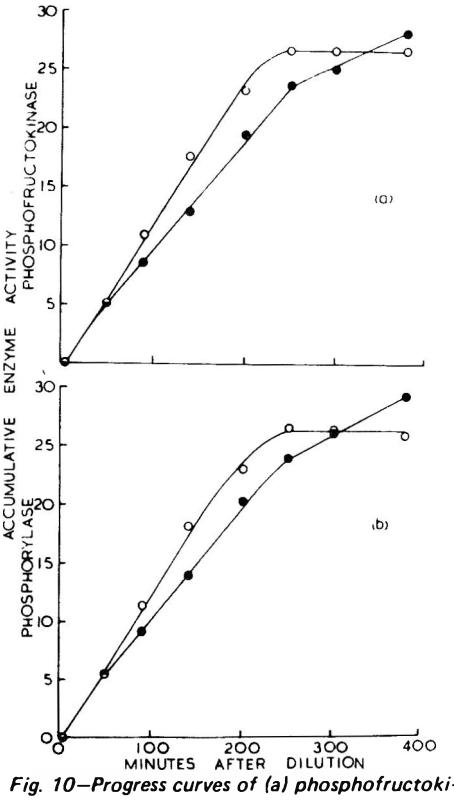

Fig. 10—Progress curves of (a) phosphofructokinase activity and (b) phosphorylase activity in 100 mM P_i minces prepared with (●) and without (○) addition of NAD. Accumulative phosphofructokinase and phosphorylase activities are expressed, respectively, as μmoles of substrate and μmoles of glucose equivalents transformed in 1g of diluted preparation or 0.5g of undiluted mince. Points are values derived as described in the text for the preparations described in Fig. 4b, 6 and 8.

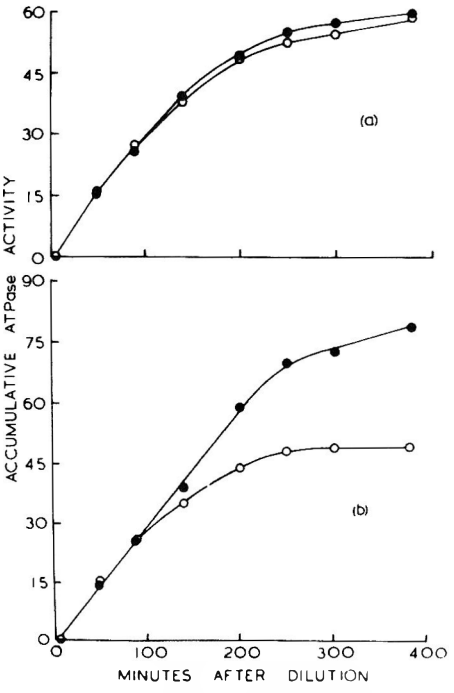

Fig. 11—Progress curves of ATPase activity in (a) 50 mM P_i minces and (b) 100 mM P_i minces prepared with (●) and without (○) addition of NAD. Accumulative ATPase activity is expressed as μmoles of ATP dephosphorylated in 1g of diluted preparation or 0.5g of undiluted mince. Points in (a) are values derived as described in the text for the preparations described in Figs. 4a, 5 and 7, and in (b) for the preparations described in Figs. 4b, 6 and 8.

possibility that the greater 'estimated' phosphofructokinase activity did not reflect greater 'actual' activity (i.e., faster phosphorylation of fructose-6-phosphate) is considered below.

The ATPase activity was initially unaffected by inclusion of NAD in the diluted preparations and, in the case of the 100 mM P_i minces but not of the 50 mM P_i minces, differences appearing after about 90 min can be attributed largely to differences in ATP concentration. During the period when fructose diphosphate was accumulating in the 50 mM P_i minces without added NAD, the ATPase activity was lower in these preparations than in the corresponding preparations with added NAD by about the same amount that the estimated phosphofructokinase activity was greater. Here, the observed effects could be explained in terms of differences in fructose-1,6-diphosphatase activity if the available AMP concentration, even when it was greatest, was less than the very small amount needed to completely inhibit this enzyme (Krebs and Woodford, 1965). Muscle fructose-1,6-diphosphatase is partially inhibited by concentrations of fructose-1,6-diphosphate such as were exceeded in the preparations without added NAD soon after the glyceraldehyde-3-phosphate dehydrogenase step became rate limiting (Krebs and Woodford, 1965). Furthermore, a decrease in fructose-1,6-diphosphatase activity without any change in 'actual' phosphofructokinase activity would result in an increase in estimated phosphofructokinase activity (Newbold and Scopes, 1967). It would also result in a corresponding decrease in the estimated ATPase activity, since less ATP would be broken down to ADP and P_i by the combined action of phosphofructokinase and fructose-1,6-diphosphatase.

The fact that both phosphofructokinase and phosphorylase kept acting for longer in the P_i minces with added NAD can be attributed to the slower exhaustion of available ATP and AMP in these preparations than in the corresponding preparations without added NAD.

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CARROT VOLATILES. 1. Characterization and Effects of Canning and Freeze Drying

SUMMARY—Volatiles in aqueous extracts of raw, canned, and freeze-dried carrots were isolated and concentrated by an on-column entrainment procedure. Twenty-three compounds were identified by GLC-MS in the raw carrot. Of these, diethyl ether, acetaldehyde, acetone, propanal, methanol, ethanol and β-phellandrene had not been previously reported in raw carrots. Several other compounds were tentatively identified. Acetaldehyde, sabinene, myrcene, and terpinolene were considered important character-impact compounds in raw carrot aroma; however, compound(s) responsible for carrot aroma remain elusive. Differences in volatile composition between canned, freeze-dried and raw carrots were found to be mainly quantitative rather than qualitative. Ethanethiol, dimethyl sulfide and dimethyl substituted styrene compounds formed with canning. Canning resulted in an approximate 50% loss of "higher boiling" compounds; however, it produced an increase in "lower boiling" compounds, particularly methanol, which increased from 0.05–60 ppm. Freeze drying resulted in an approximate 75% loss of total volatile content. Ethanethiol, dimethyl sulfide, acetaldehyde, octanal, 2-decenal and possibly dimethyl substituted styrene compounds are considered important in canned carrot flavor.

INTRODUCTION

WHILE THERE HAVE been extensive studies on the composition of carrot seed oil (reviewed by Seifert et al., 1968), there have been very few studies concerned with the volatiles present in the carrot root. In particular, the effects of processing on the volatile composition of carrots has been essentially ignored.

The earliest attempt at examining carrot volatiles was reported by Konig and Kracht (1929) who found sulfur compounds in cooked carrots. Buttery and Teranishi (1961) demonstrated that volatiles could be recovered from the headspace above cooked and frozen stored carrots, as well as other fruits and vegetables. In a survey of the low boiling compounds present in cooked foods, Self et al. (1963) reported the presence of methanal, methanethiol, propanal and/or acetone, ethanethiol, dimethyl sulfide, 2-methyl propanal and methanol in carrots that had been boiled for 30 min. Ayers et al. (1964) reported the formation of an off-odor, reminiscent of violets, in accelerated freeze-dried carrots stored in the presence of oxygen. Systematic analysis revealed the compounds mainly responsible for the off-odor to be α- and β-ionones and β-ionone-5,6-epoxide. Recently Buttery et al. (1968) reported the presence of α-pinene, camphene, β-pinene, myrcene, α-terpinene, p-cymene, limonene, γ-terpinene, terpinolene, caryophyllene, β-bisabolene, γ-bisabolene, heptanal, octanal, nonanal, 2-nonenal, terpinene-4-ol, α-terpineol, 2-decenal, bornyl acetate, 2,4-decadienal, biphenyl, dodecanal, myristicin and carotol in a carrot root oil prepared by steam distillation with a Likens extraction head. Odor thresholds of a number of these compounds present in the carrot root oil (the oil had an odor similar to cooked carrots) were measured in water solution and their total odor intensity contribution estimated.

Although aldehydes (2-nonenal in particular) appeared to make a substantial contribution to the total odor intensity of carrot root oil, key compounds responsible for raw or cooked carrot flavor remain elusive.

A knowledge of the effects of processing on a product and of what compounds cause a characteristic flavor in a product can permit the processor to make the best choice of processing conditions. In this study the identity and concentration of volatiles present in raw and processed carrots were investigated in an attempt to elucidate which volatiles are responsible for carrot flavor and to determine the effects of processing, specifically canning and freeze drying, on the volatile composition of carrots.

EXPERIMENTAL

Materials

Unless otherwise specified, the carrots (*Daucus carota* L.) used in this study were Imperator (Long Imperator Crookham) variety and were obtained from the OSU Horticulture Department during the 1969 growing season.

Following harvest, evenly sized carrots (approx 1-1/2-in. dia) were either immediately analyzed or processed. Canned carrots were prepared as follows: Carrots were washed, peeled with an abrasive peeler, and sliced (1/4 in. slices) with a Hobart unit. Slices plus an equal weight of water were placed in 303 plain body cans, steam exhausted to a center temperature of 175°F (2–2-1/2 min), sealed using C-enamel ends, and heated for 30 min at 240°F in a still retort. Canned carrots were stored at 24°C. Freeze-dried carrots were prepared as follows: Carrots were steam blanched (210°F × 3 min), peeled and diced with a Hobart unit to give 1/2 in. × 3/16 in. dices which were individually quick frozen at −30°C. Frozen dices were accelerated freeze dried in a Hull freeze drier at a vacuum of 100 microns and a temperature programmed from 230–130°F for 15 hr. The dried product was 10% of the fresh weight and was stored under nitrogen in tightly capped jars at −30°C.

Preparation of aqueous carrot extracts

Aqueous carrot extracts were prepared from raw, canned and freeze-dried carrots for on-column entrainment analysis of the volatile constituents. Raw carrot extracts (pH 6.5) were prepared from washed, peeled and diced (1/4 in. dices) carrots by blending for 30 sec in a Waring Blendor in the proportions of 200g carrots/200 ml distilled water. In an effort to obtain a representative sampling of carrot material, the 200g of carrot dices were randomly selected from approximately 700g of diced carrots. The homogenate was squeezed through four layers of cheesecloth to give a final extract volume of 250 ml. A 125-ml aliquot of the extract was immediately analyzed. Canned carrots sliced were drained, blotted and extracted in the same manner as the raw carrots except 200 ml of canned carrot liquor was used for the extraction instead of distilled water. This permitted a quantitative comparison on a fresh weight basis of the volatile content of canned and raw carrots. Freeze-dried carrots were also reconstituted so as to produce carrots equal on a fresh weight basis to the unprocessed vegetable. Freeze-dried carrots were reconstituted in a sealed flask (20 min at 50°C) in the proportions of 20g carrots/180g water. Reconstituted carrots were blended for 30 sec in the proportions of 200g carrot/200 ml water, and filtered and analyzed in the same manner as described for raw and canned carrots.

Analysis of aqueous carrot extracts

Volatiles present in 125 ml samples of aqueous carrot extracts were investigated using a rapid method (Heatherbell, et al., 1970) developed by modifying the gas-entrainment, on-column trapping technique described by Morgan and Day (1965).

For identification of compounds by combined gas-liquid chromatography-mass spectrometry (GLC-MS) water was removed from entrained volatiles using a 3-in. pre-column containing anhydrous K_2CO_3.

An Aerograph 1520 equipped with a hydrogen flame ionization detector and connected to a Speedomax H recorder (1 mv, 1 sec full scale response) was used for GLC analysis. Stainless steel columns, 10 ft × 1/8 in. O.D., packed with 7% Carbowax 20 M on 80/100 mesh Gas-Chromosorb Q, and 5% SF96-50 containing 5% Igepal CO-880 on 80/100 mesh AW-DMCS Chromosorb G were used for examining the "higher boiling" compounds. Entrainment procedure utilized a 15 min purge time (nitrogen flow = 15 ml/min) and a bath temperature of 68°C. GLC operating conditions used for the

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"higher boiling" compounds were:

Injector temperature:	180°C
Detector temperature:	225°C
Column temperature:	75° for 17 min, then 4°/min to 195°C and held
Flow rate:	25 ml/min, N_2

"Lower boiling" volatiles were examined using a TRIS column (20% 1,2,3-tris [2 cyano ethoxy]–propane on 60/80 mesh Celite 545, 12 ft × 1/8 in. O.D.); GLC conditions were the same as those used for "higher boiling" compounds except the column was operated isothermally at 37°C. Entrainment procedure was the same as for "higher boiling" volatiles except a 10-min purge time was used and a 50° bath. Various effluent splitters ranging from 3:1 (air:detector) to 19:1 were used for evaluating GLC effluent odors. GLC retention time data and comparison of mass spectra with standard spectra were used for identification of compounds. An Atlas CH-4 Nier-type mass spectrometer coupled with an F & M 810 gas chromatograph was used for GLC-MS analyses.

The concentration of individual compounds in raw and processed carrots was estimated from standard curves of peak area (measured by triangulation) vs concentration (in ppm) of authentic compound. Known concentrations of authentic compounds–dimethyl sulfide, acetaldehyde, propanal, acetone, methanol, ethanol, α-pinene, βpinene, camphene, sabinene, myrcene, α-phellandrene, limonene, γ-terpinene, p-cymene, terpinolene, octanal, 2-decenal, bornylacetate, terpinene-4-ol, β-bisabolene, γ-bisabolene, caryophyllene, and carotol–were added to reconstituted, freeze-dried, steam-stripped carrot medium which was free from volatiles. (The carrot medium was prepared by blanching, and homogenizing carrots and steam distilling the homogenate under reduced pres-

Table 1–GLC-MS identification of compounds present in raw carrots.

Peak no.[a]	Compound	tr/tr Carbowax 20M	tr/tr SF 96–50	tr/tr TRIS	M.S. Identification	M.S. Reference	Aroma (similar to authentic standards)
	Diethyl ether			0.14[d]			
1	Acetaldehyde	0.19[b]	0.14[b]	0.37[d]	+	Cornu & Massot	+
2	Acetone	0.25[b]	0.19[b]	1.00[d]	+	(1966)	
	Propanal			0.67[d]	+	ibid	
3	Methanol	0.30[b]		1.00[d]	+	ibid	
4	Ethanol	0.35[b]	0.26[b]	1.18[d]	+	ibid	
5	α-Pinene	0.46[b]	0.70[b]	0.75[d]	+	Ryhage & von Sydow	
6	Camphene	0.57[b]	0.77[b]		+	(1963)	
7	β-Pinene	0.69[b]	0.84[b]		+	ibid	+
8	Sabinene	0.76[b]	0.84[b]		+	ibid	+
9A	Myrcene	0.81[b]	0.89[b]		+	ibid	+
9B	α-Phellandrene	0.88[b]	0.93[b]		+	ibid	
10	Limonene	1.00[b]	1.00[b]		+	ibid	+
11A	γ-Terpinene	1.21[b]	1.10		+	ibid	+
11B	p-Cymene	1.38[b]	1.18[b]		+	ibid	+
12	Terpinolene	1.56[b]	1.20[b]		+	ASTM (1969)	+
13	Octanal	1.70[b]	1.17[b]		+	ibid	+
14	Unknown	0.70[c]					
15	Unknown	0.75[c]					
16	Unknown	0.77[c]					
17	Unknown	0.83[c]					
18	2-Decenal	0.85[c]			Tentative	ASTM (1969)	
19	Unknown	0.88[c]					
20	Unknown	0.93[c]					
21	Bornyl acetate	0.96[c]			+	ibid	+
22	Caryophyllene	1.00[c]	1.00[c]		+	ibid	+
23	Terpinene-4-ol	1.11[c]	0.82[c]		+	ibid	
	Sesquiterpene				+	ibid	
24	β-Bisabolene	1.14[c]	1.14[c]		+	Buttery et al. (1968)[e]	+
25	γ-Bisabolene	1.17[c]	1.18[c]		+	ibid	+
26	Unknown	1.20[c]					
27	Sesquiterpene	1.24[c]			+	ASTM (1969)	
28	Sesquiterpene	1.27[c]			+	ibid	
29	Aromatic M.W. 134	1.30[c]			+	ibid	
30	Sesquiterpene	1.35[c]			+	ibid	
31	Carotol	1.42[c]			+	Buttery et al. (1968)[e]	
32	Unknown						
33	Myristicin	1.74[c]					+

[a]Carbowax 20M; refer to Figure 1.
[b]tr/tr relative to limonene.
[c]tr/tr relative to caryophyllene.
[d]tr/tr relative to acetone.
[e]Authentic compounds.

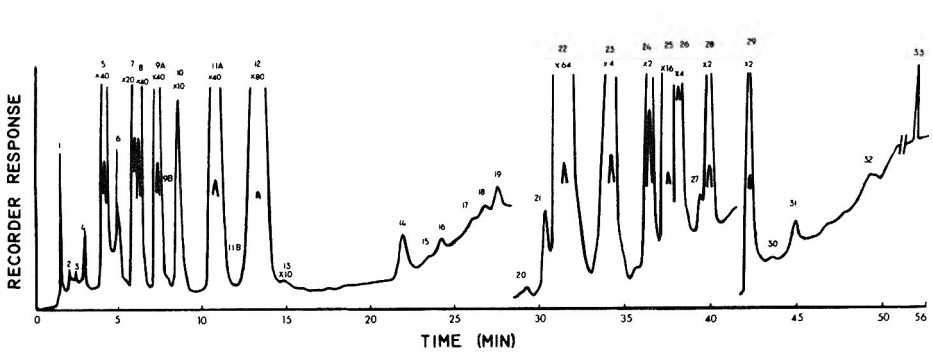

Fig. 1–On-column entrainment analysis of the volatiles present in an aqueous extract of raw carrots, using a Carbowax 20 M column.

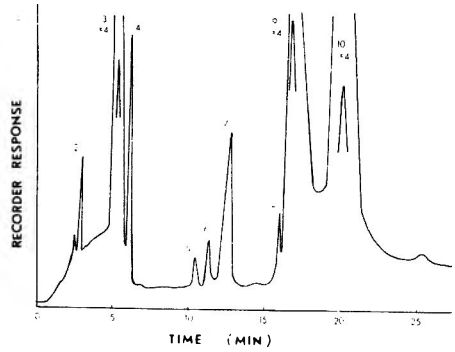

Fig. 2–On-column entrainment analysis of volatiles present in an aqueous extract of canned carrots, using a TRIS column.

sure for 8 hr at 0.5 mm pressure; the deodorized homogenate was freeze dried and stored under nitrogen.) On-column entrainment analyses were run on extracts containing various concentrations of authentic compounds; recovery of compounds from aqueous extracts was ± 7% or better. Concentration vs peak area gave linear plots in the concentration range investigated. Methyl acetate and n-heptanol at a concentration of 0.2 ppm were used as internal standards when analyzing the "lower boiling" and "higher boiling" compounds respectively. This permitted correction for possible variations in recovery.

Total essential oil content was estimated by summation of the concentration of the identified "higher boiling" compounds. This represented approximately 95% of the total "higher boiling" compounds.

RESULTS & DISCUSSION

Identification of carrot volatiles

A chromatogram of the entrained volatiles from an aqueous extract of raw carrots is shown in Figure 1. Table 1 lists the identification (mass-spectral and GLC relative retention times) of the compounds found in raw carrot. Tentative mass spectral identifications are given where spectra were either very weak or were a mixture of more than one compound so that a positive match with reference spectra was not possible.

The only previous report on the characterization of volatile constituents in the raw carrot (Buttery et al., 1968) failed to reveal the "lower boiling" compounds, diethyl ether, acetaldehyde, propanal, acetone, methanol and ethanol identified in this study. These authors used conventional methods of steam distillation and solvent extraction and "lower boiling" compounds could have been lost due to elevated temperatures over long periods or during the necessary evaporation and concentration of solvent steps. With the exception of the presence of α-phellandrene, the monoterpenes and sesquiterpenes identified confirm the findings of Buttery et al. (1968). In contrast to their reporting several long-chain aldehydes (octanal, nonanal, 2-nonenal, 2-decenal, 2,4-decadienal and dodecanal) only octanal and 2-decenal were found in this study and in very small amounts. The milder, inert gas entrainment procedure may have minimized formation of these aldehydes which can be formed from C_{18} unsaturated fatty acids by auto-oxidation (Keeney, 1962) and by heating (Buttery et al., 1968). However, trace amounts of longer chain aldehydes could have been present and remained undetected as the analysis system used did have the disadvantage of incomplete separation of some oxygenated compounds and sesquiterpenes (Fig. 1, peaks 20 and after).

A chromatogram of the "lower boiling" compounds present in an aqueous extract of canned carrots is shown in Figure 2. Table 2 lists the identification of these compounds. The presence of acetaldehyde, ethanethiol, dimethyl sulfide and methanol confirm the identification of these compounds in cooked carrots reported by Self et al. (1963). The presence of ethanol had not been previously reported in cooked carrots. The presence of propanal and/or acetone which was not resolved by these authors was resolved in this study on a TRIS column. In contrast the TRIS column did not separate methanol and acetone; these compounds were resolved on the Carbowax 20 M column.

The qualitative composition of the "higher boiling" compounds in canned carrot were essentially the same as that of the raw. Appearing in substantial quantity were three compounds having retention times coincident with peaks 14, 15, and 16 (Fig. 1) which had not been identified in raw carrot as they were present in insufficient quantity. On the basis of their mass spectra all three compounds have a M.W. of 132 and appear to be very closely related dimethyl substituted styrene isomers (ASTM, 1969). Peak 16 had the following mass spectrum: m/e 132 (molecular ion, 100%), 117 (85%), 91 (68%), 115 (36%), 92 (28%), 131 (25%), 116 (20%), 118 (13%) which agreed closely to the mass spectrum of α,p-dimethyl styrene run on the same instrument. As the relative retention time of α,p-dimethyl styrene also coincided with that of peak 16, this compound is tentatively identified as α,p-dimethyl styrene. Peak 15, based on

Table 2–GLC-MS identification of lower boiling compounds present in raw and canned carrots; Chromatogram is shown in Figure 2.

Peak no.	Compound	tr/tr acetone	M.S. identification	M.S. reference	GLC confirmation	Aroma
1	Diethyl ether	0.14	+	Cornu & Massot (1966)	+	
2	Ethane thiol[a]	0.16	+	ibid	+	+
3	Dimethyl sulfide[a]	0.30	+	ibid	+	+
4	Acetaldehyde	0.37	+	ibid	+	+
5	Unknown	0.60				
6	Propanal	0.67	+	ibid	+	
7	α-Pinene	0.75	+	ibid	+	+
8	Unknown	0.93				
9	Acetone	1.00	+	ibid	+	
	Methanol		+	ibid	+	
10	Ethanol	1.18	+	ibid	+	

[a]Compound only present in canned carrots.

Table 3—Intravarietal variation of the "lower boiling" raw carrot volatile constituents (Imperator variety).

Compound[a]	Batch I	Batch II	Batch III
Ether	Tr.[b]	Tr.	Tr.
Acetaldehyde	0.75	0.60	0.45
Acetone	0.20	0.20	0.31
Propanal	Tr.	Tr.	Tr.
Methanol	0.04	0.03	Tr.
Ethanol	80.00	65.00	5.00

[a]Concentration in ppm.
[b]Trace.

the close agreement of relative retention time and mass spectrum [m/e 117 (100%), 132 (molecular ion, 87%), 115 (40%), 91 (40%), 131 (25%), 116 (24%), 133 (15%), 92 (8%)] is tentatively identified as 2,6-dimethyl styrene. Peak 14 appears to be an aryl α-dimethyl styrene compound on the basis of its mass spectra (ASTM, 1969). The α,p-dimethyl styrene has also been reported in commercial distillates of black currants (Nursten and Williams, 1969). The volatiles present in freeze-dried carrots were the same as those reported for raw carrots.

Odor description of carrot volatiles

The raw carrot aroma apparently has a complex profile; from observations of the authors it contains predominantly a "strong" (green, earthy), "carrot tops" note and varying degrees of "soft," "sweet," "pumpkin-like," notes and "perfumy" notes. Sensory description of column effluents (using effluent splitters) indicates that several chromatographic regions (compounds) contribute to raw carrot aroma. Acetaldehyde appears to lend a "soft," sweet" note. It has been shown to be important in several foods (Amerine, 1964) and has a threshold in water of less than 1 ppm (Sheldon, 1968). Sabinene and particularly myrcene contributed "green," "earthy," and "carrot top" notes. Terpinolene and to a lesser extent, caryophyllene were "perfumy." All of these are present in concentrations far in excess of their reported thresholds in aqueous solution (Buttery et al., 1968). A soft, squash-like (cucumber) aroma was detected in the vicinity of caryophyllene on Carbowax 20 M. The retention time on Carbowax 20 M corresponded to that of the potent odorant 2,6-nonadienal (odor of cucumbers, Forss et al., 1962). The retention time of the aroma and of 2,6-nonadienal and caryophyllene were not coincident. However, there was an insufficient quantity of the compound present to permit further characterization of the compound. Buttery et al. (1968) also found tentative evidence for the presence of this compound. No single compound was found that could be claimed solely responsible for the characteristic raw carrot aroma, and it is likely that carrot flavor is an expression of a combination of compounds.

A potent odorant present in concentrations beyond the limits of detection of the method or a transient compound unstable under analysis conditions are other possibilities which have been suggested from studies on the enzymatic regeneration of carrot aroma (Heatherbell et al., 1970).

Quantitative analysis of carrot volatiles

The concentration of compounds present in aqueous raw carrot extracts were estimated from standard curves of peak area vs concentration and are given in Tables 3 and 4 (results are reported in ppm and expressed on a fresh weight basis). The three batches are of equal size carrots of the same variety (Long Imperator Crookham) at the same time (20 wk from planting date). The values obtained from a single analysis of each batch are listed. Replicate analyses of a given batch varied ± 10%, whereas the concentration of "lower boiling" compounds, apart from ethanol, are fairly reproducible, there is considerable variation in the concentration of the "higher boiling" compounds. The total essential oil concentration only varied from 24–28 ppm; however, the concentration of individual compounds could vary by as much as a factor of two or three in some instances (for example α-pinene, sabinene, limonene). As the analytical method had proven reproducible to within ± 7% in model system studies using carrot seed oil (Heatherbell et al., 1970) and as the different batches being analyzed were subjected to conditions identical to the model system studies, this variation is interpreted as representing inherent variation of the volatiles within the source material. Undoubtedly enzymatic activity is occuring during aqueous extraction. However, the belief that the observed intravarietal variation represents different levels of metabolism within the carrot source material is supported by the finding of similar variations in aqueous samples prepared from different cans of carrots where the enzymes have been inactivated. This interpretation is not unreasonable in the light of recent evidence suggesting that terpenes are subject to rapid metabolic turnover in plant tissues (Burbott and Loomis, 1969). These authors also reported variations as great as two-fold in the monoterpene essential oil content of simultaneously harvested samples of equal-sized peppermint leaves. Sugar, pectin and carotenoid concentrations in the carrot have also been reported subject to considerable intravarietal variation (Carlton and Peterson, 1963; Sistrunk et al., 1967; Bradley et al., 1967). In all subsequent quantitative studies the concentration of individual compounds are reported as one figure representing an average of not less than two independent determinations, variation in replicate analysis being ± 10% Owing to the inherent batch-to-batch variation in volatile content great care must be exercised in interpreting these results; only broad trends are considered meaningful.

Effect of processing

Tables 5 and 6 illustrate effects of canning and freeze drying on the volatile

Table 4—Intravarietal variation of the "higher boiling" carrot volatile constituents (Imperator variety).

Compound[a]	Batch I	Batch II	Batch III
α-Pinene	0.79	1.40	1.35
Camphene	0.14	0.12	0.12
β-Pinene	0.36	0.14	0.21
Sabinene	5.24	2.60	2.65
Myrcene	4.65	4.45	4.40
α-Phellandrene	Tr.[b]	Tr.	Tr.
Limonene	0.66	0.72	1.62
γ-Terpinene	0.23	0.22	0.42
p-Cymene	0.37	0.25	0.50
Terpinolene	5.52	3.90	4.15
Octanal	Tr.	0.02	0.02
2-Decenal	0.02	0.01	Tr.
Bornylacetate	0.11	0.08	0.20
Caryophyllene	5.00	4.30	6.80
Terpinene-4-ol	–	–	–
β-Bisabolene	0.34	0.44	0.25
γ-Bisabolene	4.85	4.45	2.40
Carotol	0.20	0.16	0.20
Total	28.00	24.00	25.00

[a]Concentration in ppm.
[b]Trace.

composition of carrots. Canning has had the greatest effect on the "lower boiling" compounds resulting in an increase in concentration of seven of the eight headspace components identified. Ethanol was the only compound whose concentration was relatively unaffected by the heat processing. Increases in acetaldehyde, propanal, and acetone were not large whereas the increase in methanol from 0.05 ppm to in the order of 60 ppm was striking. The heat induction of a thiol (ethanethiol) and a sulfide (dimethyl sulfide) is of particular significance. These compounds were not found in the raw carrot. (Traces were formed by the entrainment analysis method when a water bath temperature of 50°C was used; however, when the analysis was run at 25°C these compounds were not found to be present.) The Strecker degradation (Hodge, 1967) and oxidation of carotenoids (Swain et al., 1964) can result in formation of acetaldehyde. Formation of propanal could arise from lipid auto-oxidation (Day, 1965) or from non-enzymatic browning (Hodge, 1967). Acetone is also a known end product of non-enzymatic browning (Hodge, 1967). Formation of large amounts of methanol could arise from the hydrolysis of the methoxyl ester linkages in pectins. The finding of dimethyl sulfide correlates well with the reports of its formation in canned tomatoes (Nelson and Hoff, 1969), canned corn (Bills and Keenan, 1968) and in roasted filberts (Sheldon, 1969). Bills and Keenan (1968) confirmed the earlier report of Wong and Carson (1966) that dimethyl sulfide formation can result from the thermal degradation of S-Methyl methionine sulfonium salt. The flavor threshold of dimethyl sulfide is only a few ppb (Bills and Keenan, 1968) and concentrations in the range of greater than 1 ppm as reported in this study are very significant. Ethanethiol has one of the lowest recorded thresholds, less than 0.1 part per billion (Amerine et al. 1965), and must be important in any detectable amount. Acetone with an odor threshold of approximately 500 ppm (Wick, 1966) is unlikely to be important. While the threshold level of methanol is extremely high (the authors observations indicate methanol is not perceived by most people at concentrations of 1000 ppm) the large increase of this compound in canning could affect flavor by additive interactions as mixtures of sub-threshold levels of compounds have been found to initiate olfactory response (Guadagni et al., 1963).

Table 5—Effects of processing on the "lower boiling" carrot volatiles.

Compound[a]	Raw carrot	Canned carrot	Freeze-dried carrot
Ethane thiol	0.00	0.05	0.00
Dimethyl sulfide	0.00	1.55	Tr.[b]
Acetaldehyde	0.60	1.42	1.24
Propanal	Tr.	0.14	Tr.
Acetone	0.08	0.33	Tr.
Methanol	0.05	60.00	Tr.
Ethanol	50.00	45.00	7.36

[a]Concentration in ppm.
[b]Trace.

Table 6—Effects of processing on the "higher boiling" carrot volatiles.

Compound[a]	Raw carrot	Canned carrot	Freeze-dried carrot
α-Pinene	0.09	0.10	0.04
Camphene	0.04	0.02	Tr.[b]
β-Pinene	0.05	0.05	Tr.
Sabinene	0.10	0.07	0.03
Myrcene	0.35	0.21	0.04
α-Phellandrene	Tr.	Tr.	Tr.
Limonene	0.46	0.22	0.06
γ-Terpinene	Tr.	Tr.	Tr.
p-Cymene	Tr.	0.45	Tr.
Terpinolene	6.05	2.50	0.46
Octanal	0.02	0.06	Tr.
2-decenal	0.00	Tr.	0.00
α,p-Dimethyl styrene	0.00	(+)[c]	0.00
Bornyl acetate	0.22	0.08	Tr.
Caryophyllene	10.00	5.00	1.95
Terpinene-4-ol	–	–	–
β-Bisabolene	1.18	0.87	0.60
γ-Bisabolene	5.55	3.50	2.15
Carotol	0.22	0.19	0.20
Myristicin	0.30	0.35	0.25
Total	26.00	14.00	6.00

[a]Concentration in ppm.
[b]Trace.
[c](+) Increase in concentration.

Examination of the influence of canning on the "higher boiling" compounds in carrots indicates an approximate 50% reduction in total volatile content. This appears to result from a fairly uniform loss of the lower boiling monoterpenes and of the sesquiterpene caryophyllene. The concentration of the higher boiling sesquiterpenes γ- and β-bisabolene and carotol did not change significantly with canning. The loss of volatiles may occur in the exhausting step prior to retorting.

Compounds increasing with canning were p-cymene, octanal, 2-decenal and α,p-dimethyl styrene (and to a lesser extent the other two tentatively identified dimethyl substituted styrene compounds). Although the increases in octanal and 2-decenal were not large, these are potent compounds with very low thresholds of 0.7 and 0.3 parts per billion respectively (Buttery et al., 1968) and may be important in the cooked carrot aroma.

It is of interest to note the similarity in structure between p-cymene which increased on canning and α,p-dimethyl styrene (and the other dimethyl substituted styrene compounds) which was also formed on canning. The loss of two hydrogens from the p-cymene isopropyl group to form an isopropenyl group would convert p-cymene to p-methyl isopropenyl benzene (α,p-dimethyl styrene). As Wrolstad and Jennings (1965) found p-cymene can be formed from monoterpene hydrocarbons by isomerization and oxidation, it is possible this group of aromatic compounds may be formed by this mechanism during heat processing. They may also arise from dehydration of alcohols or degradation of carotenoids. Another source to be considered is the C-enamel coating on the can ends. It is felt that α,p-dimethyl styrene and related compounds contributed to stronger notes in canned carrot aromas.

The peak identified as α,p-dimethyl styrene had a strong, "weedy" (green) aromatic aroma. Whereas considerable amounts appeared in Imperator and Nantes varieties, only small amounts were present in the Chantenay and Oregon 4362.

Three other carrot varieties, Nantes, Chantenay and Oregon 4362 were also canned and the volatiles analyzed. In each instance apart from the previously discussed variation in α,p-dimethyl styrene the same trend in results as observed for the Imperator variety were obtained. These canned varieties were analyzed again after 3 months' storage at room temperature (approximately 25°C). There was no significant change in the results due to storage.

From these studies, ethanethiol, dimethyl sulfide, acetaldehyde, aldehydes such as octanal, 2-decenal and possibly dimethyl substituted styrene compounds are considered important contributors to canned carrot flavor.

The freeze-dried carrots (Imperator variety) although retaining a characteristic texture and taste only retained a moderate to weak raw carrot flavor (aroma). Examination of Tables 5 and 6 indicates freeze drying has resulted in a large loss of volatiles, in the order of a 75% reduction in the essential oil content.

Apart from the appearance of a trace amount of dimethyl sulfide there appears to be no characteristic changes induced by freeze drying in the "lower boiling" compounds. The small amount of dimethyl sulfide may arise during reconstitution or from "dry cooking" of the carrots. (An increase in low boiling compounds was noticed in stored, freeze-dried material that had been exposed to the air, particularly in acetaldehyde and acetone. This agrees with the findings of Swain, et al. (1964) who hypothesized the compounds arise from carotene oxidation).

Examination of the "higher boiling" compounds indicates there is a lesser loss of the highest boiling compounds such as the bisabolenes, carotol and myristicin. The concentration of most compounds have been reduced to the level of their estimated thresholds. (The following threshold values, in ppm, were determined in water by Buttery et al., 1968: sabinene, 0.075; myrcene, 0.013; terpinolene, 0.2; bornyl acetate, 0.07; carotol, 0.008.) The reduction of most volatiles, particularly those such as sabinene, myrcene, terpinolene, and carotol which are considered important in carrot flavor, to the vicinity of or below threshold concentration probably contributes to the substantial loss of the characteristic raw carrot flavor (aroma) in the freeze-dried carrot.

The large loss in volatiles due to freeze drying is not surprising when one considers the high vacuum (approximately 100 microns) used in the freeze-drying process. Most likely, freeze-drying conditions could be found which would reduce this loss of volatiles. However, the results do serve to illustrate the extent to which volatiles may be lost in freeze drying.

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CARROT VOLATILES. 2. Influence of Variety, Maturity and Storage

SUMMARY—The volatile composition of carrot varieties Imperator (Long Imperator Crookham), Nantes, Royal Chantenay, Autumn King, Oregon 4362, and Wisconsin 5 were analyzed by GLC on-column entrainment. Differences were quantitative rather than qualitative. Variation in concentration of individual terpenes and their summation (5–27 ppm) appeared to be consistent with descriptions of the flavor characteristics of different varieties. Studies on effects of maturity revealed that of the "low boiling" constituents, acetaldehyde and particularly ethanol increased in late season whereas acetone, propanal, and methanol remained relatively constant. Regarding "higher boiling" constituents, their total concentration remained relatively constant while the concentration of individual terpenes constantly changed. Mature carrots stored for 5 wk accumulated large quantities of acetaldehyde and ethanol. The concentration of other volatiles did not change significantly.

INTRODUCTION

AN EARLIER PAPER (Heatherbell et al., 1971) reported the characterization of carrot root volatiles and the effects of canning and freeze drying on their volatile composition. This paper reports the influence of variety, maturity and storage on the volatile composition of carrots.

An understanding of how the volatile composition of fruits and vegetables can be affected by variables such as variety, maturity and storage is of value to both the plant geneticist and the food processor. Varietal differences can be exploited by the plant geneticist who, by systematic breeding, can develop varieties with more desirable flavors for both the fresh market and for processing. The processor can apply this information in establishing harvest dates and storage conditions, and in selecting varieties and optimum processing parameters.

The influence of maturity, variety and storage on the flavor chemistry of vegetables has been neglected by horticulturalists and flavor chemists until recently. Johnson et al. (1968) reported variations of several ppm with variety and harvest in the concentrations of isoamyl alcohol, pentanol and 3-hexen-1-ol in tomatoes. Nelson and Hoff (1969) also reported varietal variations in the concentration of acetaldehyde, acetone, methanol, ethanol, isovaleraldehyde and hexanal in tomatoes. Stevens et al. (1967) not only found quantitative differences in oct-1-en-3-ol and linalool in snap bean varieties but also demonstrated the differences were inheritable. Stevens (1970) has also shown that quantitative differences in the concentration of 2-isobutylthiazole, methyl salicylate and eugenol between tomato varieties is inheritable.

EXPERIMENTAL

CARROT VARIETIES and breeding lines used in this study were obtained from the OSU Horticulture Department during the 1969 growing season, and included Imperator (Long Imperator Crookham), Nantes, Royal Chantenay, Autumn King, Oregon 4362 and Wisconsin 5. To examine the influence of maturity, Wisconsin 5 and Autumn King varieties were harvested at approximately monthly intervals from the date of planting (June 12, 1969). For the varietal study, evenly sized carrots (ca. 1½ in. dia, 150g) were harvested within a 3-day period in early November. For storage, 5–10 lb lots of carrots (Wisconsin 5, Autumn King) were washed, topped and sealed under relative humidity conditions approaching saturation in polyethylene bags which were stored at 34°F in a dark ventilated room.

2-lb lots of carrots were divided into 2 batches, diced, and analyzed as described previously (Heatherbell et al., 1971). Replicate analyses within a batch would vary ± 10%. Batch-to-batch variation, however, would show larger variation. (Refer to discussion on intravarietal variation in preceding paper, Heatherbell et al., 1971). Values are reported as one figure in this paper and are an average of at least 2 independent determinations (2 batches).

RESULTS & DISCUSSION

Influence of variety

Tables 1 and 2 illustrate results obtained by comparing the volatile constituents presented in six different carrot varieties, all harvested and analyzed within a 3-day period in November. The varieties were selected to represent a range of flavor (aroma) characteristics that had been observed in both the raw and canned carrots. Selections were based on preliminary studies on canned carrots involving preference panel subjective evaluations (G. W. Varseveld, Personal communication, 1969). It was observed that in some instances the raw carrot aroma characteristics appeared to carry through to the canned product. For instance, Autumn King was frequently described as having a strong, "green," "toppy" aroma, whereas Oregon 4362 was described as being mild and "perfumy" in aroma. Table 2 indicates the Autumn King variety has a relatively high concentration of the "green," "toppy" sabinene/myrcene compounds (approximately 30% of the essential oil throughout the growing season) which could account for the stronger nature of this carrots' aroma (flavor). In contrast, the mild, bland, "perfumy" Oregon 4362 not only has a relatively low concentration of these compounds (approximately 4% of the essential oil) but also has a relatively high concentration of the "perfumy" terpinolene (approximately 50% of the essential oil). Apart from the Oregon 4362 and the Nantes the total essential oil concentration of all varieties was reasonably constant between 24–27 ppm. The value of 5 ppm for the Oregon 4362 was dramatically lower than the other varieties and may be responsible for the much milder, blander nature of the carrot. Nantes with an essential oil concentration of 11 ppm was also noted as being a milder flavored carrot. The other varieties displayed no markedly differing flavor characteristics.

The only qualitative difference found within the six varieties was the consistent appearance of up to 2 ppm of p-cymene in the Autumn King variety. Only on very rare occasions was its presence noted in other varieties and then only in trace amounts. This strong aromatic compound

Table 1—Influence of variety on the "lower boiling" carrot volatiles.

Compound[a]	Wisconsin 5	Imperator	Nantes	Chantenay	Oregon 4362	Autumn King
Diethyl ether	Tr.[b]	Tr.	Tr.	Tr.	Tr.	Tr.
Acetaldehyde	0.75	0.60	0.85	0.66	0.80	0.40
Acetone	0.30	0.08	0.04	0.03	0.03	0.31
Propanal	Tr.	Tr.	0.02	Tr.	Tr.	Tr.
Methanol	0.06	Tr.	0.06	0.05	0.05	0.04
Ethanol	35.00	50.00	51.00	30.00	31.00	80.00

[a]Concentration in ppm.
[b]Trace.

[1]Present address: Plant Diseases Division, D.S.I.R. Private Bag, Auckland, New Zealand.

Table 2—Influence of variety on the "higher boiling" carrot volatiles.

Compounds[a]	Wisconsin 5	Imperator	Nantes	Chantenay	Oregon 4362	Autumn King
α-Pinene	1.00	0.09	1.10	0.65	0.05	0.79
Camphene	0.10	0.04	0.06	0.10	0.03	0.14
β-Pinene	0.07	0.05	0.22	0.33	0.09	0.36
Sabinene	0.08	0.10	0.68	0.68	0.13	3.24
Myrcene	2.90	0.35	0.32	1.85	0.06	4.65
α-Phellandrene	0.65	Tr.[b]	Tr.	0.06	0.03	Tr.
Limonene	0.10	0.46	0.19	0.70	0.12	0.06
γ-Terpinene	1.18	Tr.	0.75	3.51	0.22	0.03
p-Cymene	0.00	0.13	0.00	0.00	0.00	0.78
Terpinolene	5.45	6.05	0.81	5.45	2.45	4.52
Octanal	0.02	0.01	0.03	0.02	Tr.	Tr.
2-Decenal	Tr.	0.00	0.00	Tr.	0.00	Tr.
Bornyl acetate	0.05	0.22	0.02	0.12	Tr.	0.11
Caryophyllene	6.65	10.00	3.30	5.05	1.21	4.00
β-Bisabolene	0.34	1.18	0.28	0.46	0.07	.34
γ-Bisabolene	2.25	5.55	2.95	4.05	0.22	4.75
Carotol	0.15	0.22	0.38	Tr.	Tr.	0.10
Total	27.00	26.00	11.00	23.00	5.00	24.00

[a]Concentration in ppm.
[b]Trace.

may also contribute to the stronger flavored Autumn King variety. Sabinene readily breaks down to p-cymene (Wrolstad and Jennings, 1965). However, it is not believed to be an artifact in this case as other varieties containing substantial quantities of sabinene contained no p-cymene.

Influence of maturity

The determination of an index of carrot maturity is difficult as the tissue has continuous cell division and growth. In this study which used the Wisconsin 5 variety, age, size and weight are the parameters used as a guide to maturity. Great care must be taken in interpreting these results owing to the inherent variation in volatile contents within a given sample of carrots (Heatherbell et al., 1971). Only broad trends are considered meaningful.

The results in Table 3 indicate that whereas the concentration of acetone, propanal, and methanol remain relatively constant throughout the study, the acetaldehyde and particularly the ethanol concentration increases dramatically in the late season (fall, winter) carrots. The ethanol increases from a few ppm to over 1000 ppm. Accumulation of acetaldehyde and ethanol are indicative of anaerobic respiration. Accumulation of these compounds by plant tissues subjected to stresses such as low oxygen supply is well documented (Cossins and Beevers, 1963; Amerine, 1964). Fall and winter in Oregon are very wet seasons producing long periods of essentially "water-logged" soil conditions which could induce anaerobic metabolism in a storage root such as the carrot. Lemon (1962) and Lemon and Wiegand, (1962) indicate root respiration can be limited by the diffusion of oxygen to the root surface. Poorly drained soils would greatly limit the diffusion of oxygen (Klock and Brooks, 1968).

The determination of ethanol concentration produced the most variable results of all the volatiles analyzed, particularly in the early season carrots. Concentrations ranging from a few ppm to 40 ppm were detected in the early season carrots. Cossins and Beevers (1963) reported that ethanol accumulated during periods of anaerobiosis can be metabolized when the tissue gains better access to oxygen. These authors found that plant tissues, including carrot discs, rapidly metabolized labelled ethanol to carbon dioxide, organic acids, amino acids and other products, in periods of 4 hr or less. This evidence for rapid metabolic turnover of ethanol dependent on external stresses may account for the variation in ethanol content found in carrots.

Examination of the higher boiling compounds (Table 4) indicates that although the total essential oil content remains relatively constant between 21–28 ppm for the first 20 wk of growing season, the concentration of in-

Table 3—Influence of maturity on the "lower boiling" volatiles in carrots.

	Age[a]				
	6	16	20	24	28
	Average weight, g				
	20	120	150	150	150
	Average diameter, inches				
Compound[b]	0.4	1.0	1.5	1.5	1.5
Diethyl ether	Tr.[c]	Tr.	Tr.	Tr.	Tr.
Acetaldehyde	0.15	0.20	0.75	1.40	1.82
Acetone	0.10	0.30	0.25	0.15	0.40
Propanal	Tr.	Tr.	Tr.	Tr.	Tr.
Methanol	0.05	0.06	0.04	0.10	0.05
Ethanol	1.50	35.00	42.00	140.00	1200.00

[a]Weeks from date of planting.
[b]Concentration in ppm.
[c]Trace.

Table 4—Influence of maturity on the "higher boiling" volatiles in carrots.

	Age[a]				
	6	16	20	24	28
	Average weight, g				
	20	120	150	150	150
	Average diameter, inches				
Compound[b]	0.4	1.0	1.5	1.5	1.5
α-Pinene	0.65	1.00	0.25	0.65	1.18
Camphene	0.14	0.10	0.01	0.03	0.02
β-Pinene	0.46	0.07	0.65	0.61	0.55
Sabinene	0.45	0.08	2.31	0.75	1.00
Myrcene	1.30	2.90	1.28	2.30	0.61
α-Phellandrene	Tr.[c]	0.65	0.25	0.06	0.06
Limonene	0.52	0.10	0.29	0.55	0.60
γ-Terpinene	2.15	1.18	0.65	4.05	7.75
p-Cymene	0.00	0.00	0.00	0.00	Tr.
Terpinolene	5.45	3.68	3.10	13.00	7.75
Octanal	0.02	0.01	0.02	0.02	0.02
2-Decenal	0.02	Tr.	Tr.	0.00	0.00
Bornyl acetate	0.05	0.08	0.04	0.03	0.02
Caryophyllene	6.65	11.90	17.55	18.52	15.55
β-Bisabolene	0.34	0.61	0.22	0.42	5.00
γ-Bisabolene	2.25	5.00	1.00	7.45	11.65
Carotol	0.15	Tr.	0.28	0.31	0.30
Total	21.00	27.00	28.00	49.00	50.00

[a]Weeks from date of planting.
[b]Concentration in ppm.
[c]Trace.

dividual terpenes is constantly changing. This confirms the belief that terpenes undergo constant metabolic changes and are not merely being accumulated as an end-product (Burbott and Loomis, 1969). The total essential oil content increased to approximately 50 ppm at the end of the season (24–28 wk) largely due to an apparent increase in the synthesis of the sesquiterpenes β- and γ-bisabolene and to a lesser extent the monoterpenes γ-terpinene and terpinolene. The Autumn King variety gave similar trends with a lesser increase to approximately 35 ppm at the end of the season. As these results are expressed on a fresh weight basis the increase could in part reflect an anticipated decrease in moisture content with age. Similar variations in terpenes and an increase in monoterpene hydrocarbons with increasing maturity in citrus peel and leaf oils has been reported by Attaway and Metcalf (1967).

In Oregon most of the harvesting of carrots for processing occurs in November (corresponding to 20 wk from planting in this study); however, harvesting may continue through December.

Effects of storage

During the course of this study carrot samples which had been stored in polyethylene bags were observed to undergo some interesting changes on storage. Immature carrots (approximately ½ in. dia, 30g in weight, harvested 8 wk from planting) were observed to keep for approximately 5 wk under these conditions. (The carrots retained good turgor, texture and were not infected). However, within this 5-wk period the carrots had accumulated large quantities of acetaldehyde (increased from 1–8 ppm) and ethanol (increased from 30–1500 ppm) which are indicative of anaerobic respiration (Amerine, 1964; Cossins and Beevers, 1963). Evidently the polyethylene bags created a reduced oxygen tension due to the respired carbon dioxide. Apart from an increase to a few ppm of methanol the concentration of the other volatiles had not changed significantly at the end of the storage period.

With mature carrots (approximately 1½ in. dia, 150g in weight) the storage life was observed to extend to at least 3–4 months. Under these conditions, a storage life of approximately 5 wk for immature carrots and approximately 4 months for mature carrots agrees well with the values reported by Lutz and Hardenburg (1968) for commercial storage. When samples of mature carrots in storage were examined at bi-weekly intervals it was not until the eighth week that the concentration of acetaldehyde and ethanol began to increase (up to 2 ppm and 1000 ppm, respectively). It was observed that although the carrots retained good turgor and taste the aroma changed towards the end of the storage life. A softer, "sweeter" aroma, which is believed due to acetaldehyde/ethanol (Heatherbell et al., 1971) developed. A similar but less pronounced trend occurred in the late season carrots in the soil. Ethanol is only very weakly perceived at concentrations of 1000-2000 ppm (Bills and Keenan, 1968).

Accumulation of acetaldehyde and ethanol appear to be related to senescense. These compounds were found in high concentration in both late season carrots and in carrots after 2 mo storage. However, it must be remembered that the conditions were believed to be anaerobic in these instances; the effects of senescence and anaerobic growth cannot be distinguished without further studies.

An important conclusion from the maturity, variety, and storage studies is that great care should be exercised in reciting the full history of carrots, and presumably of other fruit and vegetable products, used for flavor analysis.

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EFFECT OF HEAT ON THE FLAVORING COMPONENTS OF MAPLE SIRUP: A Preliminary Study by Gas Chromatography

SUMMARY Portions of a distinctively flavored commercial maple sirup were heated by autoclaving at 15 psig (250°F) for 1.5 and 4 hr. A GLC flavor profile of the treated and untreated sirups showed that 25 components in their flavor fractions changed in relative amounts. In the light colored original sirup, acetol and compounds related to lignin (vanillin, syringaldehyde, dihydroconiferyl alcohol) predominated. As the sirup was heated, carbohydrate breakdown products began to appear and then to predominate (acetol, furanones, cyclotene, and HMF). As the carbohydrate degradation products increased the sirup darkened in color and became acrid in flavor; at this point vanillin began to decrease. From this preliminary study it would seem possible, after identifying all the compounds in the profile, to produce various levels of different flavor in maple sirup by a regulated heat process. The need is indicated for a complete study of the production of maple flavorants by heat, starting with a flavorless sap concentrate.

INTRODUCTION

THE CHARACTERISTIC flavor of maple sirup is developed during the concentration of the maple sap by atmospheric boiling (Skazin, 1930). Low temperature vacuum evaporation produces an almost flavorless sirup (Findlay and Snell, 1935; Porter et al., 1952). Early efforts to identify the chemical compounds in maple sirup contributing to its flavor were handicapped by the lack of present day methods of isolating and identifying trace amounts of flavorants. Nelson (1928), Skazin (1930), and Skazin and Snell (1928–29) have reported by classical methods the isolation of an aldehyde with a vanillin odor. Sair and Snell (1939) advanced somewhat the knowledge of maple flavorants by using chloroform to isolate the flavoring compounds from the sugars and ash constituents of maple sirup. However, this work was also limited by the techniques available for separation and identification. A vanillin-like compound was isolated but not definitely identified.

Underwood et al. (1961) attempted to extend the chloroform extract work of Sair and Snell using column chromatography; vanillin and syringaldehyde were definitely found along with evidence of many more compounds. However, it was not until the advent of gas chromatography that real progress began to be made in unraveling the secret of maple flavor. During the 1960's the author and coworkers reported on a large number of compounds found in maple sirup related to its flavor (Filipic et al., 1965; Filipic et al., 1969; Underwood and Filipic, 1963). As an accumulative result of these studies a GLC procedure was designed (Underwood et al., 1969) to produce a chromatogram depicting maple flavor (flavor profile). 25 peaks on this chromatogram represent flavorants in a chloroform extract of sirup. Other work (Willits and Porter, 1950) had revealed that the flavor can be enhanced in commercial maple sirup by additional heat treatment. The flavor changes in that work was evaluated by organoleptic and color measurements.

Using the GLC flavor profile, it is possible to determine changes in the individual flavorants with varying sirup treatments. This paper reports a preliminary study on component changes in the chloroform flavor extract of a commercial maple sirup given additional heat treatment.

EXPERIMENTAL

A TYPICAL commercial maple sirup with distinctive maple flavor was divided into three portions. The first portion was held without treatment; the second autoclaved for 1.5 hr at 15 psig (250°F), and the third autoclaved for 4 hr. A ½ gal aliquot of each portion was extracted with chloroform as described earlier (Underwood et al., 1969). These three extracts were analyzed by the GLC procedure detailed in the same paper to produce flavor profiles for the three portions of sirup. Reproductions of these profiles are shown in Figures 1, 2, and 3. A semi-quantitative evaluation of the changes in amounts of the compounds in the flavor extract in significant amounts is shown in Table 1.

RESULTS & DISCUSSION

A COMPARISON of Figures 1, 2, and 3 shows that most of the components increased as the sirup was heated. The semi-quantitative data in Table 1 showing these trends in more detail, indicate that a few compounds decreased rather than increased. Only half of the peaks recorded in the table are named, because not all of them were completely identified. Since a milder extraction was used in this study than in the exhaustive study reported earlier (Filipic et al., 1969), fewer peaks were obtained and identification procedures in addition to retention time had to be repeated. Only those peaks deemed useful to evaluate the effect of the heat treatment were completely reidentified by mass spectrographic analysis, but the heat treatment produced unpredicted changes in some peaks. Therefore, a further, more complete study of the effect of heat on maple flavorants, using the flavor profile, will require complete identification of all the peaks on the chromatogram.

The exhaustive extraction of c[l] oroform soluble flavorants from maple sirup mentioned above has shown that these compounds can be divided into two

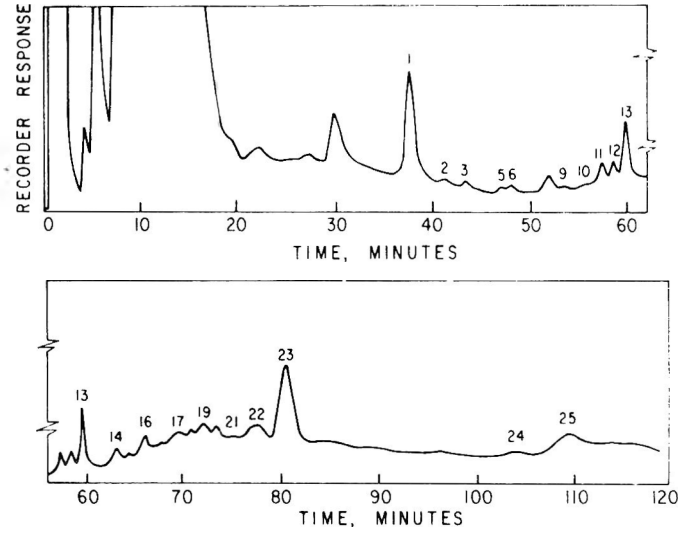

Fig. 1—Flavor profile of a typical commercial maple sirup.

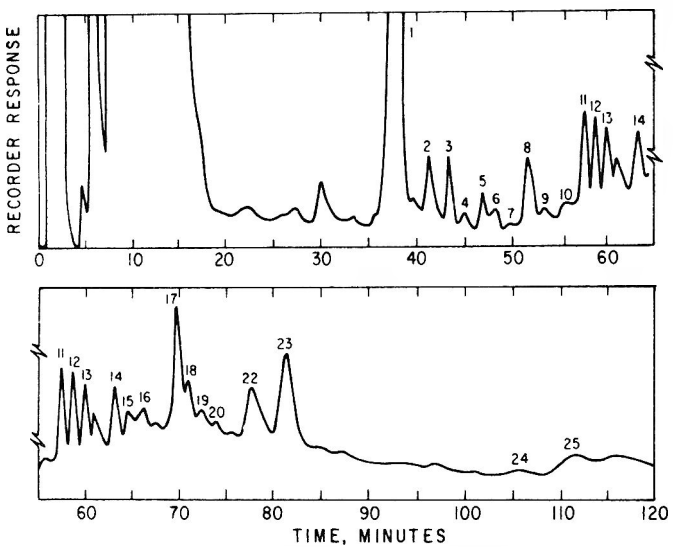

Fig. 2–Flavor profile of a typical commercial maple sirup autoclaved 1.5 hr at 15 psig.

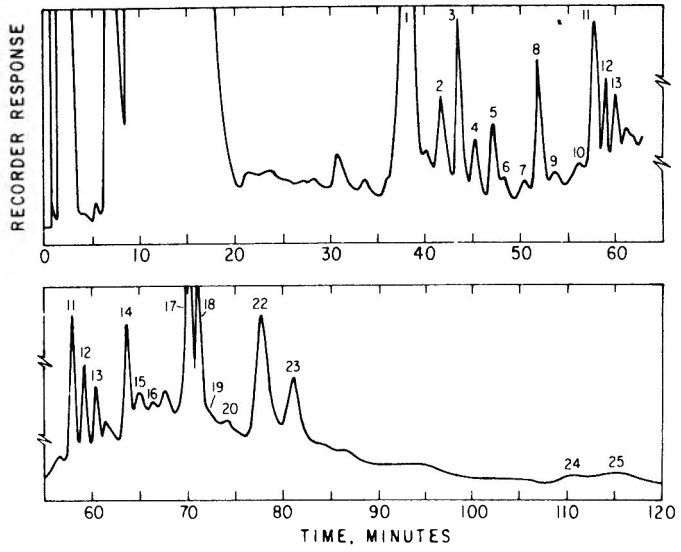

Fig. 3–Flavor profile of a typical commercial maple sirup autoclaved 4.0 hr at 15 psig.

groups according to their source. These sources are the trace of a ligneous material and the carbohydrates (almost 100% sucrose) in the sap.

Vanillin was the only measured compound of definite ligneous origin in the extract. It was probably the predominant flavorant in the original sirup. The further heat treatment caused a modest increase in the amount of this constituent at the end of 1.5 hr, but after 4 hr a significant decrease had occurred. The identity of the other compounds which decreased must be reaffirmed to determine whether other ligneous compounds respond as vanillin does.

Acetol is a major constituent in flavor extracts of maple sirup and was increased by the heat treatment. Its role in the formation of maple flavor compounds is yet unknown. Undoubtedly its increased production is from monosaccharides resulting from hydrolysis of the sucrose in the sirup. Also, Table 1 indicates that the rate of acetol formation decreases as the time of heating increases. This is probably due to the fall in pH of the sirup from the build-up of acids as the heating continues. Acetol formation from a hexose such as glucose is favored by an alkaline media (Lento et al., 1960).

The BHT (peak 13) found in this extract comes from the diethyl ether used to purify the original chloroform extract. Looked upon at first as a nuisance in our work, we now use it as an internal standard for rating the uniformity of our final concentrate of flavorants.

Finally, Table 1 lists a number of compounds that are formed from the carbohydrates in the sap and sirup. Sucrose constitutes 95% of the total solids in the exudate of the maple tree. The compounds shown make from an entirely negative to a heavily positive contribution to the desirable maple flavor in the sirup. Isomaltol and cyclotene have flavor characteristics that would help build a pleasing maple flavor. These both increased significantly during the heat treatment, especially the cyclotene. This increase in cyclotene is highly important because it is reportedly a powerful flavoring agent.

Two furanones have been identified in maple sirup. They are the "pineapple" furanone (2,5 dimethyl-4-hydroxy-3(2H)

Table 1–Peak heights[a] of components of maple sirup flavor extract.

Peak		Heating time–hours 0	1.5		4	
no.	Identity	Peak ht.	Peak ht.	% Increase	Peak ht.	% Increase
1	Acetol	68	976	1335	1224	1700
2		2.0	37	1750	49	2350
3	Acetic acid	3.5	42	1100	113	3128
4	Furfural	–	7.5	–	31	–
5	Propionic acid	2.5	25	900	48	1820
6		3.5	6.0	71	4	14
7		1.5	0.5		8	430
8		1.5	42	2700	83	5400
9	Isomaltol	0.5	4.0	700	6.0	1100
10		1.0	5.5	450	1.5	50
11	Cyclotene	10.0	56	460	88	780
12		9.5	50	426	33	247
13	BHT[b]	34	36	6	37	9
14	Pineapple furanone	8.5	36	323	66	676
15		1.5	11	633	10.5	600
16		9.5	10	5	4	–58
17	α-Furanone	2.0	71	3450	300	14,900
18		4.0	13	225	–	–
19		5.5	6	9	–	–
20		5.0	4.5	–5	4	–10
21		1.0	1.5	50	1.5	50
22	HMF	8.5	30	250	77	800
23	Vanillin	47	56	19	34	–28
24	Syringaldehyde[c]					
25	Dihydroconiferyl alcohol[c]					

[a]In millimeters.
[b]Artifact introduced by diethyl ether treatment of extract.
[c]Peaks too low and broad for heights to have any significance.

furanone), and a second furanone as yet not definitely identified. The mass spectrographic data of the latter appears to conform to the structure 4-hydroxy-2-hydroxymethyl-5-methyl 3(2H) furanone reported by Shaw et al., 1968. The "pineapple" furanone has strong caramel flavoring characteristics and certainly contributes to maple flavor, although probably modifying the more delicate flavor in an extremely fancy maple sirup to give it caramel tones. Its formation slowed with heating time and, again, this is probably due to fall in pH. The second furanone showed a large rate of increase with heating and probably contributes to the heavy caramel flavor of the middle grades of sirup.

The acids, furfural and HMF all increase with heating. These compounds impart the heavy, acrid caramel flavor to lower grade, dark, sirups. When sirups were heated at temperatures higher than reached in this study, as is possible in a heat exchanger (Willits et al., 1966), the predominant compound that forms is hydroxymethylfurfural (HMF) (Porter et al., 1952). The increase in acidity that accompanies heating causes accelerated production of HMF until the flavor imparted by this and other compounds to the sirup makes it unpalatable.

The study has answered one question that has been debated by those involved in maple sirup flavor research. Earlier work had demonstrated definitely that total flavor in maple sirup increases with heat treatment and changes in character. The flavor changes from the delicate maple to a heavy acrid caramel flavor, the latter over-riding the true maple. It has not been known whether true maple flavorants continue to increase but are masked by the relatively larger increase of the other flavorants, or the production of maple flavorants is limited. The brief data in this work indicate that there is a limit to the increase of maple flavorant and continued heating will cause a decrease. Proper regulation of heat and heating time would be required to produce a sirup of optimum maple flavor. This was discussed on a practical basis by Willits et al. (1966) in reporting on a heat process for continuously increasing the flavor in maple sirup.

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BIOCHEMISTRY OF TEA FERMENTATION: THE ROLE OF CAROTENES IN BLACK TEA AROMA FORMATION

SUMMARY—The carotenoid compounds present in fresh tea leaf were quantitatively extracted and separated by thin layer chromatography. Neoxanthin, violaxanthin, lutein, and β-carotene were identified and estimated by spectrophotometry. Quantitative studies showed that these carotenoid compounds decreased from about 0.053% (dry weight basis) in the fresh tea leaf to about 0.030% in the fermented (3 hr fermentation) leaf to about 0.026% in the fired black tea. This analytical study was followed by an investigation to determine how, and to what, these carotenoid compounds are altered during the black tea manufacturing process. The fate of β-carotene in tea fermentation was first studied in model systems. The model consisted of a crude soluble enzymes preparation extracted from fresh tea leaves, tea flavanols, and β-carotene. The model system was buffered at pH 5.4 and incubated for 30 min at 30°C. The volatile compounds formed in this system were studied by gas chromatographic analysis of headspace volatiles. It was found that β-ionone (identified by GLC retention time and mass spectrometry) was formed in this model tea fermentation system as a result of tea flavanol oxidation and drying of the reaction mixture after oxidation. Further, it was found that all three basic reaction mixture ingredients, i.e., active tea enzymes preparation, tea flavanol, and β-carotene were necessary for the production of β-ionone. While β-ionone was the major volatile product formed from β-carotene in these systems, experiments utilizing 15,15'-^{14}C-β-carotene showed that several unidentified compounds also were formed. The formation of β-ionone from β-carotene during black tea manufacture was confirmed in separate experiments. Organoleptic and GLC headspace analyses showed that black teas made in the usual way except that β-carotene was added at the leaf maceration stage (stage which initiates fermentation) contained more β-ionone than control black teas. The results obtained with β-carotene have been generalized on paper for all of the carotenoid compounds known to be present in fresh tea leaf. The results of this work indicate that many of the important black tea aroma constituents are probably formed during the tea conversion process by oxidative degradation of the carotenoid compounds present in the system. The possible mechanism for these transformations is discussed.

INTRODUCTION

IT HAS BEEN SHOWN (Tirimanna and Wickremasinghe, 1965; Nikolaishvili and Adeishvili, 1966) that the carotenoids present in fresh green tea shoot tips undergo appreciable decreases in concentration during the various stages of conversion of these tea shoot tips to the black tea of commerce. Further, it has been suggested (Tirimanna and Wickremasinghe, 1965; Sanderson, 1965; Müggler-Chavan et al., 1969) that the changes which the carotenoids undergo during the black tea manufacturing process are important in determining the quality, especially the flavor, of the black tea product produced.

This investigation was undertaken to establish that carotenoid compounds do in fact undergo degradation to black tea aroma constituents during the black tea manufacturing process and to determine the conditions and factors which cause this degradation. Further, an effort to identify the products of this degradation was made. β-carotene, one of the major tea leaf carotenoids, was used in these investigations as a model compound because of its availability.

This is part of our ongoing investigation of the mechanism by which black tea aroma is formed during the conversion of fresh tea leaf to black tea (Co and Sanderson, 1970).

EXPERIMENTAL

Materials

Epigallocatechin gallate (EGCG) and the crude tea enzymes preparation were made as previously described (Co and Sanderson, 1970).

β-carotene was purchased from Pierce Chemical Co. and it was twice crystallized from $CHCl_3$ and CH_3OH before use.

15,15'-^{14}C-β-carotene was a gift from Hoffman-La Roche, Basle, Switzerland.

The leaf used in this investigation was grown (fresh green tea shoot tips) and manufactured (black tea) at Lipton's Experimental Tea Garden in South Carolina. The commercial tea products used in this investigation were regular production samples of Lipton's current products.

Tea leaf processing

Frozen fresh Ceylon tea leaves were mixed with dry ice and hammer-milled to pass a fine herring-bone screen. The milled tea leaves were spread on aluminum trays to a depth of about ¼", thawed, covered with damp cheese cloth and allowed to undergo oxidation (tea fermentation) at room temperature (about 22°C). The tea was stirred occasionally to ensure uniform fermentation. After 3 hr of fermentation, the tea leaves were fired (dried) in a forced draft oven at 90°C for 1 hr to a moisture level of 4%. Water extracts were prepared by extracting black tea with 10 times its weight of boiling water for 15 min and repeating this procedure three times. The extracts were manufactured into instant tea.

Extraction of pigments

Approximately 10g of fresh tea leaves were mixed with a pinch of $MgCO_3$ and extracted four times with 50 ml of cold 80% aqueous acetone in a Waring Blendor. The combined acetone extracts were transferred to a separatory funnel and mixed with 100 ml of diethyl ether. Distilled water was then added until two layers formed. The aqueous layer was separated and extracted once more with 50 ml diethyl ether. The combined ether extracts were washed twice with 20 ml distilled water, dried with anhydrous Na_2SO_4, and concentrated to the desired volume.

Thin layer chromatography

An appropriate amount of the ether extract was concentrated under a stream of nitrogen gas and streaked on silica gel t.l.c. plates (Eastman Chromagram, Type K 301R2) and developed using petroleum ether (30–60°): benzene: ethanol (100:20:7). The pigments were eluted from the silica gel with acetone.

Determination of extinction coefficients of carotenoids

The carotenoid extract, after removal of chlorophyll by saponification (Goodwin, 1955), was streaked on 2 mm preparative silica gel plate (Silica Gel F-254, Brinkmann Instruments), and developed with petroleum ether (30–60°): benzene: ethanol (100:20:7). The chromatograms were developed five times; each time the plates were dried at room temperature for 2 min. The different carotenoids were eluted with acetone and transferred to petroleum ether. Xanthophylls were crystallized from methanol by adding small amounts of water. β-carotene was crystallized from benzene by adding small amounts of methanol in the cold (−15°C). The crystals were collected and dried under a stream of nitrogen gas. They were stored in a desiccator for 24 hr before they were used for extinction coefficient determination.

Methods of identification

Phase separation. The carotenoids in petroleum ether were shaken with 90% methanol. The carotenes remain in the ether layer. The xanthophylls in the methanol layer were extracted with diethyl ether after addition of water.

Color reaction with concentrated HCl. Ethanol solutions of the carotenoids were shaken with one-half their volume of concentrated HCl. Carotenoid epoxides give blue color.

Phase test for chlorophylls. An ethereal solution of chlorophyll was underlaid with an equal volume of a 30% solution of KOH in methanol. A colored ring is formed at the interface of the two phases which is characteristic of the chlorophyll being tested.

Absorption spectra. The absorption spectra of the different chlorophylls and carotenoids

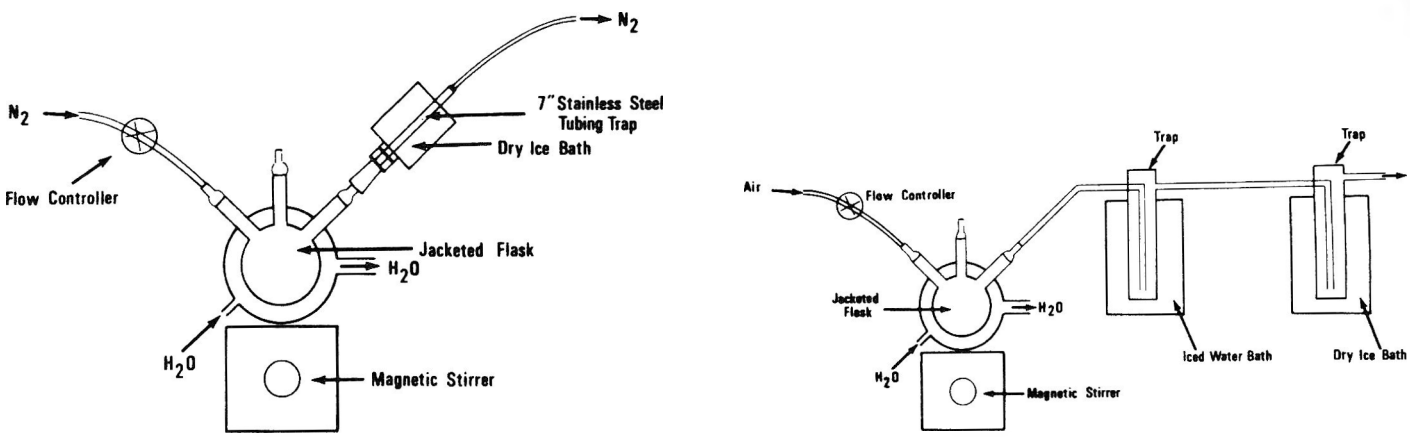


Fig. 1—Schematic diagram of headspace volatiles collection apparatus. 1A (left) Reaction vessel with headspace volatiles trap attached. 1B (right) Reaction vessel with trap system used to collect total volatiles during drying of model systems.

were obtained in acetone, diethyl ether, ethanol, chloroform and hexane using a Beckman DK-1 spectrophotometer.

Co-chromatography. The extract was co-chromatographed with lutein from egg yolk, synthetic β-carotene, chlorophylls a and b from fresh spinach, and pheophytins a and b prepared from chlorophylls a and b treated with oxalic acid.

Quantitative determination of carotenoids

Fresh and fermented tea samples were extracted as described above. Dry tea samples were rehydrated before extraction to ensure complete extraction of the pigments. The extracted pigments were separated by t.l.c. as described above and their concentration was determined in acetone using extinction coefficients obtained experimentally.

Model tea fermentation system

Oxidations were carried out in jacketed 50 ml reaction vessels (Fig. 1). The reaction mixture was made up as follows: 10.0 ml substrate/buffer solution containing 0.1 mM EGCG and 0.1 mM β-carotene in citrate-phosphate buffer (0.1M, pH 5.4); 0.2g of sodium lauryl sulfate as an emulsifier, plus 2.0 ml of the soluble tea enzymes preparation.

The enzymic oxidation was continued for 30 min during which time the reaction was aerated by stirring vigorously with a magnetic stirrer.

Collection of headspace volatiles

The method used was similar to that described by Co and Sanderson (1970). At the end of the oxidation period, a headspace volatiles trap was attached to one of the side arms of the reaction vessel which was otherwise closed (Fig. 1A). Hot water (95°C) was circulated through the jacket. The sample was stirred continuously with a magnetic stirrer. 3 min after the hot water was turned on, the headspace volatiles in the vessel were flushed into the trap with N_2 gas flowing at a rate of 50 ml/min for 5 min. The headspace volatiles trap consisted of stainless steel tubing, ¼" O.D. × 7" long, packed with 0.9g of 5% Apiezon L on Gas-chrom Q, 100–120 mesh. The trap was maintained at −80°C with dry ice.

Drying of the model system consisted of heating the reaction mixture to 95°C by circulating water through the jacket and passing a stream of dry air (100 cc/min) over the reaction mixture. The volatiles present in the model tea fermentation system, including the water vapor were condensed in two traps, kept at 0°C and −80°C, respectively (Fig. 1B). When approximately 11 ml of the initial 12 ml volume had been collected in the traps, the air stream was discontinued, and the reaction flask cooled to 0°C. The water collected in both traps was added back to the reaction flask, and the collection of headspace volatiles was carried out as described above.

Thin layer chromatography and liquid scintillation counting

After collection of headspace volatiles, aliquots of the model system reaction mixtures containing ^{14}C-β-carotene were applied to silica gel thin layer plates (Eastman Chromagram, Type K 301R2) and developed with petroleum ether (30–60°): benzene: ethanol (100:20:7).

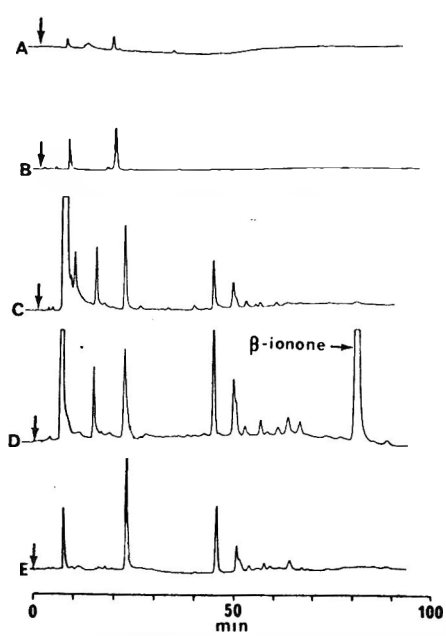


Fig. 2—Gas chromatograms of headspace volatiles of a model tea fermentation system containing β-carotene. (A) no fermentation; no drying. (B) 30 min fermentation; no drying. (C) No fermentation; dried. (D) 30 min fermentation; dried. (E) Same as (D) but no β-carotene.

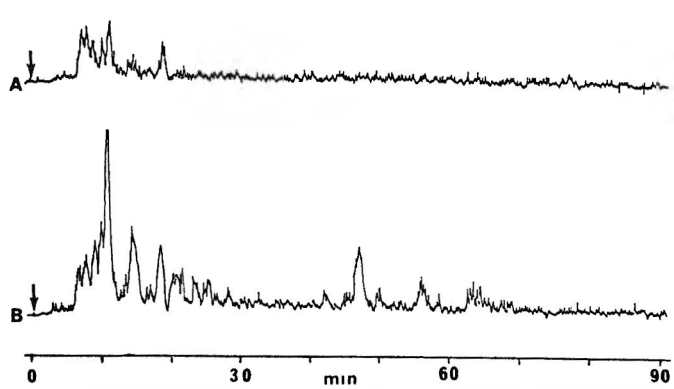


Fig. 3—Radio-gas chromatograms of headspace volatiles of a model tea fermentation system containing 15,15'-^{14}C-β-carotene. (A) Control: same as (B) but without enzyme added to reaction mixture. (B) Complete reaction mixture fermented for 30 min and dried.

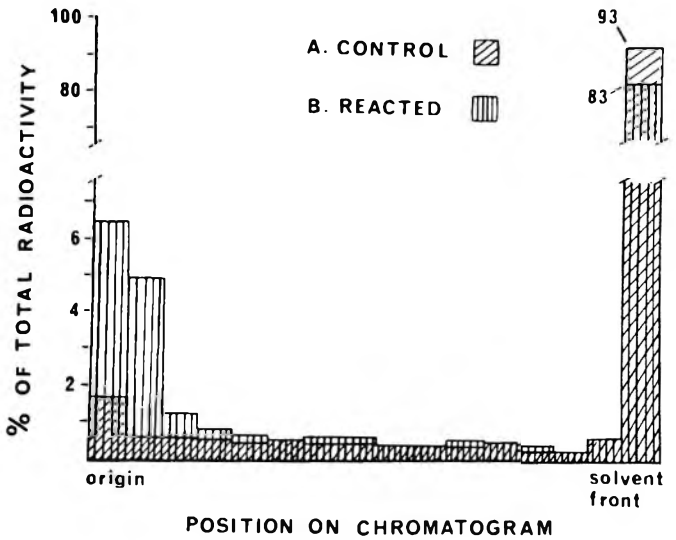


Fig. 4–Distribution of radioactivity on thin layer chromatograms of model tea fermentation systems containing 15,15'-^{14}C-β-carotene (See experimental section for details). (A) Control: ^{14}C-β-carotene only. (B) Reacted: complete model tea fermentation system, with ^{14}C-β-carotene, after oxidation.

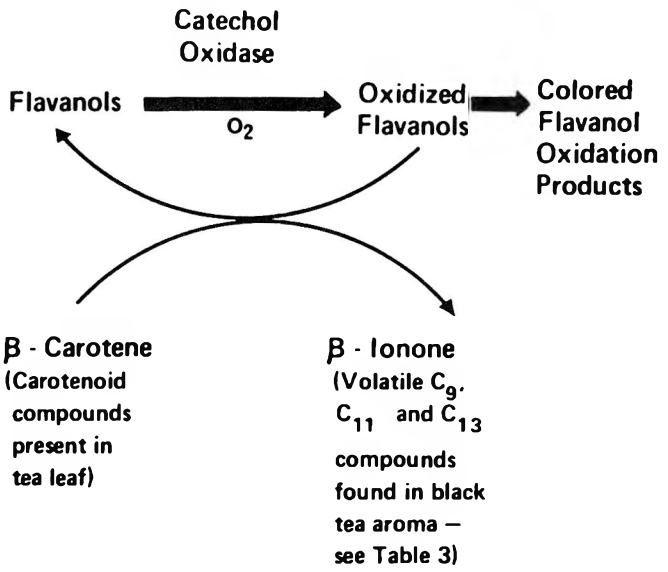


Fig. 5–Scheme showing relationship between flavanol oxidation and oxidative degradation of carotenoid compounds during tea fermentation.

After development the plates were divided into 1 cm zones and the silica gel in each zone was scraped into liquid scintillation vials.

Radioactivity in these samples was determined by liquid scintillation counting after adding 15.0 ml of toluene containing 60 mg of PPO and 0.75 mg of POPOP.

Gas chromatography

A Barber-Colman Series 5000 Selecta System with dual hydrogen flame ionization detectors was used. The carrier gas was shut off before the trap containing the headspace volatiles (see Collection of Headspace Volatiles above) was attached to the inlet of the GLC unit; a heating tape was wrapped around the trap, the carrier gas valve opened (carrier gas = argon; flow rate = 65 ml/min) and development of the chromatogram was started.

The column temperature was programmed from 80°C–200°C at 2°C/min followed by hold at 200°C. The columns used were of stainless steel, ¼" O.D. × 20' long, packed with 10% Carbowax 20M on Gas-chrom Q, 60–80 mesh. Part of the column effluent was directed to a radioactivity monitoring unit (Barber-Colman Model 5190) in experiments in which radioactive compounds were used, or to a manually operated trap at –80°C when samples of specific headspace volatiles were required for identification.

Mass spectrometry

The mass spectral data were obtained with an Hitachi-Perkin Elmer High Resolution RMU-6 Mass Spectrometer.

Preparation of black tea samples

Fresh frozen tea leaf was cryogenically milled with dry ice in a hammer mill. The dry ice was allowed to sublimate and the tea leaf was fermented for 2-½ hr at room temperature (about 22°C). The fermented tea was dried in a forced air oven at 90°C for 20 min.

β-carotene was added to designated samples of milled tea leaf prior to initiation of the tea fermentation process as follows: β-carotene equal in weight to 0.1% of the dry weight of the tea leaf was dissolved in a minimum amount of ethyl ether, this solution was mixed thoroughly with the sample of milled tea leaf, the leaf was spread in a thin (about 0.5 cm) layer on a tray, and the ether was allowed to evaporate. Fermentation and drying of these samples was carried out in the same manner as described above for samples without added carotene.

RESULTS

Identification of tea leaf carotenoids

Four carotenoid pigments were found in tea extracts using the experimental procedures described above. These compounds were identified as lutein, β-carotene, neoxanthin and violaxanthin. Their characteristics are summarized in Table 1.

The extinction coefficients of the carotenoids found in tea were determined and they were summarized in Table 2.

Changes in carotenoids during the manufacture of black tea from fresh tea leaves

The amount of carotenoids found in tea samples taken at various stages of manufacture of black tea and in samples

Table 1–Some characteristics of carotenoids in tea.

Spot	Phase separation 90% MeOH	Phase separation Pet. ether	Color reaction with HCl	Absorption maxima (nm) Observed	Absorption maxima (nm) Reported[d]	Probable identification
2	+	–	light blue	419,440,467[a]	417,438,467[a]	Neoxanthin
3	+	–	blue	426,451,479[b]	424,451.5,482[b]	Violaxanthin
4	+	–	No reaction	423,444,473[c]	420,447,477[c]	Luetin
9	–	+	No reaction	450,477[c]	451,482[c]	β-carotene

[a]In ethanol.
[b]In chloroform.
[c]In hexane.
[d]Goodwin (1955).

Table 2–Extinction coefficient of carotenoids found in tea.

Spot	Probable identification	λmax. nm, in Acetone	Experimental (in acetone)	Reported[e]
2	Neoxanthin	442	2358	2270[b]
3	Violaxanthin	441	2234	2250[b], 2250[b]
4	Lutein	445	2534	2250[b], 2580[c], 2600[d]
9	β-carotene	455	2600	2650[a], 2450[a], 2550[c]

[a]In hexane.
[b]In ethanol.
[c]In 80% ethanol – 20% ether.
[d]In ether.
[e]Goodwin (1955).

Table 3—Concentration of carotenoids in the tea samples taken at various stages of manufacture of black tea and in two commercial tea samples.

Samples	Neoxanthin		Violaxanthin		Lutein		β-Carotene	
	μg/g Dried Sample	% of Original	μg/g Dried Sample	% of Original	μg/g Dried Sample	% of Original	μg/g Dried Sample	% of Original
Fresh tea	51	100	120	100	260	100	102	100
1 hr Fermented tea	28	57	47	39	148	57	67	66
2 hr Fermented tea	30	58	53	43	154	59	64	62
3 hr Fermented tea	30	59	54	44	158	60	62	61
Fired and dried tea	23	46	36	30	154	59	61	60
Hot water extract[a]	0.6	1	0.7	0.5	8	3	3	3
Spent leaves[b]	11	22	18	15	147	57	58	57
Instant tea (prepared in this investigation)	0	0	0	0	1.2	0.5	0.3	0.3
Commercial black tea (Lipton)	18	—	0	—	141	—	33	—
Commercial instant tea (Lipton)	0	—	0	—	4	—	1	—

[a]Amounts corrected to g whole tea basis. Extract solids = 43% of whole tea.
[b]Amounts corrected to g whole tea basis. Spent leaves solids = 57% of whole tea.

Table 4—Formation of β-ionone from β-carotene in a model tea fermentation system.[a]

Treatment of reaction mixture		Headspace vapors over reaction mixture	
Oxidation period (min)	Drying treatment	Ionone formed[b]	Aroma by sensory analysis
0	None	No	No aroma
30	None	No	No aroma
0	Dried	No	No aroma
30	Dried	Yes	Strong; like violets

[a](The model system was composed of a soluble tea enzymes preparation and EGCG. See methods section for more details).
[b]Detected by gas chromatography.

of a commercial instant tea and a commercial black tea are shown in Table 3.

There was a large decrease in the amount of all the carotenoids during the first hour of fermentation. During subsequent fermentation, the xanthophylls neoxanthin and violaxanthin showed a slight increase. The firing (drying) stage resulted again in a large decrease of neoxanthin and violaxanthin.

It is noteworthy that commercial black tea has even less carotenoids than the fired and dried tea made in this laboratory (Table 3) suggesting that the processes operating to destroy carotenoids in commercial practice are even more effective than our laboratory experiments indicated.

The hot water extraction of tea resulted in further destruction of neoxanthin and violaxanthin. Most of the remaining carotenoids stayed in the spent leaves after hot water extraction. Only trace amounts of carotenoids were found in instant tea (Table 3).

Mechanism of carotene degradation and characterization of carotene degradation products

The mechanism by which carotenoid compounds are degraded during the conversion of fresh green tea leaf to black tea was studied using a model tea fermentation system composed of a soluble tea enzymes preparation, EGCG (tea flavanol) and β-carotene in a buffer system at pH 5.4.

The model system studies showed (Table 4; Fig. 2) that β-carotene is degraded to several volatile compounds when dried in the presence of oxidized tea flavanols. The major volatile oxidation product was trapped as it eluted from the gas chromatograph and it was identified as β-ionone by comparison of gas chromatographic retention time with an authentic sample, by I.R. and by mass spectroscopy. Infrared absorption bands appeared at 3.42, 6.20, 6.83, 7.33, 7.98, 8.94, and 10.30 microns. The principal fragments obtained by mass spectroscopy appeared at m/e = 39, 41, 43, 91, 117, 135, 178, and 192 (molecular ion peak). These absorption bands and mass fragments agreed with those obtained using a standard sample of β-ionone, and with values published in the literature (Bondarovich et al., 1967).

Drying of the model system prior to its oxidation did not produce β-ionone, nor did a reaction mixture which was allowed to oxidize for 30 min with no subsequent drying. Control reaction mixtures which contained no β-carotene did produce several volatile compounds when oxidized and dried (Fig. 2E) but no β-ionone was formed.

Further, it was found that all three basic reaction mixture ingredients, i.e., active tea enzyme preparation, EGCG, and β-carotene, were necessary for the production of β-ionone.

When 15,15′-^{14}C-β-carotene was oxidized in the model system, several peaks appeared in the radio-gas chromatograms obtained (Fig. 3B). These results show that volatile compounds are formed by cleavage of the center portion of the carotene molecule as well as by splitting off of the end groups (ex. ionones). A control reaction run without enzyme and under nitrogen to prevent oxidation showed (Fig. 3A) that these results were not due to volatile contaminants in the β-carotene. The organoleptic importance of these "other" products can only be guessed at (see Table 5).

It was noticed that during the drying step and collection of headspace volatiles, a malty sweet odor escaped through the dry ice cooled traps. An odor similar to this was also detected while collecting black tea headspace volatiles. In the experiment in which 15,15′-^{14}C-β-carotene was used (Fig. 3), these vapors were collected by passing the gases leaving the volatiles trapping system (Fig. 1) through 15 ml of toluene in a liquid scintillation vial. Subsequent liquid scintillation counting of the final trap showed that considerable radioactivity (19,300 dpm) was present in this toluene trap used with the complete reaction mixture as compared to only a much smaller amount of radioactivity (6,900 dpm) in the trap used with the control. These results suggest that some very volatile compounds involving the central portion of the β-carotene molecule are formed during the tea fermentation process.

The formation of β-ionone from β-carotene during black tea manufacture was confirmed in separate experiments. A solution of β-carotene in ethyl ether was added to cryogenically milled fresh tea leaf which was then allowed to ferment for 2-½ hr and dried. Analysis (organoleptic and instrumental by GLC) showed that the amount of β-ionone in the final product was significantly greater than was present in a control sample of the same

tea manufactured without the addition of β-carotene.

Separation of a reaction mixture in which radioactive β-carotene had been used, by thin layer chromatography and subsequent liquid scintillation counting of the chromatograms showed that approximately 10% of the β-carotene was converted to more polar compounds. This is shown by the increase in radioactive material of low Rf value (Fig. 4). No effort was made further to resolve this polar material.

DISCUSSION

RESULTS OF THIS investigation show clearly that a considerable number of volatile compounds (Fig. 2 and 3) are formed from β-carotene in a model tea fermentation system when the following two requirements have been met: (a) the tea flavanol has undergone oxidation; and (b) the oxidized reaction mixture has been taken to dryness.

Degradation of β-carotene did not take place to an appreciable extent in these model systems unless both of the above condition were met. Thus, the oxidation of β-carotene by oxidized tea flavanols appears to be potentially less favorable than the conversion of l-amino acids to the corresponding Strecker degradation (i.e., decarboxylated and deaminated) aldehydes which is catalyzed by oxidizing tea flavanols in dilute solution, i.e., no drying of the reaction mixture is required (Co and Sanderson, 1970).

However, the results of this investigation (Table 3), and others (Tirimanna and Wickremasinghe, 1965; Nikolaishvili and Adeishvili, 1966) show clearly that appreciable losses of all the carotenoid compounds present in fresh tea leaf takes place within the tea leaf during the fermentation stage of the black tea manufacturing process and it was found in this investigation that an obvious β-ionone odor developed in fermenting tea leaf to which small quantities of β-carotene had been added. These results suggest that the oxidation potentials developed in the more dilute model tea fermentation system are too low to effect appreciable oxidative degradation of carotenoids while the oxidation potentials developed in concentrated tea fermentation systems (i.e., withered and macerated tea leaf, or almost dried model tea fermentation systems) are sufficient to bring about these changes.

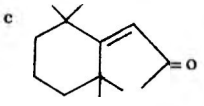
β-ionone was identified (Fig. 2) as the major volatile product formed from β-carotene degradation in the tea fermentation system. β-ionone is known to be a constituent of black tea aroma (Müggler-Chavan et al., 1969; Bondarovich et al., 1967) and the results of this investigation confirm contentions (Müggler-Chavan et al., 1969) regarding the possible link between β-carotene and β-ionone in tea.

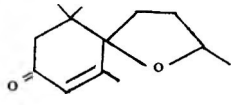
Table 5—Black tea aroma constituents supposed to be derived from carotenoid compounds.[a]

Carotenoids found in tea leaves (Tirimanna & Wickremasinghe, 1965)	Primary Oxidation Products	Secondary Oxidation Products
β-Carotene	β-Ionone + TAK[b]	Dihydroactinidiole[c] 2,2,6-Trimethyl cyclohexanone 5,6-Epoxy ionone 2,2,6-Trimethyl-6-hydroxycyclohexanone
α-Carotene	α-Ionone + β-Ionone + TAK	
Lutein	[3-Hydroxy-β-ionone] + [3-Hydroxy-α-ionone] + TAK	Theaspirone[d]
Neoxanthin[e]	3-Hydroxy-5,6-epoxyionone + 3,5-Dihydroxy-6-hydroionone + TAK	
Phytoene	Linalool + TAK	
Phytofluene	Linalool + TAK	
Lycopene	Linalool + TAK	
γ-Carotene	β-Ionone + Linalool + TAK	
Cryptoxanthin	β-Ionone + [3-Hydroxy-β-ionone] + TAK	
Violaxanthin	[3-Hydroxy-5,6-epoxy ionone] + TAK	
Zeaxanthin	[3-Hydroxy-β-ionone] + TAK	

[a](Known reaction based on results of this investigation, ➡ ;
Highly probable reaction based on results of this investigation, ⟹▷ ;
Probable reactions, ⇢ ;
Supposed reactions, ⩬▷ ;
Compounds shown in brackets have not yet been identified in tea).

[b]TAK = Terpenoid-like aldehydes and ketones. Oxidation products of all the carotenoids listed.

c 

d 

[e]Co et al., 1971.

Confirmation of the degradation of β-carotene to β-ionone during the tea conversion process provided by the results of this investigation adds reliability to the supposition that several other similar reactions involving carotenoid compounds as precursors of black tea aroma constituents do occur during the black tea manufacturing process. These related reactions are shown in Table 5 and a diagrammatic scheme which outlines the mechanism for the transformations undergone by carotenoid compounds during the tea conversion process is shown in Figure 5. Some further oxidation of primary carotene degradation product, i.e., epoxidation of the ionones, is proposed in Table 5. These secondary oxidations were not studied in this investigation but there is evidence that these reactions do occur under certain conditions in natural products (Ayres et al., 1964; Chichester and Nakayama, 1965). Therefore, it is reasonable to suggest that these reactions occur during the tea conversion process as outlined in Figure 5 and Table 5.

The possible importance of these reactions (Table 5) is obvious when one considers the large number of compounds which are black tea aroma constituents and which can now be presumed to originate, in part at least, from transformations of carotenoid compounds during black tea manufacture.

It is noteworthy that enzyme systems capable of oxidizing carotene to other products have been reported (Booth, 1960) to be present in green leaves of several plants but that the likely presence of phenolic compounds in the test systems used raises the possibility that a

mechanism similar to that operating in fermenting tea leaf operates in these other plant leaf systems. The oxidation of carotenes by lipoxidase systems which have been reported (O'Reilly et al., 1969; Grossman et al., 1969) appears to be analogous to the polyphenol oxidase system which effects carotene oxidation in fermenting tea leaf.

The interrelationship which exists between the fresh tea leaf constituents and constituents to be found in the black tea made from the leaf studied in this investigation, and others (Sanderson, 1964; Sanderson and Kanapathipillai, 1964; Co and Sanderson, 1970), point out again (Sanderson, 1965) the importance of the chemical composition of the tea leaf in determining the organoleptic properties of the finished black tea. Unfortunately, it must be stated that all too little is known as yet about what the composition of a tea leaf should be to enable the best black tea to be produced. And, of course, much remains to be learned about how the black tea manufacturing conditions (Eden, 1965; Harler, 1963; Hainsworth, 1969; Keegel, 1958) affect the organoleptic properties of the final product.

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NONVOLATILE ORGANIC ACIDS IN GUAVA

SUMMARY—The nonvolatile organic acids of guava were extracted and isolated. TLC of the acids showed the presence of 6 acids, 5 of the acids identified as lactic, malic, citric, ascorbic and galacturonic. GLC of the methyl esters of the guava acids confirmed the presence of malic, citric and lactic acid. Quantitative determinations using succinic acid as an internal standard showed citric and malic acid to be present in almost equal amounts and lactic acid in much lesser amount in cultivated guavas. In wild guavas, citric acid was the predominant acid, with lesser amounts of malic and lactic acids.

INTRODUCTION

GUAVA (*Psidium guajaba*), native to tropical America, is widely distributed through the tropics (Kennard and Winters, 1960). Introduced to Hawaii in about 1790, it flourishes in nearly all parts of the Islands at elevations below 3,000 ft. The fruit is consumed mainly in processed form in the United States, although it is eaten fresh in many tropical areas of the world. Guava is an excellent source of ascorbic acid, ranging well over 100 mg per 100g; it is a good source of niacin, the edible portion containing more than 1 mg per 100g (Wenkam and Miller, 1965; Watt and Merrill, 1963). Puree and juice from preferred cultivated Hawaiian varieties are quite acidic, pH 3.0–3.2; wild guavas vary from pH 3.0–3.5.

Guava puree and juice are used in production of beverages, dairy products, jams and jellies and many specialty products. Addition of edible organic acids such as citric, malic or fumaric is commonly employed for pH control in a number of these products. Thus, it has become necessary to know the kinds and amounts of nonvolatile organic acids normally present in mature guava fruits used for food products.

Santini (1953; 1956) reported citric, malic and tartaric acids present in guavas; he referred to earlier work (Gumarals and de Abreau, 1939) which showed malic and tartaric but no citric acid in guava. Buch (1960) refers only to Santini's work, and further search of the literature reveals no subsequent work to resolve these conflicting reports on nonvolatile acids in guava. Thin-layer chromatography (TLC) and gas-liquid chromatography (GLC) were employed in this laboratory for separation, identification and quantitative estimation of the nonvolatile organic acids in guava produced in Hawaii.

MATERIALS & METHODS

Preparation of guava puree

Guavas of the Beaumont variety and its seedlings were harvested at the full-ripe stage from plots at the University of Hawaii Agricultural Experiment Station. This variety and its seedlings account for most of the cultivated guava in Hawaii. Puree was prepared as follows: several hundred pounds of fruit were washed, macerated in a Fitzmill with a 4-B screen, seeds removed in a Langsenkamp pulper with .045-in. screen and stone cells removed by passing the pulp through a finisher with a .020-in. screen. The puree was stored at 0°F in hermetically sealed plain tin cans. Frozen wild guava puree was obtained from a commercial processor. Wild guavas are from trees growing in the wild in virtually every part of the State at elevations below 3,000 ft. This fruit varies widely in physical characteristics, but is nevertheless utilized in guava products.

Total acidity and ascorbic acid analyses

The colorimetric method of Loeffler and Ponting (1942) with slight modifications was used for ascorbic acid assay. Total titratable acidity was determined by the method of the AOAC (1960).

Extraction of organic acids

100g of guava puree was blended with 600 ml of 70% methanol (v/v) for 1 min in a Waring Blendor. The mixture was filtered in vacuo through Whatman No. 2 filter paper. The filtrate was percolated through a regenerated column of Dowex 50 w × 4 cationic resin, and then through a column of Amberlite IRA 400 anionic resin. The column was rinsed with 500 ml of distilled water to remove the sugars.

The acids were eluted from the anionic column with 100 ml of 6N formic acid followed with water until approximately 250 ml of eluant was obtained. The eluant was concentrated to about 10 ml in vacuo at 59°C and further evaporated to a syrupy consistency under a stream of N_2 for 16 hr at room temperature, to remove formic acid.

For thin-layer chromatography (TLC) the extract was taken up in 2 ml of water and for gas-liquid chromatography (GLC) in 2 ml of methanol.

Methylation

The acids were esterified to their corresponding methyl esters using the method described by Mazliak and Salsac (1965). 2 ml of BF_3-methanol 14% (w/v) was added to 2 ml of guava extract in methanol and allowed to react for 16 hr at room temperature in tightly capped vials. Samples of the reaction mixtures were injected directly into the gas chromatograph.

Gas-chromatographic analysis

A Varian Aerograph Model 204 gas chromatograph with a flame ionization detector was used. Nitrogen, partially saturated with water, was the carrier gas at a flow rate of 25 cc/min. The hydrogen gas flow rate was 44 cc/min. Two different columns were used for retention time studies: a 9-ft, .093-in., i.d. stainless steel column packed with 15% DEGS (diethylene glycol succinate) on Chromosorb W HMDS 60/80, and a 2.5-ft, .093-in. i.d. stainless steel column packed with 5% NPGS (neopentylglycol succinate) on Chromosorb W HMDS 60/80 (Gee, 1965). For the qualitative determination of dimethyl malate and trimethyl citrate the DEGS and NPGS columns were operated isothermally at 170 and 145°C, respectively, with an injection port temperature of 180°C and a detector temperature of 220°C. For determination of methyl lactate the NPGS column was operated isothermally at 90°C with an injection port temperature of 170°C and detector temperature of 225°C.

Table 1—R_f values (× 100) of organic acids on cellulose developed in Solvent I (BFW).

Compound	Known	For guava
Lactic	75	75
Malic	55	56
Citric	45	46
Ascorbic	27	26
Galacturonic	11	13
Unknown	–	06
Tartaric	26	–

Table 2—R_f values (× 100) of organic acids on cellulose developed in Solvent II (AFW).

Compound	Known	For guava
Lactic	70	71
Malic	39	39
Citric	25	26
Ascorbic	09	11
Galacturonic	03	03
Tartaric	13	–

Table 3—R_f values (× 100) of organic acids on silica gel developed in Solvent I (BFW).

Compound	Known	For guava
Lactic	60	59
Malic	45	43
Citric	34	34
Galacturonic	12	11
Unknown	–	05
Ascorbic	40	–
Tartaric	23	–

Table 4–R_f values (× 100) of organic acids on silica gel developed in Solvent II (AFW).

Compound	Known	For guava
Lactic	62	62
Malic	40	40
Citric	29	30
Unknown	–	12
Galacturonic	04	05
Ascorbic	24	–
Tartaric	27	–

Table 5–R_f values (× 100) of organic acids on silica gel developed in Solvent III (BBIFW).

Compound	Known	For guava
Lactic	66	63
Malic	55	55
Citric	43	46
Galacturonic	22	22
Ascorbic	12	13
Unknown	–	07
Tartaric	37	–

Table 6–Retention time (minutes) of methyl esters of known organic acids and guava acid–two columns.

Compound	Known	Beaumont Guava	Wild Guava
		15% Degs at 170°C	
Dimethyl malate	9.57	10.45	10.10
Trimethyl citrate	46.90	47.46	47.71
Methyl lactate	.94	1.08	.74
		5% NPGS	
Dimethyl malate[b]	2.45	2.41	2.49
Trimethyl citrate[b]	13.53	12.21	13.13
Methyl lactate[a]	.96	1.01	.93

[a]Column temp, 90°C.
[b]Column temp, 145°C.

Table 7–Quantitative determination of organic acids in wild and Beaumont guavas by GLC.

Acid	Beaumont (g/100g)	Wild (g/100g)	Beaumont (meq)	Wild (meq)
Malic	0.469	.182	8.31	2.71
Citric	0.532	.541	6.99	8.46
Lactic	0.025	.012	0.28	0.12

The NPGS column was used for the quantitative determination of the acids. Succinic acid was used as the internal standard. Known quantities of succinic acid (0.5–1.0g) were added to 100g of guava puree. The extraction, methylation and gas chromatography of the acids were performed as just described. Quantitative data were calculated from the peak area and weight relationships of 12 or more replications.

Thin-layer chromatography of organic acids

Silica gel (Eastman chromagram sheet K301R) and cellulose powder MN 300 (Macherey, Nagel and Co.) coated plates, 250 μ thick, were used. The plates were activated at 110°C for 30 min and stored in a desiccator. The organic acid extract in water was applied directly to thin-layer plates with a micropipette in volumes of 1–8 μliters. The cellulose plates were developed in the organic phases of the following solvent systems: I. BFW (n-butyl alcohol-formic acid-water 4:1:5, (v/v). II. AFW (n-amyl alcohol-formic acid-water 4:1:5, (v/v). The silica gel plates were developed in these same solvents and also in: III. BBIFW (benzyl alcohol-tert butyl alcohol-isopropyl alcohol-formic acid-water 24:8:8:1:8, (v/v). After development, the plates were dried overnight. The yellow acid spots were located on a blue-green background by spraying with bromocresol green (0.4% w/v in 95% ethanol) solution adjusted to pH 5.5. At least 6 plates of each adsorbent and each solvent system were developed; the R_f values in Tables 1–5 each represent averages of 6 or more replications.

RESULTS & DISCUSSION

Thin-layer chromatography of organic acids

Guava acids chromatographed on cellulose and developed in Solvent I showed 6 spots, indicating the presence of 6 acids. 5 of the spots had R_f values corresponding to those of lactic, malic, citric, ascorbic and galacturonic acids (Table 1). One of the spots had an R_f value of 26; the R_f values of ascorbic and tartaric acids are 27 and 26, respectively. This could be interpreted to confirm earlier reports, Santini (1953; 1956), of the presence of tartaric acid. However, R_f values of spots on other adsorbents with other solvent systems, as will be seen below, failed to substantiate the presence of tartaric acid. When the acids were chromatographed on cellulose in Solvent II, 5 spots appeared with R_f values indicating lactic, malic, citric, ascorbic and galacturonic acids (Table 2).

Guava acids on silica gel developed in Solvent I indicated the presence of 5 acids, 4 of which were identified as lactic, malic, citric and galacturonic. The fifth was not identified by comparative R_f value. Ascorbic acid was not separated from malic acid by this system and did not appear as a discrete spot (Table 3). Extracts spotted on silica gel and developed in Solvent II also indicated the presence of 5 acids, with 4 identified as lactic, malic, citric and galacturonic (Table 4). The fifth was not identified. Extracts on silica gel with Solvent III showed 6 acids to be present, 5 identified as lactic, malic, citric, galacturonic and ascorbic. Of the various systems used here, silica gel with Solvent III appeared to give the best results in terms of resolution and definition of spots (Table 5).

To determine whether lactic acid might be an artifact due to spoilage, extracts from selected, prime, unblemished fruit and from over-ripe, partly rotten fruit were prepared. Lactic acid was detected by TLC in the extract from prime, fresh fruit just as in the previously described material. No evidence of greater amounts of lactic acid in the over-ripe fruit was seen.

Gas-liquid chromatography of methyl esters of organic acids

The GLC retention times of known organic acids and of acids extracted from guava are shown for DEGS and NPGS columns in Table 6. These data confirm the presence of lactic, malic and citric acids. Tartaric acid was not detected in either wild or cultivated guava. Galacturonic and ascorbic acids could not be confirmed by GLC of their methyl esters, due to their high degree of hydroxylation.

The quantitative determination data are shown in Table 7. Malic and citric acid were found in Beaumont guavas in approximately equal amounts, 0.47 and 0.53% by weight, respectively. These acids are about 20 times greater in abundance than lactic acid, present in the amount of only 0.025%. In wild gauva extract we measured 0.18% malic, 0.54% citric and 0.012% lactic acid.

The total titratable acidity of purees of cultivated (Beaumont) and wild guavas were 18.4 and 14.08 meq per 100g, respectively. The Beaumont fruit had 0.73 meq/100g of ascorbic acid (128 mg/100g) and the remaining 2.1 meq/100g we attribute to galacturonic, volatile and unidentified nonvolatile acids. In wild guava, we measured 32 meq/100g of ascorbic acid (56 mg/100g). This, together with the malic, citric and lactic acids, totaled 11.62 meq/100g for acids determined quantitatively. Here again, the remaining 2.47 meq are attributed to galacturonic, volatile and unidentified nonvolatile acids.

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ORGANIC ACID PROFILES OF THERMALLY PROCESSED, STORED SPINACH PUREE

SUMMARY—Deaerated spinach puree was packed into TDT tubes, flushed with nitrogen and sealed. The tubes were divided into batches and processed with an Fo = 4.9 at temperatures ranging from 240–300°F with 10°F increments. Analyses were carried out immediately after processing and after storage for 3 months at 75°F in the dark. Organic acids were analyzed by means of an Automatic Organic Acid Analyzer (AOAA) with confirmation by paper chromatography. Oxalic acid showed poor resolution on the AOAA; therefore, a chemical method was used for analysis. pH measurements were taken and color measured on a Hunterlab Model D25 Color Difference Meter. Catalase determinations were done on the stored samples. After processing, the greatest changes were noted at 240°F, with only minor changes above 280°F. Acetic and pyrrolidone-carboxylic acid (PCA) showed the most change after processing. After storage, the concentration of acids, color and pH was similar for all packs and all proved catalase negative. During storage succinic, PCA and acetic acids showed the greatest increase with the latter two increasing the most at higher temperatures. Also, during storage α-ketoglutaric acid disappeared and pyruvic, glutaric, oxaloacetic and malonic acids were formed at all processing temperatures.

INTRODUCTION

THE MAINTENANCE of color in thermally processed, stored green vegetables has been and remains a problem in food processing. Many attempts have been made to stabilize the color in past years with limited success (Clydesdale and Francis, 1968; Clydesdale et al., 1970).

One of the major problems has been the increase in total acidity caused by processing, which also occurs in products other than green vegetables. El Miladi et al. (1969) reported an increase in total acidity in tomato juice processed at 220°F for 20 min. Shallenberger et al. (1959) found that pyrrolidone-carboxylic acid (PCA) was found during the production of beet puree and Luh et al. (1969) found a decrease in pH of retort-processed strained carrots along with formation of PCA. Crean (1966) reported that the breakdown of carbohydrate molecules, when heated in acid solution, gives rise to various acids and suggested that this reaction occurs in thermally processed canned vegetables.

Clydesdale (1966) found that when spinach puree was processed by conventional and high-temperature short-time (HTST) the conventional packed showed a decrease in pH of about 10% while the HTST-packed remained virtually unchanged. Lin et al. (1970) found similar results and reported that acetic acid and PCA showed the most striking changes with increases of 129 and 132%, respectively, at 240°F.

It was also noted by Clydesdale (1966) that the pH of HTST-processed samples decreased more rapidly than the pH of those conventionally processed during storage, producing approximately equal values after 3 months. These results correspond to observations that HTST produces a superior product in terms of color after processing but rapidly loses such improvement during storage.

Information concerning the relationship between pH, color and organic acid changes during storage of conventional and HTST processed packs is scant. Formation of acids is an important parameter in the final quality and safety of a processed fruit or vegetable. Lin et al. (1970). Therefore, this investigation was initiated to study changes in these parameters during storage of packs processed at different temperatures. Spinach puree was processed with Fo = 4.9 at temperatures ranging from 240–300°F with increments of 10°F. Half of the samples were analyzed for color, pH and organic acids immediately after processing and the other half stored at 75° for 3 months in the dark prior to analysis. Catalase determinations were done on all samples to obtain an index of possible enzyme regeneration. It was hoped that this investigation would provide some insight into the cause of the greater pH decreases in HTST samples during storage as compared to conventionally processed packs.

MATERIALS & METHODS

SPINACH purchased from a local market was comminuted, deaerated and packed into TDT tubes with a syringe immediately after the tubes had been flushed with nitrogen. After filling, the headspace in the tubes was flushed with nitrogen and then sealed with an oxygen flame.

The tubes were divided into batches of 50 each and processed with an Fo = 4.9 at temperatures ranging from 240–300°F with 10°F increments as calculated by Gupte et al. (1964). After processing, ½ of the tubes were frozen at −20°F until analyses for organic acids, color and pH were performed; the other half stored at 75°F in the dark for 3 months prior to analysis.

Organic acid analyses

Organic acids were quantitatively analyzed by an automatic organic acid analyzer (AOAA) (Waters Associates, Inc., Framingham, Mass.). Development of this analyzer is described by Kesner and Mutwyler (1966).

Acidified, oven-dried (110°C, overnight) silica gel (Mallinckrodt, SilicAR cc-4, 200–325 mesh) was used along with a five-chamber gradient elution system employing chloroform and tertiary amyl alcohol in various proportions as the eluant. The indicator used was made up of 1g of the sodium salt of o-nitrophenol (Eastman Organic Chemicals) in 2 liters of anhydrous methanol. The conditions and calibration procedures used in this analysis are described in detail by Lin et al. (1970).

Identification

Tentative identification of all acids was based on retention times on the AOAA in comparison with standard acids as described by Lin et al. (1970).

Confirmatory identification

Further confirmation of the identification of organic acids was accomplished by means of paper chromatography. Fractions of the eluant stream were collected from the AOAA, concentrated, redissolved in 50% methanol and spotted on Whatman No. 1 sheets as described by Lin et al. (1970). The chromatograms were run according to the method of Markakis et al. (1963), in which the spotted papers were irrigated descendingly by the upper phase of a mixture of 1 butanol:3N formic acid 50:50 by volume. The lower phase of the mixture was used for vapor equilibration. After 12 hr the papers were dried in an air draft and sprayed with a 0.05% solution of bromphenol blue (Na salt) in 50% ethanol.

Oxalic acid analysis

It was found in a previous study by Lin et al. (1970) that the conditions used for the analysis of the other acids on the AOAA produced a diffuse peak for oxalic acid. Therefore, a chemical analysis based on the method of Baker (1952) was used. This method depends upon precipitation as calcium oxalate from a depro-

Table 1—Color and pH in spinach puree over the processing temperature range 240–300°F with Fo = 4.9 and stored for 3 months.

Process	pH		$\tan^{-1}$ a/b	
(°F)	PP	SP	PP	SP[a]
Fresh	6.40	6.40	−37.9	−37.9
240	6.12	6.10	− 5.9	− 9.7
250	6.29	5.95	−11.6	−11.6
260	6.35	5.90	−15.1	−10.9
270	6.40	5.90	−19.4	−11.3
280	6.41	5.92	−26.6	−11.9
290	6.41	5.90	−27.8	−15.2
300	6.41	5.90	−29.0	−14.6

[a]PP— processed puree; SP—processed puree stored for 3 months at 75°F in the dark.

Table 2–Concentration of organic acids in spinach puree over the processing temperature range 240–300°F with an Fo = 4.9 and stored for 3 months.

	Acid concentration (μeq/g dry weight of spinach)																	
	Acetic		Formic		Fumaric		Lactic		Succinic		PCA		Malic		Citric		Oxalic	
Process	PP	SP	PP	SP	PP	SP	PP	SP	PP	SP	PP	SP	PP	SP	PP	SP	PP	SP
240	16.1	38.2**	19.2	23.6	27.8	23.8*	9.0	9.6	20.7	58.3**	56.6	72.6*	76.6	99.3*	50.0	72.3*	704.4	677.4
250	10.9	35.8**	18.0	26.3*	29.1	27.7	9.6	9.5	21.0	58.4**	36.6	64.2**	74.8	100.1*	50.1	71.8**	694.1	683.1
260	7.8	35.7**	14.3	15.5	29.6	23.0*	10.2	7.4*	18.0	56.7**	29.3	57.2**	66.9	90.7**	46.2	67.0*	676.4	690.7
270	7.9	36.2**	18.6	21.2	38.3	27.7*	14.1	8.3	28.1	50.0*	33.5	48.5*	85.0	84.4	52.1	61.2*	704.8	608.8
280	6.8	38.6**	16.8	18.7	38.6	29.5*	12.4	6.2*	22.0	48.3**	23.7	54.6**	77.7	93.9*	48.6	64.3*	712.9	747.0
290	8.0	37.6**	19.2	24.6	38.1**	30.1	14.6	10.5*	24.2	54.2**	28.5	49.8**	80.1	96.0*	51.1	68.8*	661.3	704.0
300	7.8	40.7**	18.6	24.6*	37.6	24.5**	13.7	10.7*	23.2	58.4**	27.6	56.7**	81.1	88.6*	51.7	63.3*	782.7	729.1
Fresh	7.9	7.0	16.9	16.9	36.0	36.0	12.2	12.2	24.2	24.2	23.1	23.1	75.3	75.3	43.8	43.8	795.2	795.2

[a]Same as Table 1.
*A significant difference at the 5% level between processed and processed stored spinach.
**A significant difference at the 1% level between processed and processed stored packs.

teinized extract and subsequent titration with potassium permaganate.

Color measurement

Instrumental color data were obtained from a Hunterlab Model D25 Color Difference Meter (Hunter Associates Laboratory Inc., Fairfax, Va.). The data were reduced to the function $\tan^{-1}$ a/b as suggested by Clydesdale and Francis (1969).

pH Measurements

These were obtained with a Radiometer, Model 25, pH meter.

Statistical evaluation

Statistical analysis of the organic acid data was done by the Range Method (Kramer and Twigg, 1966).

Catalase determinations

Catalase determinations were done by the method of Gagnon et al. (1959). This method involved dipping a paper disk (No. 57-GH, Schleicher and Schuell Co., Keene, N.H.) into a slurry of the puree and then dropping it into a test tube (i.d. = 20 MM) containing 3% hydrogen peroxide solution. The flotation of the disk within a given time constitutes a positive test. Blanks were run on reagents and paper disks to establish the time in which the disk would rise in the absence of catalase.

RESULTS & DISCUSSION

TABLE 1 shows a comparison of color and pH of the processed puree (PP) which underwent processing at different temperatures with the same Fo, and the same samples (SP) following storage at 75°F in the dark for 3 months. The color data are reported in terms of $\tan^{-1}$ a/b, which is an angular function of hue. Use of this function correlates very well with visual judgments of the color of processed spinach puree (Clydesdale and Francis, 1969). Larger negative values denote better color retention. In the case of the PP, as expected, the HTST process provided the best color retention and processing at 240°F, the worst. However, after 3 months' storage the color was similar for all process temperatures, showing that the initial advantage gained by an HTST process is rapidly lost on storage. From Table 1, it appears that there was a slight improvement in color for the samples processed at 240°F, but the effect was very small.

As noted previously by Lin et al. (1970) the PP showed the greatest changes in pH at 240°F and the least at temperatures above 280°F. However, consistent with the color results, the pH values were similar after storage for all processing temperatures. Therefore, during storage, the HTST samples showed greater color degradation and a greater pH decrease than the conventionally processed pack after storage, in line with results of Clydesdale (1966).

Table 2 shows the organic acid profiles of the PP and SP samples. Acetic, succinic and pyrrolidone-5-carboxylic acid (PCA) showed the greatest increase in concentration during storage at every process temperature. However, in the samples processed at 240°F there was an increase in acetic acid of about 137% during storage as compared to a 420% increase in the samples processed at 300°F. Similarly, PCA showed an increase of 28 and 105% during storage for process temperatures of 240 and 300°F, respectively. It has been shown in some products that PCA is formed from glutamine during processing (Shallenberger et al., 1959; Rice and Paderson, 1954) and possibly the same pathway is followed during storage. Succinic acid showed a large similar increase at all temperatures.

Malic and citric acid showed lesser increases in acid concentration at all temperatures. However, they showed less of an increase during storage in the HTST packs than in the packs processed at a lower temperature (240°F), in contrast to PCA and acetic acid.

Fumaric acid showed a decrease in acid concentration at all temperatures during storage, with a greater decrease in the pack processed at 300 than at 240°F. Lactic acid showed a slight decrease at the elevated temperatures, but no significant decrease was noted at 240 and 250°F. Oxalic acid showed no significant changes during storage at any temperature.

Table 3 shows the concentration of α-ketoglutaric acid after processing and the concentration of pyruvic, glutaric, oxaloacetic and malonic acids after storage. This table shows that α-ketoglutaric acid disappeared during storage at all temperatures. The other acids were formed only during storage and were not present after processing or in the fresh sample. In Table 3 the concentration of

Table 3–Destruction and formation of organic acids during storage after processing over the temperature range 240–300°F with an Fo = 49.

	Acid concentration (μeq/g dry wt spinach)[a]									
	α-Ketoglutaric		Pyruvic		Glutaric		Oxaloacetic		Malonic	
Process	PP	SP	PP	SP	PP	SP	PP	SP	PP	SP[b]
240	31.1	0	0	6.8	0	33.7	0	41.1	0	21.0
250	31.1	0	0	6.7	0	30.5	0	37.8	0	24.2
260	29.2	0	0	5.3	0	17.6	0	33.8	0	19.1
270	34.0	0	0	6.0	0	25.4	0	44.3	0	14.4
280	32.8	0	0	4.5	0	20.7	0	58.8	0	18.3
290	34.5	0	0	4.7	0	31.0	0	33.7	0	17.1
300	32.6	0	0	6.3	0	31.8	0	25.2	0	20.0
Fresh	30.4	0	0	0	0	0	0	0	0	0

[a]Except for oxaloacetic acid, which is expressed as area under its curve in cm^2.
[b]Same as Table 1.

oxaloacetic acid is reported in terms of area under the curve (CM^2). This was because oxaloacetic acid is extremely unstable on the acidified silica gel column and it was impossible to create a standard curve or to predict destruction of this acid from the puree as it passed through the column. Therefore, it can only be stated that it was formed, but absolute quantities cannot be given.

Mechanisms for the increase, decrease, disappearance and formation of these acids during storage are not known at this time. Perhaps the degradation of some compounds such as sugar (Crean, 1966) and the formation of PCA from glutamine (Shallenberger et al., 1959) may explain the increase in acid concentration in some cases.

It appears from this study that the cause of a decreased pH and the concomitant decrease in color which occurs during storage of HTST packs of spinach puree is due mainly to the increase in concentration of PCA, acetic and succinic acids and also the formation of pyruvic, glutaric, oxaloacetic and malonic acids which are not present after processing and form only during storage. It would seem that the problems associated with the storage of HTST-processed spinach puree will not be resolved until more is known about the mechanisms of formation of these acids. If this information were available, steps might be taken in an attempt to prevent such formation and increase during storage.

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EFFECT OF GAMMA IRRADIATION ON THE POSTHARVEST PHYSIOLOGY OF FIVE BANANA VARIETIES GROWN IN INDIA

SUMMARY—Gamma irradiation to 20–40 krad inhibits the ripening changes in preclimacteric bananas without affecting the fruit quality. Both fruit maturity at harvest and post-irradiation storage temperature markedly influence the response to irradiation. The optimum dose and the maximum tolerable dose varied among the five varieties screened. Ability of the banana fruit to withstand higher doses of gamma irradiation depends on the physiological status of the fruit at time of irradiation. Irrespective of varietal differences, irradiation of preclimacteric bananas to doses above 50 krad resulted in severe skin discoloration and fruit splitting. Irradiation under anoxia did not markedly reduce the radiation injury, suggesting that factors other than ozone formed during irradiation in air may contribute the radiation damage. Fruits on the climacteric could tolerate up to 200 krad but no effect on ripening rate was observed. Ethylene or 2,4-D could reverse irradiation-induced inhibition of ripening in bananas. Irradiation seems to decrease the sensitivity of banana fruit to the ripening action of exogenously added ethylene.

INTRODUCTION

BANANA occupies an important place among the fruits cultivated in India. The total area under banana in India is estimated to be 158,000 hectares, with a production of 2,131,000 metric tons per year (F.A.O., 1962), most of which is consumed within the country. It is estimated that about 20–30% wastage occurs during transport and marketing, partly due to the prolonged exposure of fruits to the high tropical temperatures, which initiates ripening changes resulting in increasing susceptibility to mechanical and microbial spoilage. Any treatment which can slow down the ripening in bananas under the existing conditions will be of advantage to the banana trade.

Inhibition of ripening in bananas by gamma irradiation has been reported by Amezquita et al., 1965; Brownell, 1952; Ferguson et al., 1966; Hannan, 1955; Kahan et al., 1966; Luse et al., 1966; Maxie et al., 1968 and Teas et al., 1962. However, there seems to be marked disagreement in the literature with regard to the optimum dose and maximum tolerable dose. This may be attributed to the variation in varieties, physiological status of the fruit at the time of irradiation and the time interval between harvesting and irradiation. Few reports are available on the effect of gamma irradiation on freshly harvested bananas of specific maturity.

Our findings on the effect of gamma irradiation on the postharvest physiology of five banana varieties of commercial importance grown in India are discussed in this paper.

MATERIALS & METHODS

BANANA varieties (local names are given in parentheses), Giant Cavendish (Harichal), Red (Lal Kela), Fill Basket (Velchi or Poovan) and French Plantain (Rajeli) of known maturities, were harvested from a banana plantation in Bassein, 40 miles away from Trombay and brought to the laboratory the same day, either by rail or road. Dwarf Cavendish (Basrai) was obtained through a local banana dealer, after being transported 200 miles by truck, and was a day old. Only fruits free from abrasions and mechanical bruises were chosen.

Maturity of the fruits at harvest was determined on the basis of pulp-to-skin ratio and fullness of the fingers as judged by angularities (Paul Thomas, 1969). Odor of the pulp and respiration rate before irradiation treatments confirmed that the bananas were in the preclimacteric stage.

Irradiation of banana fruits

Bananas were irradiated either as hands (cluster of fruits) or as individual fruits (fingers). The cut ends of the hands and fingers after drying were smeared with a fungicidal paste containing pentachloronitrobenzene to check fungal rot during storage. Equal numbers of fingers from the various hands were taken in each treatment to nullify any variation in maturity between hands of the same bunch.

Irradiation of fingers was carried out in a gamma cell 220 (Atomic Energy of Canada Ltd., 22,500 curies) in air at an ambient temperature of 25–26°C. The dose rate was 14.5 krad per minute. The irradiation chamber, 6 by 8 in., showed axial respective dose-rate variations of 0.856, 0.931 and 0.832M Mrads/hr in the bottom, middle and upper 2.5-in. regions.

Hands were irradiated in a Package irradiator (Kumta and Sreenivasan, 1966) in air at an ambient temperature of 25–26°C. Dose rate was approximately 0.25 krad per minute. The ratio of maximum to minimum dose received within the container box of 12 by 6 by 6-in. size was 1.4. Dosimetry was performed either by the ferrous sulfate or the ceric sulfate aqueous dosimeter. In all the experiments, fruits were irradiated within 20–30 hr of harvesting.

Irradiation of banana fruits in different gaseous atmospheres

Banana fruits were kept flushed under an atmosphere of nitrogen, or CO_2 in glass desiccators for 1.5–2 hr to equilibrate with the respective gases. The fingers were quickly transferred to polyethylene bags of 2.5-mil thickness and heat sealed, with appropriate gas being simultaneously blown in and exhausted from the bags, this whole operation taking only a few seconds. Irradiation was carried out in the gamma cell 220 and the bags were cut open after irradiation and the fruits stored in air.

In another set of experiments, bananas were irradiated under nitrogen in the glass desiccator itself in the package irradiator. In all these experiments the nitrogen gas from cylinder was bubbled through 20% alkaline pyrogallol to remove traces of oxygen present and a Beckman oxygen analyzer was used to monitor the oxygen level in the desiccators.

Determination of ripening rate

Ripening rate was recorded by observing each fruit in the samples at regular intervals. A numerical value of color: 1–green; 2–greenish-yellow; 3–yellowish-green; 4–yellow with green tip; 5–full yellow; 6–yellow with brown flecks; 7–yellow with brown or black patches (over-ripe) was assigned to each fruit in a given sample at each observation time. The above index was found suitable for all the varieties except Red, for which fruits were given numerical values from 1–6 based on skin color and fruit firmness, as follows: 1–greenish-red and hard; 2–reddish-green and hard; 3–red with green tip; 4–full-red and slightly soft; 5–red and soft; 6–red with brown or black patches (over-ripe). A coefficient of ripening or mean color index was calculated as follows:

$$\text{Coefficient of ripening (Mean color index)} = \frac{\text{Total score for a sample}}{\text{No. of fruits in the sample}}$$

Fruit respiration. Respiration rates of individual fruits (taken from the same hands) in duplicate were determined at 24-hr intervals till the fruits ripened. Individual fruits were placed in 2-liter glass desiccators fitted with rubber corks containing air inlet and outlet tubes. A continuous flow of carbon dioxide free air at the rate of 50 cc per minute was maintained and the carbon dioxide evolved by the fruits during a 2-hr experimental setup was trapped in a Pettenkoffer tube containing 50 cc of 0.1N $Ba(OH)_2$. The CO_2 output was calculated by titrating the excess alkali against 0.1N HCl and expressed as mg CO_2/kg fruit/hr.

Chemical constituents

Reducing and total sugars were determined colorimetrically by Somogyi's method as modified by Nelson, 1944. Ascorbic acid was estimated by visual titration, against 2,4-dichlorophenol indophenol dye (AOAC, 1960). For total titratable acids and pH, 10g fresh tissue was homogenized in a Waring Blendor with 100 cc glass-distilled water for 3–4 minutes. The pH of the slurry was determined using a Beckman pH meter. After centrifuging the slurry, a suitable aliquot from the supernatant was titrated against 0.1N NaOH using phenolphthalein as indicator to an end point of pH 8.0 and expressed as cubic centimeters of 0.1N NaOH required to neutralize 100g fresh tissue.

Total soluble solids were determined with a Bellingham and Stanly pocket refractometer

Table 1—Optimum dose, maximum tolerance dose and extension in storage life of bananas irradiated at the preclimacteric stage.

Variety	Optimum dose (krad)	Maximum tolerance dose (krad)	Extension in storage life in days over controls at 24–29°C, R.H. 75–80%	
			In preclimacteric condition	Total
Dwarf Cavendish	30	40	4	8
Giant Cavendish	35	40	3–4	7–8
Fill Basket	25	35	4–5	8–9
Red	40	50	4–5	7–8
French Plantain	20	30	8	14

Banana fruits harvested at 75% maturity were irradiated in the preclimacteric stage. Maturity was determined based on pulp-to-skin ratio at harvest. Fruits with pulp-to-skin ratio of 1.40:1.0 to 1.50:1.0 were classified as 75% mature. The ratios were 1.49:1.0 for Dwarf Cavendish; 1.45:1.0 for Giant Cavendish; 1.5:1.0 for Fill Basket; 1.5:1.0 for Red and 1.48:1.0 for French Plantain.

Table 2—Effect of fruit maturity and post-irradiation storage temperature on irradiation-induced inhibition of ripening in bananas.

Variety	Fruit maturity at harvest (%)	Dose in krad	Extension in storage life in days over controls			
			At 29–32°C		At 21°C	
			In preclimacteric condition	Total	In preclimacteric condition	Total
Giant Cavendish	75	35	2	4–5	5–6	9–10
	85	35	1	3	4	7–8
Red	70	40	4–5	7–8	6–7	9–10
	85	40	2–3	4–5	4–5	7–8
Fill Basket	75	25	–	–	7–8	11–12
	90	25	–	–	3–4	7–8

Fruits of different maturities were irradiated in the preclimacteric stage. In each experiment 25 fruits in duplicate from each maturity stage were irradiated to the optimum dose levels. Pulp-to-skin ratios at harvest were as follows: Giant Cavendish: 75% mature, 1.45:1.0; 85% mature, 1.7:1.0; Red: 70% mature, 1.3:1.0; 85% mature, 1.7:1.0; Fill Basket: 75% mature, 1.45:1.0; 90% mature, 1.80:1.0.

and expressed as percent sucrose. Texture of whole fruit and pulp was measured with a Magness Taylor pressure tester.

Organoleptic evaluation

For organoleptic evaluation, irradiated and control fruits were ripened at 21°C with or without ethylene treatment. Fruits of color stage 5 were peeled and slices of pulp were placed for assessing flavor, texture and taste. For evaluating skin color and over-all appearance of fruits, whole fruits were kept. A panel of 10 judges scored on a 9-point Hedonic scale, from 1, extreme dislike, to 9, extreme liking. The final rating was obtained by averaging out the marks, with 9 as the highest. A score of 5.5 or above was considered acceptable.

Ethylene and 2,4-dichlorophenoxyacetic acid treatments

2,4-D was dissolved in water and 1% Tween-80 was added as an adjuvant. Fruits were immersed in the above solution for 10 min and then dried under a fan at ambient temperature.

For ethylene treatment, fruits were exposed to 200 ppm of ethylene gas for 24 hr at 21°C.

RESULTS & DISCUSSION

IN THE initial experiments, 10 fingers each from different varieties of bananas harvested at 75% maturity were irradiated to doses of 5, 10, 15, 20, 25, 30, 40, 50, 75, 100 and 200 krad. The optimum dose range for each variety was assessed from this experiment as the range showing maximum delay in ripening and minimum skin injury. Based on these results two subsequent experiments were conducted using banana fingers of similar maturity. In each experiment 20 fingers each were irradiated to 15, 20, 25, 30, 35, 40 and 50 krad to determine the optimum dose for each variety.

In general, delayed ripening was observed in all irradiated fruits, irrespective of the dosage employed or variety. At doses up to 35 krad, a linear relationship seems to exist between dose and rate of ripening. It is discernible from Table 1 that the optimum dose and the maximum tolerable dose varied from variety to variety. Variety Red could withstand doses up to 50 krad without much skin damage, whereas varieties Fill Basket and French Plantain were highly susceptible to irradiation dose exceeding 30 krad. The Cavendish group could tolerate up to 40 krad of gamma rays.

Irrespective of varietal differences, doses exceeding 50 krad caused deleterious effects on bananas, when irradiated in the preclimacteric stage. 100 krad and above caused extensive splitting and complete blackening of the skin during subsequent storage. Such fruits had soft and mealy pulp with slight cooked flavor, breaking into lumps on mild application of pressure. Ferguson et al., 1966, observed skin splitting and discoloration in Gros Michel bananas irradiated at 150 krad and above in small closed chambers. They attributed this to ozone formed during irradiation rather than radiation damage. They further stated that this radiation damage could be overcome by irradiating the bananas in well-ventilated rooms where ozone formed during irradiation could be removed. However, Maxie et al., 1968, reported that aeration of the irradiation chamber during treatments did not influence radiation injury on bananas at higher doses.

Our studies on irradiation of preclimacteric bananas to 100 and 200 krad under different gaseous atmospheres showed that anoxia did not reduce the magnitude of radiation damage considerably. Bussel (1965) reported that the internal atmosphere of Bartlett pears could be completely freed of oxygen in about 40 min when the fruits were held in nitrogen flowing at the rate of 200 ml per minute. Thus, it is unlikely that in our experiments, the ozone formed from traces of oxygen present would have contributed to radiation damage. Studies in our laboratories have shown that increased polyphenol oxidase activity on irradiation may contribute to skin discoloration in banana fruits (Paul Thomas and Nair, 1970).

The physiological state of the banana fruits at the time of irradiation influences the fruit's capacity to withstand higher doses of irradiation. Thus, Giant Cavendish banana fruits irradiated at color stage 3 (yellowish-green) could tolerate up to 200 krad without much pronounced radiation damage except the fruit tips remained brownish-green at the fully ripe stage. However, no delay in ripening rate was observed as judged by skin color and total soluble solids. The reported observation of Ferguson et al., 1966, that Gros Michel bananas could tolerate doses up to 200 krad may be because the bananas were no longer in the preclimacteric stage at the time of irradiation. Their data show that the fruits were at the color stage 2–3 (light-green to 50% yellow) at the time of irradiation.

The extreme susceptibility of bananas in the preclimacteric stage to doses above the threshold level was evident when banana hands were irradiated to optimum dose levels in a package irradiator, where the overdose ratio, i.e., the ratio of maximum to minimum dose, was 1.4. Some fingers in the hand which had received doses above the threshold level showed skin discoloration, whereas, the rest of the fingers receiving the optimum dose level ripened to give good yellow color. This shows that, in large-scale irradiation programs, especially with bananas, the dose distribution has to be kept reasonably uniform to avoid radiation damage to the fruits.

The earlier report from this laboratory that gamma irradiation did not signifi-

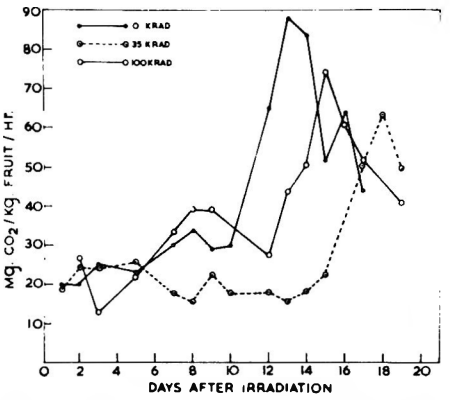

Fig. 1–Effect of gamma irradiation on respiration rate of preclimacteric Giant Cavendish banana fruits of 75% maturity (pulp-to-skin ratio at harvest–1.45:1.0).

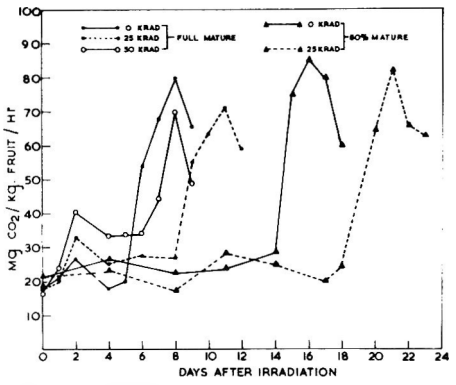

Fig. 2–Effect of gamma irradiation on respiration rate of preclimacteric Fill Basket banana of different maturity. (Pulp-to-skin ratio at harvest–fully mature, 1.95:1.0; 80% mature, 1.62:1.0.)

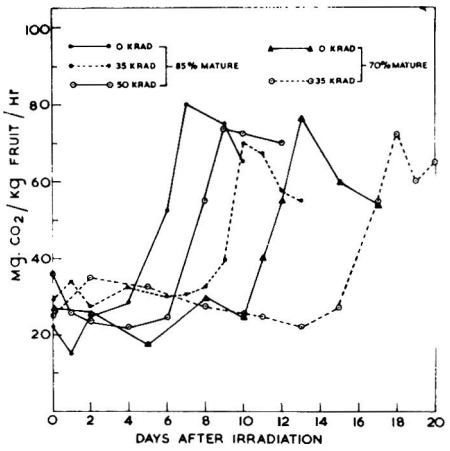

Fig. 3–Effect of gamma irradiation on respiration rate of preclimacteric Red bananas of different maturity. (Pulp-to-skin ratio at harvest–85% mature, 1.7:1.0; 70% mature, 1.3:1.0.)

cantly delay ripening in four varieties of bananas, irradiated at fully mature but unripe stage (Dharkar and Sreenivasan, 1966), was because the bananas used in the above experiments were no longer in the preclimacteric stage.

Effect of fruit maturity at the time of irradiation and post-irradiation storage temperature on rate of ripening

In these studies bananas were harvested at different stages of maturity and were irradiated to optimum dose levels in the preclimacteric stage. Results are shown in Table 2. Two important conclusions have been drawn from these studies, namely 1) the maturity of the fruit at the time of irradiation considerably influences the post-irradiation storage behavior, the maximum response to irradiation in terms of delayed ripening being observed with less-matured fruits, and 2) on the influence of post-irradiation storage temperature on the ripening rate, the maximum benefit was obtained when fruits were stored at 21°C rather than at 29–32°C. This observation is contrary to the report of Luse et al., 1966, stating that 10–40 krad stimulated ripening in Monte Cristo bananas stored at 68°F, whereas at 75 and 85°F, 20–40 krad retarded ripening.

Fruit respiration

Figure 1 shows the respiratory pattern of Giant Cavendish bananas irradiated to 35 and 100 krad at 75% maturity. The climacteric onset in fruits irradiated to 35 and 100 krad was delayed by 5 and 2 days, respectively, over the controls. However, the time required to reach the respiratory peak after the climacteric onset was about the same for both irradiated and control fruits. The respiratory CO_2 evolution at the climacteric peak was found to be comparatively low in irradiated bananas.

Figures 2 and 3, respectively, show the respiratory pattern of Fill Basket and Red bananas irradiated at two stages of maturity. It is clear from the data that the extent of delay in the onset of climacteric varied with fruit maturity at the time of irradiation.

Textural changes. Figure 4 shows the immediate effect of gamma irradiation on whole fruit and pulp texture. The extent of radiation-induced softening was found to vary among the varieties. Up to 50 krad there was little effect on either whole fruit or pulp texture. In the range of 50–100 krad, there was a marked loss in texture both in whole fruit as well as in the pulp. Above 100 krad the loss in tissue firmness was not as marked, but continued with increase in dose given.

Examination of the pulp tissues from Dwarf Cavendish bananas immediately after irradiation to doses of 30–200 krad failed to reveal any increases in reducing sugars, suggesting that in bananas it is unlikely that starch degradation by radiation contributes much to losses in texture. However, the possibility of random hydrolytic cleavages of starch molecule, to fragments of lower molecular weight like dextrins and oligosaccharides cannot be ruled out. The data suggest that most of the textural changes take place during or immediately after irradiation, which may be attributed to depolymerization and conversion of protopectin into soluble pectic substances as noted for many other fruits (Glegg et al., 1956; Kertesz et al., 1964; McArdle and Nehemias, 1956; Rouse and Dennison, 1968; Somogyi and Romani, 1964).

Ascorbic acid. The immediate effects of gamma irradiation of preclimacteric bananas in the range of 0–200 krad on ascorbic acid content are shown in Table 3. Loss in ascorbic acid was minimal up to 100 krad and maximum loss occurred at 200 krad. It can be seen that the extent of loss varied among varieties. Maximum loss occurred in the variety French Plantain, as high as 30% loss at 200 krad as against only 1.3% in Fill Basket at the same dose level. However, doses above 50 krad have no practical application, because of the severe injury to skin and pulp.

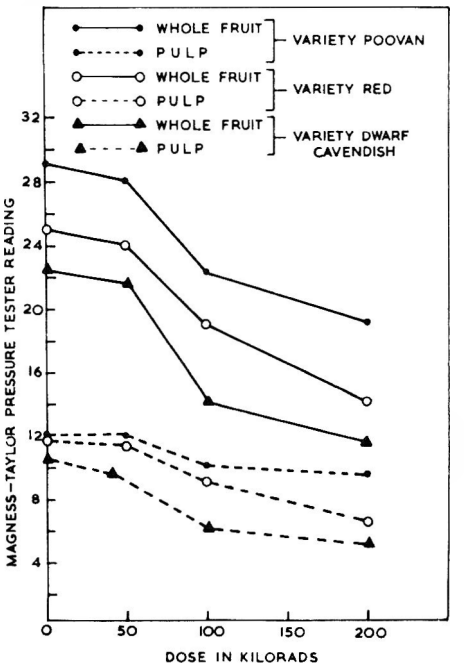

Fig. 4–Immediate effect of gamma irradiation on whole fruit and pulp texture of preclimacteric bananas.

Table 3—Immediate effect of gamma irradiation on ascorbic acid content of preclimacteric bananas.

Variety	mg ascorbic acid per 100g pulp[a]				Percent loss over control		
	0 krad	30 krad	100 krad	200 krad	30 krad	100 krad	200 krad
Dwarf Cavendish	11.12	12.04	10.75	10.59	0	3.3	4.8
Giant Cavendish	11.89	11.20	11.30	9.97	5.8	5.0	16.2
Fill Basket	12.07	12.07	12.60	11.92	0	0	1.3
Red	9.54	8.99	8.88	8.97	5.7	6.9	5.9
French Plantain	24.0	22.50	22.50	16.72	6.3	6.3	30.3

[a]Average value of three independent estimations on triplicate fruits.

Table 4—Effect of gamma irradiation on some chemical attributes of ripe bananas.

Variety	Treatment	T.S.S.	pH	Total titratable acids (ml 0.1N NaOH 100g pulp)	Ascorbic Acid (mg/100g pulp)	Sugars	
						Red. sugar	Total sugar
						(%)	
	Control	23.0	5.13	50.0	7.6	5.80	19.37
Giant Cavendish							
	35 krad	21.0	5.09	52.5	9.4	5.99	16.25
	Control	24.0	4.91	86.6	10.8	13.5	18.0
Fill Basket							
	25 krad	24.0	4.77	88.0	9.6	13.0	17.5
	Control	25.0	5.09	49.5	8.2	3.5	18.0
Red							
	40 krad	24.0	4.95	56.1	8.1	4.25	19.0

Banana fruits were irradiated at 75% maturity in the preclimacteric stage and allowed to ripen at 21°C; RH 85–90% without external ripening stimuli. Analyses were carried out at color stage 5 in all cases. Values reported are average of three independent estimations.

Effect of irradiation on chemical composition of ripe fruits

Table 4 shows the effects of irradiation on some chemical attributes of ripe bananas. On ripening, no differences were noticed in ascorbic acid levels between controls and irradiated fruits. In varieties Red and Fill Basket, reducing and total sugar values showed close agreement in irradiated and control fruits. However, in the variety Giant Cavendish the irradiated fruits recorded lower sugar and total soluble solids than controls, suggesting that though the color stages of both irradiated and control fruits were the same at the time of analysis, the hydrolysis of starch to sugars had not progressed at the same rate in the irradiated bananas. Ferguson et al. (1966) and Maxie et al. (1968) observed similar decreased rates of starch hydrolysis in irradiated Gros Michel bananas. Total titratable acids recorded slight increase and pH slight decrease in irradiated bananas.

Effect of quality of ripe bananas

Results of organoleptic evaluation of ripe fruits given in Table 5 indicate that bananas irradiated up to a dose of 40 krad compared favorably with unirradiated fruits. Some judges remarked that irradiated fruits were either starchy or slightly unripe. This suggests that color changes alone will not give an accurate index of the ripeness of irradiated bananas. Despite this, irradiated bananas were acceptable to the taste panel.

Antagonistic effect of ethylene and 2,4-dichlorophenoxy acetic acid on irradiation-induced delay in ripening

Ethylene or 2,4-D treatment either before or after irradiation offset irradiation-induced inhibition of ripening (Table 6). The observed difference in the ripening rates between 2,4-D and ethylene-treated fruits is not surprising. Ethylene is a more potent ripening stimulant and concentrations as low as 0.1–10 ppm are known to hasten ripening if applied to banana fruits in the preclimacteric stage (Biale, 1960). 2,4-D application is known to stimulate ethylene production (Morgan and Hall, 1962; Holm and Abeles, 1967) and this 2,4-D-induced ethylene may be responsible for the enhanced ripening in bananas.

The rate of ripening was comparatively slower in bananas, when irradiation preceded ethylene treatment as against irradiation after ethylene treatment. This suggests that the decreased sensitivity of irradiated fruits to the ripening action of ethylene may be the possible cause for irradiation-induced inhibition of ripening in bananas. That irradiation of bananas in

Table 5—Average hedonic ratings for irradiated and unirradiated bananas.

Dose in krad	Giant Cavendish	Dwarf Cavendish	Red	Fill Basket
0	6.0	6.0	7.0	6.5
20	6.8	7.0	7.0	6.7
25	6.5	6.8	6.8	6.2
30	7.0	6.8	6.3	5.6
35	6.8	6.2	7.0	6.0
40	6.5	6.0	6.9	6.3

Based on a 9-point hedonic scale as described under Materials & Methods. Values are the means of 20 evaluations each, for the various doses and varieties. A score of 5.5 or above is considered acceptable.

Table 6—Antagonistic effect of ethylene and 2,4-D on irradiation-induced delay in ripening of preclimacteric Giant Cavendish bananas.

Treatment	Mean color index									
	Days after treatment									
	2	4	6	8	11	13	14	16	18	20
Control	1.0	1.0	1.0	3.0	5.5	7.0				
2,4-D 1,000 ppm	1.0	3.5	4.7	5.6	7.0					
2,4-D 1,000 ppm + 35 krad	1.0	1.0	2.0	3.5	4.7	6.6	7.0			
35 krad + 2,4-D 1,000 ppm	1.0	1.0	1.5	3.0	4.2	6.5	7.0			
Ethylene 200 ppm	2.0	4.0	5.0	7.0						
Ethylene 200 ppm + 35 krad	1.0	3.0	5.0	7.0						
35 krad + 200 ppm ethylene	1.0	3.0	4.0	6.0						
35 krad	1.0	1.0	1.0	1.0	1.0	2.5	3.5	4.8	5.5	6.0

Giant Cavendish bananas of 75% maturity were subjected to various treatments as detailed above in the preclimacteric stage. Fruits were allowed to ripen at 21°C; RH 85–90%. Mean color index was recorded as detailed under Materials & Methods. The values are based on 20 fruits each in the various treatments.

which the ripening had already been initiated, as in the ethylene treatment prior to irradiation, did not result in inhibition of ripening changes, suggests that irradiation, like other treatments (gas storage, refrigeration, etc.) should be given when fruits are still in the preclimacteric stage.

The present investigations clearly indicated the necessity for establishing the optimum dose and maximum tolerable doses for each variety under study. Thus, an average dose range cannot be fixed for all banana varieties to obtain the desired effect by gamma irradiation.

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SOLUBILIZATION OF AQUEOUS SOLUTIONS IN NONPOLAR LIQUIDS

SUMMARY—Water was solubilized in orange oil, soybean oil, benzyl alcohol, n-heptane, n-hexane and cottonseed oil. Using dioctyl sodium sulfosuccinate, aqueous ascorbic acid was solubilized in orange oil, soybean oil, benzyl alcohol, n-heptane and n-hexane. Triglycerol monooleate and decaglycerol dioleate, or caprylic acid and ethoxylated stearic acid solubilized aqueous ascorbic acid in cottonseed oil. Solubilization applications are suggested.

INTRODUCTION

A SURFACTANT in solution can associate and form micelles above a certain concentration. The critical micelle concentration (CMC) is characteristic for a given surfactant-solvent system. Micelles vary in respect to size, shape and complexity.

The surfactant molecules of a micelle in water solution are generally oriented with their polar ends, or "heads," on the exterior surface of the micelle. The hydrophobic ends, or "tails," are in the interior. The interiors of such micelles can be considered minute pools of organic solvent. When the micelle can enclose molecules of a water insoluble substance to form a single phase system, solubilization is said to have occurred. This is not emulsification; that is, the solubilized substance is not dispersed in the water as a separate phase.

The technology of solubilization deals mostly with that of hydrophobic matter in water (Elworthy et al., 1968). However, water can be solubilized in hydrophobic liquids (Mathews and Hirschhorn, 1953; Reerink, 1958; Demchenko, 1960). Here, the surfactant molecules of the micelles are oriented with their nonpolar ends, or tails, on the exterior. The polar ends are in the interior. The interiors of such micelles can be considered minute pools of polar solvent.

Very little has been published on secondary solubilization. Aebi and Wiebush (1959) studied the solubility of sodium chloride in organic solvents in the presence of surfactant and moisture. Kitahara and Kon-no (1966) found solubilization of water by ionic surfactants to decrease by the minor presence of "every kind of electrolyte." Wentz et al. (1969) studied oil-soluble surfactants as solubilizing agents for FD&C Violet No. 1 in perchloroethylene.

Our purpose in the work reported here was to develop a technology to include water soluble nutrients in nonpolar, ingestible liquids.

EXPERIMENTAL

Materials

All materials were commercially available, food grade, used without further purification since we were looking for simple industrial procedures.

Sodium dioctyl sulfosuccinate was Aerosol OT 100%, solid, obtained from American Cynamid Company. Triglycerol monooleate (Drewpol 3-1-OC), decaglycerol dioleate (Drewpol 10-2-0), ethoxylated stearic acid (Lipol 4S) and caprylic acid (Wecoline 895) were obtained from Drew Chemical Corporation. Orange oil, U.S.P. was obtained from Dodge & Olcott, Inc. Benzyl alcohol, n-hexane and n-heptane were reagent grade.

Soybean oil was refined edible grade and cottonseed oil was prime winter yellow, U.S.P. These were obtained from Welch, Holme & Clark Co., Inc.

Analysis for ascorbic acid

Ascorbic acid was determined by dichlorophenolindophenol titration (AOAC, 1950).

Procedure

Our general procedure was to dissolve appropriate surfactant in the nonpolar liquid to form a clear, one-phase system, using a magnetic bar and stirrer. We titrated this with water or an aqueous solution, slowly with stirring, until a persistent cloud resulted. No measurable error, within the range of accuracy employed in this work, was introduced by solubility of water in the various nonpolar liquids.

RESULTS

Solubilization of water

Water was added to 10.0g orange oil and 1.0g sodium dioctyl sulfosuccinate (Aerosol OT or AOT). 11.4% water was solubilized before a persistent cloud formed. The clarity of the system is evident in Figure 1. A one-phase system results.

AOT did not form water solubilizing micelles in soybean oil. Benzyl alcohol added to the soybean oil provided a medium in which micellization occurred. The solubilizing capacity of the solvent system increased as the quantity of ben-

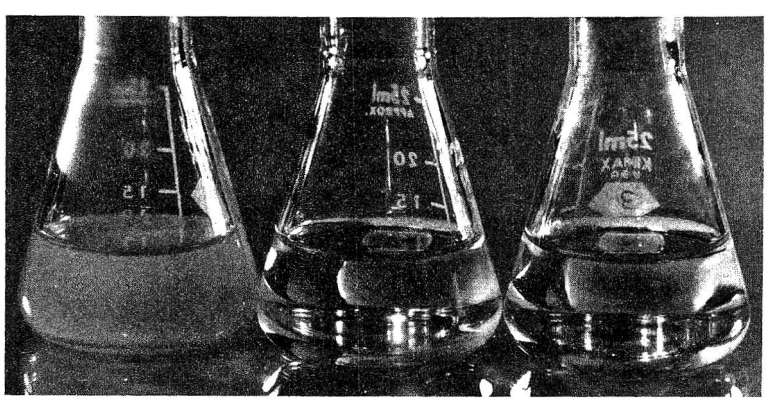

Fig. 1—Solubilization of water forming a one-phase system. Flask at left contains 10.0g orange oil + 0.57 ml water; center, 10.0g orange oil + 1.0g AOT; right, 10.0g orange oil + 1.0g AOT + 0.57 ml water.

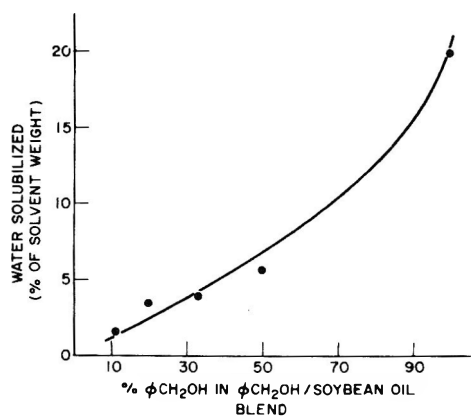

Fig. 2—Relationship between quantity of benzyl alcohol added to soybean oil and quantity of water solubilized. 1.0g AOT was used as solubilizer in 10.0g benzyl alcohol/soybean oil blend.

Table 1–Stability of ascorbic acid, solubilized as 1.0 ml of 20% aqueous solution in 10.0g orange oil containing 1.0g AOT.

Time (weeks)	Ascorbic acid (mg/ml of total system)
Initial	18.75
1	15.40
2	14.15
3	12.70

Table 2–Stability of ascorbic acid, solubilized as 1.10 ml of 20% aqueous solution in 10.0g n-heptane and 10.0g n-hexane, respectively, using 1.0g AOT, as solubilizer.

Time (weeks)	Ascorbic acid (mg/ml of total system)	
	n-Heptane	n-Hexane
Initial	14.92	14.62
1	13.6	13.5
2	12.6	12.3

Table 3–Stability of ascorbic acid, solubilized as in Table 2, but replacing water with 80% glycerol in water.

Time (days)	Ascorbic acid (mg/ml of total system)	
	n–Heptane	n-Hexane
Initial	14.35	14.38
10	13.20	13.20
24	–	12.35

zyl alcohol increased, as shown in Figure 2.

Solubilization of aqueous solutions

The next question was, could aqueous solutions of nutrients be solubilized as such? Would the aqueous solutions be taken up by appropriate micelles?

We dissolved 1.0g AOT in 10.0g orange oil. This took up 1.0 ml of 20% aqueous ascorbic acid yielding a clear, one-phase system as shown in Figure 3. Analysis of the system for ascorbic acid verified its presence and gave a measure of its stability stored at room temperature. (Refer to Table 1.)

When we added the ascorbic acid as a solid to the water solubilized in orange oil, it was taken up to yield a one-phase system. However, orange oil containing the AOT but no water did not take up ascorbic acid.

The flask on the left in Figure 4 contains 1.0 ml water solubilized in 10.0g orange oil, using 1.0g AOT; the flask on the right contains 1.0 ml 20% aqueous ascorbic acid. The flasks were stored overnight in a refrigerator. The first flask contains two phases, while the second contains a single phase. We observed this phenomenon repeatedly. The micelles incorporating solution appear to be more stable than the micelles incorporating only water.

10.0g n-heptane and 1.0g AOT took up 1.10 ml 20% aqueous ascorbic acid to yield a single phase. We prepared a similar system using 10.0g n-hexane. (Youngs and Gilles, 1970, report these hydrocarbons to be present in wheat flour.) We found ascorbic acid in the systems with stabilities as shown in Table 2.

The water can be replaced with 80% glycerol in water, with results as shown in Table 3.

2.0g triglycerol monooleate and 6 mg decaglycerol dioleate, dissolved in 10.9 ml cottonseed oil, solubilized 0.26 ml 20% aqueous ascorbic acid. 0.3g ethoxylated stearic acid and 1.35 ml caprylic acid, dissolved in 10.9 ml cottonseed oil, solubilized 0.08 ml 20% aqueous ascorbic acid.

DISCUSSION

USING the technique of solubilization, water soluble materials can be added to liquids with which they are otherwise incompatible. This should become a valuable tool in food technology. Possible applications are:

.... water soluble nutrients into essential oils for inclusion in beverages;

.... water soluble nutrients into vegetable oils for inclusion on the surface of crackers and dry cereals, and in peanut butter;

.... water soluble curing agents in oils for injection into meats for increased tissue distribution;

.... water soluble taste enhancers solubilized in oils for protection from enzymes and for greater compatibility with, for example, meats and flour.

Unpleasant tasting materials solubilized in bland lipophilic liquids are taste masked.

Solubilization can be considered submicroencapsulation. Traditional microcapsules are of an order of magnitude of as low as 20–50μ. Micelles incorporating solubilized material are of the order of 50 Angstroms. The micellar material is a barrier, or retainer, in the manner of capsular wall material.

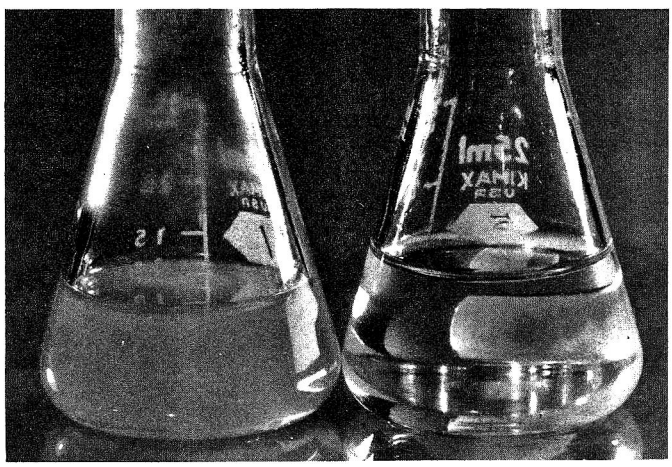

Fig. 3–Solubilization of aqueous solutions. Left flask contains 10.0g orange oil + 1.0 ml 20% aqueous ascorbic acid; right flask containing 10.0g orange oil + 1.0g AOT + 1.0 ml 20% aqueous ascorbic acid yielded a clear, one-phase system.

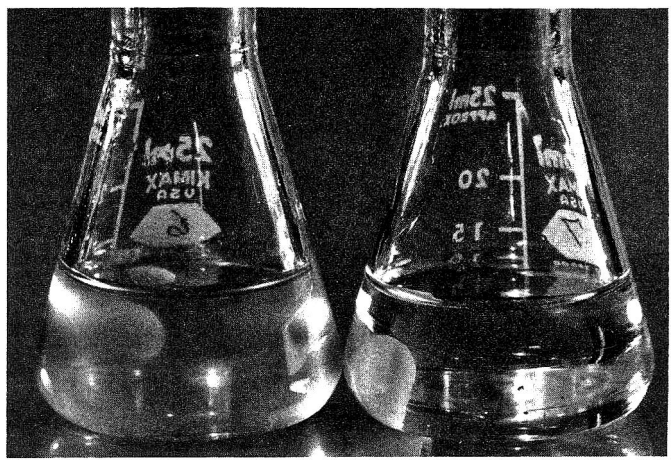

Fig. 4–Solubilization of aqueous solutions. Left flask with 10.0g orange oil + 1.0g AOT + 1.0 ml water contains two phases; right flask with 10.0g orange oil + 1.0g AOT + 1.0 ml 20% aqueous ascorbic acid contains a single phase. Both flasks were held overnight at 40°C.

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REDUCTION OF INTESTINAL GAS-FORMING PROPERTIES OF LEGUMES BY TRADITIONAL AND EXPERIMENTAL FOOD PROCESSING METHODS

SUMMARY—The propensity of legumes to promote formation of intestinal gas was evaluated in healthy young men by measuring flatus passed from the rectum and the amount of bacterially formed gas in the exhaled breath. Previous experience has shown that flatulent test meals fed in the morning cause elevation of breath hydrogen concentration, flatus volume and flatus hydrogen and carbon dioxide content about 5–7 hr later. Test responses were measured against a baseline derived from feeding a bland, low-residue formula diet. In the present study, mature, dry lima beans were found to be as high in flatulence-inducing factor(s) (FF) as are California small white beans. Mung beans and soybeans caused the same excretion of hydrogen in the breath as did white beans, but only about 2/3 as much flatus. Peanuts were not gas-forming. When the dosage of soybeans was doubled, so that the carbohydrate contribution from the two varieties was more nearly equalized, soybeans caused as much passage of flatus as did white beans. Several products processed by methods that might be expected to alter FF concentration or activity were evaluated in the same way. Soybean and mung bean sprouts appeared to retain most of the FF present in the whole bean. Soybean curd (tofu) had little residual FF but another high-protein food, MPF, made from toasted soy grits, caused as much gas formation as an equal weight of soybean carbohydrate fed as whole beans. Enzymatic treatment of comminuted soybeans, designed to hydrolyze the constituent oligosaccharides, raffinose and stachyose, frequently cited as gas-formers, had only negligible effect on production of intestinal gas. Tempeh, made from soybean grits by mold fermentation, did not increase gas production over baseline values and caused a significant delay in the time of gas formation, suggestive of temporary suppression of intestinal bacteria. Ethanolic extraction of whole white beans reduced but did not eliminate their gas-forming quality.

INTRODUCTION

LEGUMES are notorious producers of flatulence, some more so than others (Murphy, 1964; Steggerda and Dimmick, 1968; Hellendoorn, 1969). In man, common, mature, dry white beans reportedly produce more intestinal gas than do mature lima beans, soybeans, peas or lentils. Peanuts have not been tested for this property but peanut flour is said to cause gas formation (Rackis et al., 1967). On the other hand, immature green lima beans have little gas-forming quality (Murphy, 1964; 1969), indicating that the flatulence factor(s) (FF) develop during ripening and in different concentrations according to species, variety or growth conditions.

Several investigators have attempted to characterize the FF in beans. Murphy (1963; 1964) reported 10-fold concentration of FF from cooked dry white beans by a series of steps involving successive extractions with diethyl ether and hot 60% ethanol. The active fraction, low in molecular weight, contained polypeptides and sugars such as the oligosaccharides, stachyose and raffinose, and sucrose. Steggerda et al. (1966) subsequently reported the presence of an alcohol-soluble FF in soybeans. Although Hellendoorn (1969) found that alcohol-extracted Dutch brown beans "more or less maintained their flatulence activity," his procedure was less exhaustive, involving only double-extraction of cooked, dehydrated bean powder with 60% ethanol.

Most investigators ascribe flatulence to the action of intestinal anaerobic microflora on the oligosaccharides present in mature beans and their alcoholic extracts, which can be degraded by bacterial but not mammalian enzymes. Favoring this view is the fact that the oligosaccharides accumulate in beans during later stages of ripening (Gould and Greenshields, 1964). Also, Murphy (1969) has reported that flatulence production begins when lima beans reach the green ripe stage, about 2 wk before harvest, and increases steadily thereafter. However, white beans have less oligosaccharides than do soybeans, yet they provoke more flatulence; therefore, more than one factor may be involved in the final experience.

Some traditional methods of processing might be expected to alter bean FF or its behavior. The Indonesian soybean product, tempeh, is made by fermentation with a species of *Rhizopus* (Hesseltine, 1965); during fermentation, oligosaccharide content is diminished (Shallenberger, 1967) and an antibacterial compound is added (Wang et al., 1969). The manufacture of soybean curd, tofu, involves precipitation with calcium or magnesium salts from soybean milk and results in a product that includes most of the initial protein and fat but very little carbohydrate (Harris et al., 1949).

Among other compositional changes that occur during germination, the oligosaccharides, verbascose, stachyose and raffinose, disappear early from the embryo but are present in the cotyledon until the lateral roots appear at 6–7 days (Gould and Greenshields, 1964). This is a more advanced stage than is customary for bean sprouts intended for food use, and in an experimental preparation full germination of white beans failed to affect their gas-forming attribute (Murphy, 1969). However, sprouts from the usual food species, soybeans and mung beans, have not been evaluated.

In the present study, the gas-forming potential of intact legumes and these traditional bean products was evaluated by analysis of bacterially produced gases in the breath and flatus of healthy men. Experimental products, involving enzymatic treatment of soybeans and alcoholic extraction of whole white beans, also were examined. The results indicate improved quality in some of the products.

MATERIALS & METHODS

VOLUNTARY subjects of this study were healthy young men drawn from the University community. All subjects were free of known food allergy and had no recent history of gastrointestinal disorders or antibiotic usage. Tests were conducted in random sequence several months, and individual tests were separated by at least one nontest day. The number of subjects per treatment varied but no fewer than four men received each test item.

The subjects were requested to fast from 11 PM of the night preceding each test day and were given the test food at 9 AM (zero time). They were allowed 30 min to complete the meal. With few exceptions, made necessary by the nature of the products, content of each test meal was adjusted to 800 kcal and similar proximate composition by addition of anhydrous butterfat, sucrose, Swiss cheese, French bread, apple jelly or orange-flavored beverage base. (By our criteria none of these foods is gas-forming.) Decaffeinated coffee (2.5g dry solids) and enough deionized water were given to bring the total water intake from fluid-plus foods to 1,000g. A 600-kcal serving of a nutritionally balanced bland formula (Calloway and Murphy, 1968) was given at 1 and 5 PM and beverages were equalized between meals. This formula was also fed as a separate 800-kcal test meal, to determine baseline intestinal gas production throughout a test day.

Breath and flatus collection and analysis

Breath samples were obtained before and at 30-min intervals after the test meal (p.c.) until either 5 or 7:30 PM (8–10.5 hr p.c.). Rectal flatus was collected from 10 AM through 5 or 6 PM (8–9 hr p.c.). This was done by affixing to the shaved buttocks a colostomy patch with attached Tygon tube. Both breath and flatus

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Table 1—Calculated composition of test foods.

Test item	Weight[a] (g)	Composition of test item[b] kcal	Pro	Fat (g)	CHO	Approx bean equiv (g)
Bland formula[c]	382	800	23.2	26.5	130.0	–
Soybeans[d]	200	806	68.2	35.4	67.0	Same
	100	403	34.1	17.7	33.5	Same
Soybean sprouts[e]	300	138	18.6	4.2	15.9	50[m]
Tofu[e]	300	216	23.4	12.6	7.2	70[m]
Tempeh[f]	199	501	39.6	35.0	17.2	100
Tempeh control[f]	200	384	39.6	22.2	15.0	100
Comminuted soybeans[g]						
Enzyme treated and control	344	302	25.6	13.3	25.1	70
MPF[h]	136	340	68.0	1.4	21.1	200[m]
Peanuts[i]	165	960	43.2	80.4	34.0	Same
Mung beans[i]	100	340	24.2	1.3	60.3	Same
Mung bean sprouts[i]	300	105	11.4	6.0	19.8	50[m]
Baked navy beans						
SRI alcohol extracted[j]	352	464	23.3	5.3	83.7	100
SRI control[j]	306	(464)	(23.2)	(5.3)		100
Canned[k]	450	681	28.1	21.3	95.8	100
Lima beans[l]	100	345	20.4	1.6	64.0	100

[a]Weight of that item as purchased. Test foods were fed as all or part of 800-kcal meals. (See text.)

[b]Calculated from Watt and Merrill (1963) except MPF (label declaration), tempeh (Wang et al., 1968) and formula (our laboratory analysis).

[c]Composed of spray-dried egg white; sucrose, dextrimaltose and cornstarch; corn oil and hydrogenated vegetable fat; citric acid and minerals (Calloway and Murphy, 1968).

[d]Yellow variety, Quong Fat Co., San Francisco.

[e]Wo Chang Co., San Francisco.

[f]Northern Utilization Research and Development Division, USDA, Peoria, Ill.

[g]Rohm and Haas Co., Philadelphia.

[h]General Mills, Inc., Minneapolis.

[i]Shing Chong Co., San Francisco.

[j]Stanford Research Inst., Menlo Park, California. Values for treated beans, in parentheses, are approximate.

[k]S & W brand.

[l]Seaside brand.

[m]Equivalence based on protein. (See text.)

were collected in evacuated multilaminate metallized plastic bags impervious to diffusion of gases. Breath samples were analyzed chromatographically for concentration of hydrogen and methane, as described previously (Calloway and Murphy, 1968). The total volume of flatus collected was measured manometrically and the percentages of oxygen, nitrogen, carbon dioxide, methane and hydrogen determined chromatographically as before.

Breath values from 2.5 to 10.5 hr p.c. were evaluated by: 1) time of occurrence and magnitude of peak hydrogen production, 2) mean of the five highest hydrogen values and 3) the total area under the hydrogen production curve for each subject. Areas were calculated by the parabolic rule, using an odd number of points and an even number of intervals. Breath methane values are not included here, since they have been found to be characteristic of each individual and to show cyclic variation rather than to vary with the test meal (Calloway, 1966).

Flatus production was evaluated by comparing the total flatus volumes during the 4th through the 10th hr p.c., and by noting the changes in flatus composition as different test items were fed.

Foods

Mature soybeans (*Glycine max.*), mung beans (*Phaseolus mungo*), lima beans (*Phaseolus lunatis*) and white beans (*Phaseolus vulgaris*) were all fed cooked, in amounts equal to 100g weight of dry beans as purchased. At this dosage. soybeans provided about 34g of carbohydrate and the other beans about 60g (Table 1). A 200-g dose of soybeans was also fed to provide more nearly equivalent carbohydrate for comparisons among the legumes. Testing peanuts (*Arachis hypogaea*) at anything like this carbohydrate dosage was not feasible calorically; therefore, 165g of this legume was given, providing the same amount of carbohydrate as the 100-g dose of soybeans and exceeding the usual meal level by about 160 kcal.

Conventional commercial products were tested in which carbohydrate content would be modified so dose equivalents had to be computed from protein content. Amounts fed were judged to be three times a standard serving weight. These products were: sprouts from soybeans and mung beans (300g wet raw weight, ≃50g of dry beans); soybean curd (tofu, 300g wet weight, ≃70g of dry beans) and MPF, a food supplement formulated from toasted soybean grits (136g as purchased ≃200g beans). Our standardized gas-producing meal of canned oven-baked beans was included for comparison. The men were fed the entire contents of a No. 303 can (approximately equal to 100g dry weight of small white beans).

Tempeh was manufactured by C. W. Hesseltine and H. L. Wang of the Northern Utilization Research and Development Division of the USDA at Peoria, Illinois. Mechanically dehulled and cracked soybeans (Hawkeye variety) were washed many times with excess water to remove all broken seedcoats and foreign matter, soaked for 0.5 hr and inoculated with a suspension of *Rhizopus oligosporus* spores. After incubation at 31° for 24 hr the product was steamed for 5 min to destroy the mold, sliced and frozen at −20° for shipment. Control soybean grits were treated similarly except for the fermentation step. Weight served of both products was 200g. equal in protein to about 100g dry beans.

Comminuted soybeans (Kanrich variety) were treated enzymatically, to hydrolyze oligosaccharides into the component mono- and disaccharides, by F. J. Schindler of the Rohm and Haas Company. The bean slurry was autoclaved for 1 hr at 121°, adjusted to pH 5.3 with lactic acid, treated with the enzyme Diastase 80 at 50° for 16 hr and concentrated for 2 hr at 60° under reduced pressure to 20% solids content. The control sample, processed in the same way except for the enzyme step, contained typical amounts of raffinose (1.3% of dry solids) and stachyose (5.0%). The enzymatically treated sample contained negligible amounts of these sugars and increased amounts of mono- and disaccharides. Doses given, 344g, were equal to 70g of soybeans.

Ethanol-extracted white beans were prepared by R. D. Mathews of the Stanford Research Institute. Details of the processing method are subject to patent. but the product was heated just below boiling temperature for 50–60 min, extracted with 95% ethanol at 65–70° and dried in a forced-air oven. Both the extracted and control beans were oven-baked with addition of sugar, salt and flavorings according to good commercial practice, and frozen for storage.

Food preparation

Weighed portions of raw dry beans were soaked overnight, cooked at 15 lb pressure for 40 min and served with the cooking liquor, all of which was consumed by the subject. Precooked bean products were heated over boiling water and served in the heating container. Shelled raw peanuts with skins were oven roasted for 8–10 min at 150°.

Weighed portions of bean sprouts were sauteed in anhydrous butterfat for 4 min and steamed with added water for 12 min. MPF was prepared as a hot cereal, individually weighed dry portions being blended with 2.1 times the weight of water.

Tofu was sauteed in anhydrous butterfat. Tempeh was lightly browned in butterfat and then baked for 15 min at 175°; the tempeh control soybean grits were baked at this temperature for 20 min.

The enzymatically treated preparation of comminuted soybeans was of granular, semifluid consistency. It was extremely unpalatable and defied all our attempts at flavor enhancement. The men drank the unmodified material as a beverage. They had decaffeinated coffee but no other food in the test meal, which thus contained only 40% of the usual caloric allowance.

Balanced formula diet was served cold, as a beverage of milk-shake consistency.

RESULTS & DISCUSSION

Formula baseline

Intestinal gas production following

Table 2–Breath and flatus gases of men fed legume products.

Test food	Am't fed (g)[a]	No. S's	Breath hydrogen, 2.5–10.5 hr p.c. Peak Time hr p.c.	Peak Conc ppm	Avg 5 Highest values (ppm)	Area[b] under curve	Flatus Total[c] (ml/6 hr)	CO_2 (ml/6 hr)	CH_4 (ml/6 hr)	H_2 (ml/6 hr)	Corrected[c] volume (ml/hr)
Formula	382	28	6 ± 2[d]	14 ± 9	11 ± 7	142 ± 61	166 ± 79	4 ± 3	0.4 ± .8	2 ± 1	9
Cn. white beans[e]	450	12	6 ± 2	39 ± 19	30 ± 12	303 ± 201	323 ± 201	45 ± 67	26 ± 99	36 ± 48	36
Soybeans	100	6	5 ± 1	35 ± 9	27 ± 6	299 ± 80	252 ± 105	12 ± 12	3 ± 6	33 ± 42	24
Soybeans	200	6	7 ± 2	43 ± 11	35 ± 8	329 ± 57	368 ± 230	50 ± 42	4 ± 6	28 ± 31	43
Lima beans	100	4	5 ± 1	51 ± 17	38 ± 10	336 ± 100	361 ± 294				42
Mung beans	100	6	6 ± 2	30 ± 10	24 ± 7	292 ± 95	258 ± 96	17 ± 14	7 ± 9	25 ± 29	25
Peanuts	165	4	4 ± 3	13 ± 8	17 ± 9	89 ± 54	194 ± 17	2 ± 1	0	3 ± 2	14
Soybean sprouts	300	6	5 ± 2	27 ± 6	21 ± 5	175 ± 29	214 ± 46	6 ± 6	0.6 ± .9	5 ± 8	17
Mung bean sprouts	300	5	6 ± 1	23 ± 7	14 ± 9	181 ± 58	216 ± 78	7 ± 6	6 ± 13	3 ± 2	18
Tempeh	199	6	9 ± 1	20 ± 5	16 ± 4	139 ± 36	169 ± 50	2 ± 2	0.4 ± .6	0.8 ± .7	10
Grits for tempeh	200	5	5 ± 1	18 ± 6	15 ± 5	175 ± 68	205 ± 73	6 ± 4	2 ± 2	4 ± 3	16
Tofu	300	6	8 ± 2	18 ± 7	13 ± 5	141 ± 59	240 ± 62	5 ± 2	1 ± 2	3 ± 1	22
MPF	136	4	7 ± 3	20 ± 11	16 ± 13	184 ± 78	300 ± 78	21 ± 4	1 ± 2	10 ± 12	32
Enzeme-treated soy	344	6	8 ± 2	24 ± 20	18 ± 14	190 ± 115	245 ± 75	9 ± 9	2 ± 3	6 ± 8	22
Proc. control	344	6	7 ± 1	28 ± 6	23 ± 6	226 ± 62	217 ± 60	7 ± 4	4 ± 4	9 ± 14	18
Alcohol-extracted beans[e]	100	7	7 ± 2	25 ± 8	20 ± 7	194 ± 81	174 ± 112	12 ± 25	13 ± 35	2 ± 3	11
Control beans[e]	100	8	6 ± 2	25 ± 9	21 ± 7	206 ± 87	335 ± 252	54 ± 88	17 ± 38	55 ± 67	38

[a]Weight as weighed. Dry beans, formula and MPF were served with added water. Equivalence to dry bean weights of the processed products is indicated in the text and Table 1.
[b]Integrated area under 8-hr hydrogen curve. (See text.)
[c]Total includes air due to voids in collection devices, averaging 110 ± 27 ml. Corrected flatus volume has had this sum subtracted.
[d]Values are mean and standard deviation.
[e]California small white beans.

administration of the bland formula as a test meal gives a baseline against which can be compared values obtained after administration of test items. The mean peak concentration of the bacterial gas, hydrogen, in the breath of 28 subjects fed this diet was 14 ± 9 ppm (Table 2). This was identical to the mean pretest, fasting value. Morning breath hydrogen values usually decrease until about 10 AM, irrespective of the nature of the test meal (Calloway, 1966). The peak hydrogen concentration during the formula tests was 9 ppm above the mean of the lowest morning values and occurred 6 ± 2 hr after the morning meal.

Occasionally a subject had unusually high breath hydrogen values with the formula, i.e., a peak of 30–35 ppm, which is reflected in the large error term. This was often an indication that the particular subject could be expected to show erratic responses to the items to be tested. We have no explanation for this phenomenon, but study of the microflora characteristic of these men and of their tolerance to the various components of the formula might prove enlightening.

The remaining indices of intestinal gas production show the same variability within the group but confirm the benign nature of the product (Table 2). As collected, the volume of flatus passed from the rectum during the 6 hr after the meal when production is usually at its height was 166 ± 79 ml. Most of this volume is air, due to the approximately 110 ml of voids in the tubing and collection devices; the true volume of flatus was about 9 ml per hour. The gases attributable to bacteria–hydrogen, methane and some carbon dioxide–account for 10% of the total volume, or 6 ml for the 6-hr period.

California small white beans

Breath and flatus values following a meal of commercially canned baked beans, our standard gas-producing test food, showed a doubling of breath hydrogen excretion and a four-fold increase in total flatus volume, as compared with the formula. Hourly flatus volume was 36 ml, 50% of which was carbon dioxide, hydrogen and methane. Thus, the total flatus volume increased four times but contained 17 times as much bacterial gases (counting all the carbon dioxide as being bacterial even though some is from endogenous sources). Flatus hydrogen was 36 ml and respiratory exchange accounted for about another 50 ml of hydrogen during the 6-hr period, in contrast to 2 and 25 ml, respectively, with the formula diet. The wide day-to-day variation observed within and between subjects again resulted in large standard deviations, comparable to those reported previously (Calloway and Burroughs, 1969).

Soybeans

Data from soybeans fed in 100- and 200-g amounts can be used to determine if the intestinal gas response is precisely dose-dependent within a variety (soybeans) or as regards total carbohydrate (100g white beans vs. 200g soybeans). The peak breath hydrogen concentration following consumption of 100g soybeans was 35 ± 9 ppm and that following 200g was 43 ± 11 ppm. The means of the five highest breath hydrogen values, total hydrogen production and flatus volume showed a similar pattern: an increase but not a linear response to doubling the test dose. Flatus component gases behaved dissimilarly, in that carbon dioxide increased four-fold with the doubled dose of beans but methane and hydrogen were unchanged. Four subjects included in this series were also fed 150g of soybeans, but flatus analyses are not available for comparison; therefore, the data are not tabulated. However, mean breath hydrogen was 30 ± 16 ppm at peak production and 19 ± 7 ppm taking the average of the five highest values. These means are lower than the average of the six men fed the

100- and 200-g doses. The erratic response emphasizes the importance of accounting for intra- and interindividual variation in any assay of FF.

Flatus volume with the 100-g meal of soybeans was about 24 ml per hour, or 2/3 of the volume recorded for the standard bean meal, but total breath hydrogen excretion was the same as with the canned beans. The 200-g dose of soybeans caused an insignificant increase in breath hydrogen and flatus volume as compared with the bean standard, confirming that soybeans are less flatulent than California white beans.

Other legumes

The lot of lima beans used in this study was equally as flatulent as canned white beans, in conflict with the earlier reports. Such divergence is not unreasonable, considering the opportunities for variation in composition due to bean variety, maturity at harvest and storage conditions. Response to mung beans was the same as that for soybeans and substantially less than the standard bean response.

Peanuts were absolutely nonflatulent. There was little variation within the small group of men given this treatment, and both breath hydrogen excretion and bacterial gases passed as flatus were equal to or less than the formula responses. The only noticeable difference was that the peak gas production time was shortened from 6 ± 2 to 4 ± 3 hr after the test meal. If defatted peanut flour is flatulent, as reported, then one of the following must be true: FF is remarkably concentrated by the defatting process; digestibility is adversely affected by the process; or a suppressant of gas formation is removed during lipid extraction. A comparison of extracted peanut flour and presscake might help to clarify these points.

Bean sprouts

The two types of bean sprouts fed, soybean and mung bean, gave almost identical reactions. Both induced slightly more breath hydrogen, total flatus and bacterial gases in the flatus than the baseline treatment but somewhat less than the 100-g doses of the beans. Thus, intestinal bacterial action was reduced in comparison to the intact beans but not less than would be anticipated from the decreased weight of bean cotyledon consumed (protein equivalent to 50g beans). Since the sprouts meals were as large in proportion to normal serving sizes (about 3×) as were the 100-g dry bean meals, and gas production was low, in all probability sprouts will not commonly be cited as problem foods which, in fact, they appear to be.

Tempeh

The mold-fermented food, tempeh, was essentially nonflatulent. There was a negligible increase over baseline values of breath hydrogen at peak production, but the total amount passed as flatus and in the breath and the total flatus volume were identical with the formula value. The grits from which the tempeh was made were not nearly as flatulent as our equivalent dosage of soybeans. However, there is evidence, both in total breath hydrogen excretion and in flatus, of the presence of somewhat more flatulence activity in the control grits than in the fermented product.

The time of peak gas production with tempeh was significantly delayed (about 4 hr) as compared with the control grits. The delayed peak in gas formation could be due to delayed gastric emptying of the fried tempeh, but this seems unlikely because both meals contained the same amount of fat and protein. Wang (personal communication) suggests that the delay could well be due to the presence of an antibiotic substance formed by the *Rhizopus*. Although this compound does not exhibit a broad spectrum of activity, it is said to be very active against many gram-positive bacteria, including some of the typical gas-forming inhabitants of the intestinal tract. The delayed peak would coincide with a theory of temporary suppression of gut flora.

Tofu

Low breath and flatus values were recorded after a meal of soybean curd, corresponding with the very low carbohydrate content of this product. Ingestion of 300g of tofu, an amount providing as much protein as 70g of soybeans, caused no difference in breath values over the baseline level and only a minor difference in bacterial gases in the flatus. Flatus volume was slightly larger than the baseline volume, but the difference was due to air components rather than to fermentation and is not clearly relatable to the food.

This traditional and simple method of food processing eliminates most of the FF from soybeans while conserving most of the protein and fat. As a point of interest, content of the mineral used for coagulation, either calcium or magnesium, is increased in the product (Harris et al., 1949). Since all diets are commonly below the recommended levels of calcium and magnesium and the diets of Oriental peoples are especially likely to be low in milk products and, therefore, calcium, this product is particularly advantageous.

MPF

MPF is a high-protein (50%) multipurpose food with toasted soy grits as the only protein source. The size of the test dose (136g) contained the same amount of protein (68g) as 200g of soybeans. This amount of MPF contains only 1/3 the carbohydrate content found in soybeans (21 vs. 67g) and very little fat (1.4g).

The peak breath hydrogen (20 ± 11 ppm) and the mean of the five highest breath values (16 ± 13 ppm) following the ingestion of MPF were less than half the values following a 200-g dose of soybeans and about the same as a 100-g meal of whole soybeans. Although the total flatus volume was nearly as high with MPF as soybeans, the flatus component bacterial gases following the ingestion of MPF were again less than half the carbon dioxide, methane and hydrogen values following a 200-g dose of soybeans. The data indicate that the gas-forming property of the soybean was retained in proportion to the amount of carbohydrate present and was otherwise unchanged by the processes applied.

Experimental enzymatic treatment

The enzymatically treated comminuted soybeans with accompanying control were fed in an amount equivalent to 70g of dry soybeans. The flatus and breath values observed following ingestion of both the control and enzymatically treated beans were approximately 75–85% of those observed with the 100-g soybean meal, with the exception of a greatly reduced flatus hydrogen component, 9 ± 14 ml vs. 33 ± 42 ml. The enzymatic treatment reduced the flatus and breath hydrogen values somewhat, but not significantly, despite the stipulated absence of stachyose and raffinose.

The extremely poor palatability of this product would deter its usage. However, on theoretical ground it might be worthwhile to attempt an improved process, perhaps beginning with defatted soybean meal.

Experimental alcoholic extraction

Previous attempts to remove FF from beans have utilized powdered or ground beans as being more satisfactory for extraction than whole ones. A commercially attractive method would attempt to retain typical bean structure and other characteristics.

Application of such an experimental method to white beans resulted in a product that was somewhat less flatulent than the processing control sample. There was no difference between the two samples of beans as evaluated by breath hydrogen production, but both were very much less stimulatory of hydrogen in the breath than was the standard canned bean treatment. The alcohol-extracted beans resulted in flatus bacterial gases three times as high as the baseline levels but only 1/5 as high as the amount produced with the control beans from which the extracted lot was made. In terms of flatus production, the processing control was identical to the standard bean meal.

The treatment appears not to have removed the FF from beans completely

but offers promise for further development.

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APPLIED SCIENCE and ENGINEERING

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EVALUATION OF CERTAIN PHYSICAL PROPERTIES OF MEAT USING A UNIVERSAL TESTING MACHINE

SUMMARY—The Instron tester served to evaluate physical properties of uncooked rabbit and beef muscle including work of rupture, breaking strength, break elongation elasticity and stress relaxation. These methods measure variations in muscle type, aging and post-mortem treatments comparably with shearing instruments. Shank showed higher tensile properties than tenderloin, less elasticity and lost more applied stress. With rabbit, the breaking force of longissimus dorsi unrestrained during rigor was .237 lb/g ± 7.5% for samples 5.0 cm by 0.2–0.5 cm² while restrained muscle gave .168 ± 9.9% and also exhibited higher elasticity and break elongation. Post-mortem aging decreased tensile properties and elasticity. Psoas muscle, characterized by more coextensive fibers, had higher tensile properties than longissimus dorsi.

INTRODUCTION

MEAT TEXTURE is an important factor in consumer acceptance and, as such, should be able to be assessed accurately. Texture, used here to connote mechanical properties rather than coarseness or fineness, can be measured objectively while tenderness implies subjective evaluation and must ultimately be organoleptically determined through appraisal of characteristics such as juiciness, ease of tooth penetration and residual fibers.

Presently, the most widely used objective method used for evaluation of meat texture involves various means by which force is applied via a blunt edge and the amount of force required to shear the sample is observed. This procedure is open to criticism on several grounds. Szczesniak and Torgeson (1965) emphasize the importance of such variables as orientation of muscle fibers, sample temperature, speed of shearing and blade dullness. They report variations in correlation coefficients between Warner-Bratzler shear values and taste panel reports varying from no significance to very high significance. Usually only the maximum shear force is measured and not the slope of the shear force curve which has been suggested to be more meaningful (Szczesniak and Torgeson, 1965). Shearing devices are inherently empirical in nature and, as pointed out by Sharrah et al. (1965), it is not clear that these instruments measure the same characteristics in meat as do sensory panels. Another common complaint is large standard deviations, over 20% of the mean in some cases, for replicates. Attempts have been made to correct shear force values for variations in sample dimensions by both Davey and Gilbert (1969b) and Pool and Klose (1969). Both groups found that shear force was more accurately expressed as a function of linear dimension rather than cross-sectional area and it may now be possible to mathematically adjust data to account for this variable. Perhaps the most serious objection from a theoretical viewpoint has been raised by Pool and Klose (1969) who suggest that meat samples subjected to shearing stress are distorted to the point that part of the applied shear force is altered to a tensile stress of the stretching fibers. The separation of fibers is due more to tensile force perpendicular to the blade than shear parallel to the blade. Another point would seem to apply to the Kramer-type shear press which supposedly measures compression as well as shearing. Only the fibers in immediate contact with the shearing bars receive the entire stress. Other areas obtain less and are compressed so that the total area is much smaller prior to rupture of any fibers. It appears that most of the work done is required to express fluid from the sample.

Clearly a method for assessing meat texture free from the above complications would be welcome. The first step in finding a better method is to study various physical properties of muscle to see which might be likely to correlate with sensory ratings. In an effort to choose a versatile test that might be explained theoretically and for which instrumentation already existed, it was decided in this work to apply a stretching force parallel to the muscle fibers. One criterion that can be measured using this technique is extensibility or break elongation which is the length muscle fibers must be stretched to produce breakage. This method was applied to single beef muscle fibers by Wang et al. (1956) and a highly significant negative correlation was obtained when extensibility was compared to organoleptic tenderness.

Elasticity may also be measured by this procedure. Muscle, along with many other biological tissues, can be regarded as viscoelastic in that it resembles a combination of an elastic solid and a viscous fluid. Viscoelasticity can be recognized by stress relaxation which is the decay of stress with time if a material is stretched to constant extension.

The source of elasticity in muscle is not yet completely clear. Muscle seems truly elastic up to about 3% extension of muscle length (Bate-Smith, 1939) but beyond this point the stress-strain curve is nonlinear. Work done on glycerinated muscle by Hoeve and Willis (1963) indicates that at the molecular level elasticity is related to a phase change of the fibrous proteins from an oriented crystalline state to a randomly coiled, amorphous state.

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Under certain nonphysiological conditions of high temperature and strong salt solutions an essentially rubber-like elasticity, characteristic of the amorphous state, was observed. This was thought due to melting of the original crystalline fibrous proteins. Perhaps this explains the observation of Guth (1947) that the stress-strain curves of resting muscle and rubber are significantly different in that muscle corresponds to rubber stretched out so much that the chain molecules are markedly oriented.

More recently Hoyle (1968) postulated a mechanism of muscle elasticity. It was once thought that the sarcolemma or cell membrane was mainly responsible for elasticity in muscle, but Hoyle reports that elasticity is present at lengths which do not stretch the sarcolemma and that elasticity is present in fibers from which the sarcolemma has been dissected away. Also, in some kinds of fibers the sarcolemma contributes only 15–20% of the total elasticity. The role of tendons in elasticity of muscle is discounted and Hoyle concludes that the individual sarcomeres must be the major source of elastic material. Both myosin and actin filaments are reported as inelastic and a new sarcomere component is postulated, the T-filament or thin filament, which has been seen in electron micrographs of the gap region between the actin and myosin of heavily stretched fibers. It is proposed that these T-filaments run from Z-line to Z-line and besides playing a passive role as an elastic element may also be involved in contraction.

The purpose of the research reported herein was to investigate physical properties of meat and develop methods for their evaluation. The results show that the instrument used is capable of discerning variations in physical properties of uncooked muscle. It is possible that one or more of these tests will prove useful as a predictor of meat tenderness.

EXPERIMENTAL

Apparatus

The Instron universal tester (Instron Engineering Corporation, 2500 Washington Ave., Canton, Mass.) was selected to perform various tests upon uncooked meat all of which applied force parallel to the fibers. The Instron has been described in detail by Bourne et al. (1966) and White (1970) as a research tool to study the rheological properties of food materials. Basically, this instrument is used by mounting the sample in a gripping mechanism which is connected to a strain gauge. Changes in force applied to the sample cause the beam of the strain gauge to deflect. The output of the gauge is fed to a strip-chart recorder which draws a force-distance curve for every test. In these experiments a Model TT was used with the following operating parameters: crosshead speed-12 in./min; chart speed-12 in./min; cell-tension load cell 'C', 1 lb full deflection; jaws-type 2A fiber clamps.

Procedure

Breaking strength of a sample was determined by mounting it in the jaws of the Instron, initially 3.5 cm apart, and applying force by the downward movement of the crosshead. Jaw slippage was held to a minimum by wrapping the ends of the sample in moistened strips of fabric prior to mounting. Breaking anywhere along the sample except at the jaws was found to be acceptable. To measure elasticity, or the ability to recover after deformation, the Instron was programmed for extension cycling. At maximum jaw separation the sample was elongated to 115% of its rest length (i.e., 3.5–4.0 cm). The muscle bundle was tested for 1 min which involved about 40 extension cycles. Relaxation or the loss of stress at constant extension was measured in a similar manner; the crosshead was moved downward and the sample was held at the same maximum separation for one minute.

The measurements obtained from these methods are summarized in Figure 1. The following tensile properties were evaluated; breaking strength or breaking load in lb force/g sample; break elongation or strain required to rupture the sample as a percentage of the original 3.5 cm sample between the jaws; specific work of rupture or the area under the stress-strain curve in inch-lb/g sample. Time effects measured included elasticity which was taken as the area under the stress-strain curve following 1 min of cycling to 115% elongation as a percentage of the initial area, and relaxation expressed as the amount of stress loss in 1 min at 115% elongation as a percentage of the original stress.

Materials

The samples used in these experiments consisted of commercially obtained, uncooked tenderloin and shank beef muscle. Rabbit samples were paired longissimus dorsi (LD) muscles from young female animals treated during rigor to produce one muscle that had been excised, unrestrained, and allowed to contract freely while the control was restrained on the carcass at rest length. Both samples were refrigerated at 0–5°C for 24 hr post-mortem. For details of the post-mortem method and procedures used to measure sarcomere length and fractionate protein extracts consult Buck et al. (1970). Following rigor the contracted muscle was excised and both muscles subjected to physical testing.

Samples were prepared by cutting the meat to 5.0 cm length and about 0.2–0.5 cm^2 cross-sectional area. The latter was only approximate due to the irregularities of biological material and the samples were also weighed prior to testing. Experiments were conducted in a conditioned room at 70°F. Samples were kept in an ice bath prior to use.

RESULTS & DISCUSSION

THE IRREGULAR SHAPE of the stress-strain curve for muscle, an example of which is depicted in Figure 1, is thought to represent physical rupture of muscle fibers occurring at different loads. The fibers with the lowest break elongation will fracture first. Once a few fibers have ruptured, breaking is accelerated since the increasing load is spread over the remaining fibers, increasing the specific load per fiber at an ever increasing rate. The point is finally reached where the increase in load due to the breaking of a fiber will cause another fiber to break immediately and this process continues until the whole specimen ruptures. This happens at a load which is less than the sum of the breaking loads of the individual fibers (Morton and Hearle, 1962).

Very small psoas muscle fiber bundles, less than 0.5 mm diameter, were used in an attempt to obtain a stress-strain curve free from the effects of connective tissue and fat. Even small amounts of connective tissue running the length of the sample could have a noticeable effect on breaking strength and break elongation because of this material's very high breaking strength (Abrahams, 1967). These results were similar to those found for larger muscle bundles and did not resem-

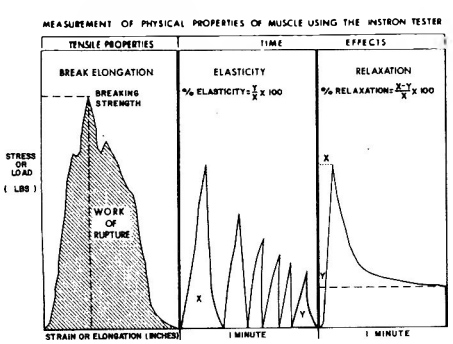

Fig. 1–Measurement of physical properties of muscle using the Instron tester.

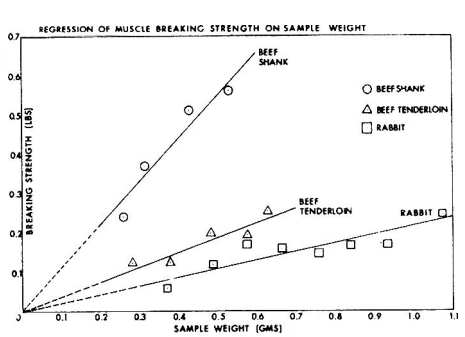

Fig. 2–Regression of muscle breaking strength on sample weight.

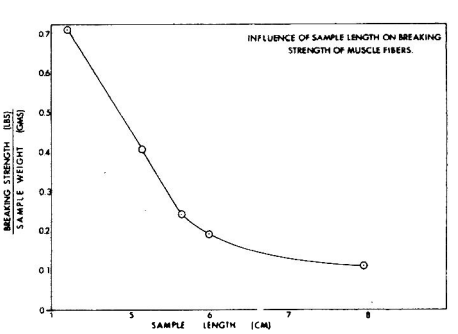

Fig. 3–Influence of sample length on breaking strength of beef tenderloin fibers.

Table 1—Physical properties of beef muscle.

Muscle	Tensile properties			Time effects	
	Specific work of rupture (inch-lb/g)	Breaking strength (lb/g)	Break elongation (%)	Elasticity (%)	Stress relaxation (%)
Shank	.327	.912	28	28	70
Tenderloin	.067	.341	20	36	60

Table 2—Characteristics of post-mortem rabbit muscle.

	Rigor treatment	
	Unrestrained	Restrained
Sarcomere length (μ)	1.90	2.34**
Total extractable protein (mg/g)	58.0	79.4*
Actomyosin (mg/g)	24.5	40.7*

**Treatments significantly different at 1% level.

*Treatments significantly different at 5% level.

ble the biphasic curves characteristic of fibrous proteins such as wool (Chapman, 1969) or spun soy fibers (Stanley, unpublished data). This may be interpreted as meaning that the stress-strain curve for muscle reflects other constituents than the fibrous proteins, perhaps the sarcolemma.

Averaged data for all animals are presented in Figure 2 and indicate that for the range of sample sizes used, breaking strength is proportional to sample weight at constant length and hence to cross-sectional area. The lines shown are regression lines plotted through the origin. Larger samples of rabbit LD muscle were required to obtain consistent results because in this muscle, in contrast to the beef samples and rabbit psoas muscle, microscopic examination showed the fibers are not completely parallel to one another. Tenderloin, a quite tender muscle, has a somewhat higher breaking strength per unit weight than rabbit. This may also be explained by a failure of the rabbit fibers to be completely parallel or run the entire length of the sample which leads to fibers being pulled over one another rather than breaking. Standard deviations for replicates of a single animal were between 15 and 20% of the mean for beef muscle and slightly higher for rabbit. Again, it is felt that this is due to irregularity in the rabbit muscle.

The effect of sample length on breaking strength of beef muscle is shown in Figure 3. These results may be explained by the so-called "weak-link" effect which predicts a fiber will break at its weakest point and the longer the fiber, the greater the statistical possibility of its having a "weak-link" (Morton and Hearle, 1962). This leads to the breaking strength of a specimen decreasing as the test length is increased. It is possible that over the region where the curve is linear the fibers are mainly running the entire distance but that at greater lengths the force is utilized more to cause the fibers to slide over one another.

The physical properties of uncooked, commercially obtained beef muscle are presented in Table 1. Each number represents the average of 28 determinations on two animals. The magnitude of difference between the specific work or breaking strength for two muscles is about what might be expected using a shear press (e.g., see Wang et al., 1956). Wang's results were supported in that a greater break elongation was found for the tougher sample. Since an extensiometer was not used with this work, it is possible that jaw slippage may have affected these results, but because of the rather large elongation found, it seems likely that this factor is of slight importance. Average standard deviations for elasticity and relaxation measurements were about 10% of the mean. The more tender cut had a greater ability to recover after deformation and lost less stress during mechanical conditioning. It was found that when the log of stress is plotted against relaxation time, an initial fast decay occurs, followed by a linear decrease. Thus, stress decay in muscle is an exponential function of time.

These initial results with beef were indicative that the instrument was capable of discerning gross differences in physical properties of meat. Next we investigated the rabbit system in which the post-mortem conditions could be better controlled. In all trials the unrestrained muscle had shorter sarcomeres than its restrained twin. This difference in sarcomere length proved highly significant. Total extractable protein soluble at an ionic strength of 0.55 was measured for both treatments and the protein extracted from unrestrained or contracted muscle was significantly less than that extracted from restrained muscle. Lowering the ionic strength of the protein extract to 0.23 precipitated a crude actomyosin fraction. Significantly more actomyosin was precipitated from restrained muscle. These results are summarized in Table 2 and are similar to those found by other investigators using these post-mortem treatments (Herring et al. 1965, 1967a; Buck and Black, 1967, 1968; Buck et al., 1970).

The physical properties of rabbit muscle were evaluated and the results in Table 3 are the average of 12 determinations on one or two animals. The difference in breaking strength for the two treatments is comparable to that found for shear force values of cooked muscle by Buck et al. (1970). Break elongation was higher for all rabbit trials than for the tough shank muscle which indicates that a simple inverse relationship between extensibility and tenderness does not hold in all cases. Again, elasticity was higher and relaxation lower for the presumably more tender or restrained muscle. To measure animal variation 7 pairs of LD muscles were evaluated for breaking force only. A total of 140 tests were performed for both treatments. The contracted muscle averaged .237 lb/g and the restrained muscle .168. Standard errors of the mean for the two treatments were 7.5% and 9.9% respectively. A paired "t" test showed the difference in the means to be highly significant ($P < 0.01$).

Since the degree of contraction is the only difference between the muscle pair, the theory has been proposed that contraction causes a greater degree of overlap between the thick and thin muscle filaments and leads to a higher concentration of actomyosin in the unrestrained sample (Herring et al., 1967a, b). This is the concept of actomyosin toughening and is supported by electron microscopic evidence (Carlsen et al., 1961). Experiments attempting the direct measurement of actomyosin by salting out have been unsuccessful in demonstrating a higher concentration of actomyosin in the contracted sample (see also Buck et al., 1970). However, this appears to be a fault of the method used rather than the theory (H. O. Hultin, private communication).

Although at one time it was thought that post-mortem stretching was effective because of thinning of connective tissue (Buck and Black, 1968) this now seems unlikely considering the results we have obtained. These clearly show a greater breaking strength for the unrestrained muscle even when the force is applied parallel to the fibers.

Table 3 also presents data showing the effect of post-mortem aging on the physical properties of rabbit muscle. Measurements made 2 hr, 1 day and 8 days

Table 3—Effect of muscle type, aging and post-mortem treatment on physical properties of rabbit muscle.

Muscle	Aging period	Post-mortem treatment	Tensile properties: Specific work of rupture (inch-lb/g)	Tensile properties: Breaking strength (lb/g)	Tensile properties: Break elongation (%)	Time effects: Elasticity (%)	Time effects: Stress relaxation (%)
LD	2 hr	–	.092	.344	34	41	41
LD	1 day	restrained	.045	.195	35	39	47
		unrestrained	.053	.253	31	32	50
LD	8 days	restrained	.038	.159	31	17	52
		unrestrained	.065	.241	33	28	48
Psoas	1 day	restrained	.078	.293	16	20	66
		unrestrained	.191	.572	21	16	69

Table 4—Correlation between methods used to evaluate physical properties of meat.

Comparison	Correlation coefficient
Breaking strength vs specific work of rupture	+0.99**
Breaking strength vs break elongation	−0.22
Breaking strength vs elasticity	−0.17
Breaking strength vs stress relaxation	+0.74**
Break elongation vs elasticity	+0.26
Break elongation vs stress relaxation	−0.61*
Elasticity vs stress relaxation	−0.40

**Coefficient significant at 1% level.
*Coefficient significant at 5% level.

post-mortem showed a greater than two-fold reduction in breaking force and specific work of rupture over this period for restrained rabbit samples along with a similar decrease in elasticity. Aging generally had less effect on unrestrained samples. Elasticity decreased to a greater degree in the restrained muscle, perhaps as a consequence of sustained stretching. Relaxation increased over this period but not to the extent of the other indices. Recently it has been reported that aging causes changes in the microscopic appearance of the myofibril. The Z-lines disappear completely and the A bands lengthen at the expense of the I zone (Davey and Gilbert, 1967, 1968, 1969a; Fukazawa et al., 1969). The breaking strength of a muscle fiber may be related to the propensity of the Z-line area to rupture and thus be a valid measurement of tenderness in aged meat.

The results obtained when rabbit psoas was treated similarly to LD may be found in Table 3 as well. After one day post-mortem both restrained and unrestrained samples had consistently higher tensile properties than were found with LD. Break elongation was lower and approximated that found in beef. Following the general pattern elasticity was lower and relaxation higher in the unrestrained treatment. Also, removing the muscle and allowing it to undergo the rigor period without restraint increased the tensile properties measured in these experiments.

Microscopic examination of rabbit psoas revealed that its oriented fibrous structure closely resembled the beef muscle rather than the more random fiber alignment of LD. That the fiber orientation can influence mechanical properties is evident from the results of Corey (1970) who, working with a model system constructed from spun soy fiber, found networks composed of parallel fibers held together with a gelatin binder exhibited higher plastic response, total strain and stress relaxation but a smaller elastic region when compared to random or perpendicular networks. These results agree well with the data given here for psoas and LD muscle.

After the results had been obtained, it was of particular interest to note the degree of independence of the tests used. Correlations were calculated and the coefficients are presented in Table 4. Work of rupture and breaking strength are so strongly related that it should not be neccessary to measure work directly. Elongation seems independent of either of the other tensile properties while elasticity appears independent of the tensile properties. Stress relaxation, while only marginally related to elasticity, shows a significant positive correlation with breaking strength and negative correlation with elongation. This might be related to structure if stress relaxation can be thought of as the proclivity for fibers to rupture and slide over one another while break elongation is a measure of the resistance of the sample toward elongation.

These results suggest there may be several advantages to the type of tests described. Several different and independent values may be obtained from one instrument as compared to the less versatile shearing devices. The methods seem as reproducible as those currently employed. It is possible that physical properties can be related to muscle structure as has been done for other protein fibers so that the technique will develop into more than an empirical tool. It is evident that tenderness in meat is due to the subtle interaction of many factors, one of which is texture. An important question is which of the many parameters available should be measured as a predictor of tenderness. Correlations of physical properties with subjective evaluations should be helpful in this regard. It seems unlikely that one test would be adequate for all cases.

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EFFECTS OF TYPES AND LEVELS OF FAT AND RATES AND TEMPERATURES OF COMMINUTION ON THE PROCESSING AND CHARACTERISTICS OF FRANKFURTERS

SUMMARY—Frankfurter emulsions containing either 25% or 35% beef fat, pork fat, or cottonseed oil were prepared by comminuting at 1500, 2500, or 5000 rpm to temperatures ranging from 45°–85°F. Data were obtained on the viscosities of the emulsions; except for initially high viscosities for which unmelted fat was responsible, the viscosities of emulsions containing the fats, or oil, were similar: viscosities tended to decrease with increasing time and temperature of chopping. The frankfurters were stuffed, smoked, and cooked, and data were obtained on shrinkage, fat retention, ease of peeling, specific gravity, and texture. Shrinkage was inversely related to content of fat. Fat separation mainly occurred in processing frankfurters containing beef fat; the data suggest that emulsions containing beef fat should be comminuted to 65°–75°F to avoid possible under or overchopping: the results show that optimum conditions were time as well as temperature dependent. The air content of frankfurters varied inversely with the maximum temperature attained during comminution. Frankfurter skin strength was lessened on increasing the temperatures to which emulsions were comminuted; elasticity, the equivalent of rubberiness, decreased under these conditions.

INTRODUCTION

THE SCIENCE of meat emulsions has been referred to as one of the youngest areas in food science (Saffle, 1968). It is because of this, and not any lack of recent effort, that research on many factors involved in the production of emulsion-based products is lacking or incomplete. Priority has been given to solving the problem of producing emulsions which can be heat processed during production and possibly reheated by consumers without loss of fat. Meat proteins have been identified as emulsion stabilizers and factors affecting this function established as detailed recently in a review by Saffle (1968).

On the other hand, while the properties of meat fats are known to affect emulsification, the effect of variations on emulsion stability and processing requirements are not well understood. Results of earlier work of Townsend et al. (1968a; 1968b) suggested that the melting characteristics of meat fats could be the basis for differences in the maximum temperatures at which meat formulas should be emulsified. In later work the stability of emulsions of two series of fractionated beef and pork fats was studied with results suggesting that the melting characteristics of fats, rates and extent of temperature rise, and rates of dispersion (shear forces) interrelate in determining emulsion stability (Swift et al., 1968).

Subsequently, to obtain additional information on this relationship, an investigation of the effect of varying formulas and processing on emulsion and frankfurter characteristics was undertaken. Emulsions containing 25% or 35% beef fat, pork fat, or cottonseed oil and chopped at 1500, 2500, or 5000 rpm to temperatures ranging from 45°–85°F were prepared, stuffed, and then smoked and cooked. The effects on the time required in chopping, viscosity of emulsions, and the shrinkage, peeling characteristics, specific gravity and texture of frankfurters are reported in this paper.

EXPERIMENTAL

Materials and formulas

Two Commercial grade beef and eight market weight hog carcasses were stored at 37°F for from 3–5 days after slaughter. All tissues from the carcasses were removed and separated into distinctly lean and fat portions after which the lots of lean beef, beef tissue fat, lean pork, and pork tissue fat were cut into pieces several cubic inches in size (no internal fats, i.e., kidney, caul, or ruffle fats, were used). After each lot was thoroughly mixed, samples were removed for analyses and approximately 12.5-lb portions were stored under vacuum in Cry-O-Vac bags at 0°F. A winterized cottonseed oil was stored at 37°–38°F. Based on the results of analyses of raw materials, formulas designed to produce frankfurters containing approximately 9–10% added moisture and either 25% or 35% fat were calculated (Table 1).

Appropriate quantities of frozen fat and lean meat were removed from the freezer 2 days before each experiment and allowed to partially thaw. Lean meats were ground once through a 3/16-in plate and tissue fat once through a 1/2-in plate in a room maintained at 55°F and comminuted immediately thereafter. Cottonseed oil was used immediately upon withdrawal from a 37°–38°F cooler. The weight of lean beef, lean pork, and fats, or oil, in each batch comminuted was 50 lb to which were added appropriate weights of ice and a curing and spice mixture which provided 2.5 lb NaCl, 0.25 oz $NaNO_2$, 2 oz $NaNO_3$, 0.86 oz Na ascorbate, 1.98 lb cane sugar, and 8.5 oz of commercial spices per 100 lb meat.

Table 1—Formulas comminuted and the composition of finished frankfurters.

Series no.	Fat: Approx. content %	Fat: Type[1]	Emulsification rpm	Meat[2]: Lean beef %	Meat[2]: Lean pork %	Fat or oil %	Ice lb/100 lb meat	Composition of frankfurters[3]: Moisture %	Fat %	Protein %	Added moisture %
1	25	BF	1500	46	20	34	32	57.6	25.7	12.3	8.4
2	35	BF	1500	42	12	46	27	50.4	34.0	10.9	6.8
3	25	BF	2500	46	20	34	33	56.8	25.6	12.5	6.8
4	35	BF	2500	42	11	47	30	49.4	34.2	10.7	6.6
5	25	BF	5000	46	20	34	33	56.8	25.8	12.8	5.5
6	35	BF	5000	42	12	46	27	49.5	34.5	11.3	4.3
7	25	PF	1500	45	25	30	28	57.1	25.8	12.8	5.9
8	35	PF	1500	46	10	44	25	49.3	34.6	10.8	6.1
9	25	PF	2500	45	25	30	28	55.9	25.9	12.7	5.1
10	35	PF	2500	46	10	44	25	48.5	35.6	10.4	6.9
11	25	PF	5000	45	25	30	28	55.6	26.7	12.4	6.0
12	35	PF	5000	46	10	44	25	49.3	35.0	10.7	6.5
13	25	CO	1500	46	27	27	34	55.6	25.9	12.9	4.0
14	35	CO	1500	52	11	37	26	47.1	34.9	11.2	2.4
15	25	CO	2500	46	27	27	34	55.8	26.9	12.6	5.4
16	35	CO	2500	48	12	40	30	48.6	35.8	10.8	5.4
17	25	CO	5000	46	27	27	34	55.4	27.2	12.5	5.4
18	35	CO	5000	52	11	37	26	49.3	34.0	11.1	4.9

[1] Type of fat or oil. BF – beef fat; PF – pork fat; CO – cottonseed oil.
[2] 5.2 lb cure and spices added per 100 lb meat.
[3] Mean of analyses on all lots of each series.

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Processing

The emulsions were prepared in a 90-lb capacity Model KA 110 Koch-Alpina silent cutter fitted with six knives which were operated at 1500, 2500, or 5000 rpm, with bowl speeds of 6.5, 13, or 22 rpm, respectively, depending on the current level and/or the pulley ratio selected. The cutter was fitted with a thermocouple and emulsion temperatures were continuously recorded to within ± 2°F. All ingredients, except fats or oil, were placed in the silent cutter and the machine operated for 1-1/2 to 2 min at reduced speed, except that in comminuting at 1500 rpm this rate was maintained throughout. The tissue fats, or the oil, were then added and the machine was operated at the selected maximum rate.

Samples weighing approximately 11 lb each were removed when the emulsions became 45°, 55°, 65°F and higher temperatures, in some cases as high as 85°F. Elapsed time was recorded. Small portions of these samples were used in determining viscosity. Small portions of emulsions were vacuumed: both vacuumed and non-vacuumed were stuffed into 23 mm No-Jax casings. Hereinafter the samples withdrawn and stuffed by the above described procedure are termed lots (designated by temperature of sampling) which make up series (designated by level and type of fat or oil and the rpm during comminution).

The lots of frankfurters were weighed and then cooked and smoked in an air-conditioned smokehouse operated to produce 130°F DB for 10 min; 145°F DB-135°F WB for 30 min; 165°F DB-140°F WB for 12 min, and 190°F DB-163°F WB until, in approximately 10 min, internal temperatures became 155°–157°F. Internal temperatures were determined by means of thermocouples inserted when the frankfurters became sufficiently heat coagulated. The frankfurters were showered with cold water until internal temperatures decreased to 90°F and were held in the smokehouse an additional 10 min to dry. They were weighed and stored overnight at 37°–39°F in plastic tubs covered with polyethylene film. After an examination during which "fat-caps" and the relative ease of peeling were observed the frankfurters were vacuum packaged in Kapak pouches; those containing samples intended for determinations of specific gravity and texture were stored at 37°–39°F, and those for histological examination at 0°F.

Methods

The percentages of moisture, fat, and protein in meat raw materials and finished frankfurters were determined by A.O.A.C. methods (A.O.A.C., 1965). The mean apparent viscosity of each lot of emulsion was determined immediately upon withdrawal from the chopping bowl from duplicate measurements with a Model HBT Brookfield viscosimeter mounted on a Helipath stand (designed for use with plastic and thixotropic materials) and equipped with a special bar-type spindle (shaft length, 11.5 cm; shaft diameter, 3 mm; cross bar length, 25.8 mm; cross bar diameter, 1.5 mm; rotation at 50 rpm in air).

From the data on weights of lots before and after cooking and smoking the percentage of shrinkage of each lot and the mean percentage of shrinkage of each series were calculated. The presence of any "fat-caps" and the relative ease of peeling were determined by examining 55–65 frankfurters from each lot. Judgments were made on manually removing casings rang-

Table 2—Effects of different levels and types of fat and rates and temperatures of comminution on the production of frankfurters.

Series no.	Fat content %	Emulsification		Smoking-cooking		Peelability[2,4]	Specific gravity[5]
		Rpm	Temp. °F	Shrinkage[1] %	Fat separation[2,3]		
Beef fat							
1	25	1500	45		++	G–	0.949 a
			55	10.2 ± 0.2	++	G–	0.973 b
			55		0	G (W)	0.993 c
			71		0	G (W)	1.000 c
2	35	1500	45		++	G +	0.963 a
			55	7.8 ± 0.3	++	G +	0.970 b
			65		0	G +	1.006 c
			70		0	G +	1.003 c
3	25	2500	45		++	G +	0.951 a
			55		++	G +	0.967 b
			65	10.7 ± 0.1	++	G +	0.980 b c
			75		0	G +	0.990 c
			82		++	G +	1.005 d
4	35	2500	45		++	E	0.935 a
			55		++	E	0.954 b
			65	9.2 ± 0.3	+	G +	0.970 c
			75		0	G +	1.000 c
			83		+++	P	1.007 c
5	25	5000	45		++	E	0.970 a
			55		+	E	0.974 a
			65	10.1 ± 0.2	+	G +	0.994 b
			75		0	G +	1.003 b c
			85		0	P	1.005 c
6	35	5000	45		++	G +	0.953 a
			55		+	G +	0.960 b
			65	8.8 ± 0.2	+	G –	0.964 c
			75		0	G (W)	0.997 d
			85		0	G (W)	1.002 d
Pork fat							
7	25	1500	45		0	G +	0.973 a
			55	9.7 ± 0.1	0	G +	0.972 a
			65		0	G +	1.007 b
			69		0	G +	1.008 b
8	35	1500	45		++	G –	0.961 a
			55	7.8 ± 0.4	0	G +	0.970 b
			65		0	G +	1.002 c
			72		0	G +	1.004 c
9	25	2500	55		0	G +	0.968 a
			65	9.0 ± 1.0	0	G +	0.999 b
			75		0	G +	1.014 b
			79		0	P	1.015 b
10	35	2500	55		0	G +	0.948 a
			65	6.4 ± 0.6	0	G +	0.983 b
			75		0	G +	0.996 c
			78		0	P	1.003 d
11	25	5000	55		0	G –	0.959 a
			65	9.6 ± 0.2	0	G +	0.987 b
			75		0	G +	0.999 b c
			85		0	G +	1.007 c
12	35	5000	55		0	G +	0.964 a
			65	7.5 ± 0.7	0	G +	0.971 b
			75		0	G +	1.005 c
			85		0	G +	1.001 c

(continued)

Table 2–continued

Series no.	Oil content %	Emulsification Rpm	Emulsification Temp. °F	Smoking-cooking Shrinkage[1] %	Smoking-cooking Fat separation[2,3]	Peelability[2,4]	Specific gravity[5]
			Cottonseed oil				
13	25	1500	45		0	E	1.009 a b
			55	12.9 ± 0.4	0	E	1.005 b
			65		0	E	1.012 a
			74		0	E	1.015 a
14	35	1500[6]	45		0	G +	0.991 a
			55	9.4 ± 0.8	0	G +	1.001 a b
			65		0	G +	1.006 a b
			69		0	G +	1.008 b
15	25	2500	45		0	E	0.995 a
			55	12.8 ± 0.1	0	E	0.993 a
			65		0	E	1.001 b
			75		0	E	1.009 c
			85		0	E	1.011 b c
16	35	2500	45		0	E	1.004 a
			55		0	E	1.005 a
			65	10.0 ± 0.4	0	E	1.004 a
			75		0	E	1.005 a
			85		0	E	1.009 a
17	25	5000	45		0	E	0.978 a
			55		0	E	0.987 a b
			65	13.1 ± 0.2	0	E	1.000 b c
			75		0	E	1.007 c
			85		0	G	1.006 c
18	35	5000	45		0	G +	0.974 a
			55		0	G –	0.989 a b
			65	9.4 ± 0.3	0	G –	0.998 b
			75		0	G –	1.001 b c
			85		0	P (W)	1.007 c

[1] Mean of percentages of shrinkage of all lots in each series ± standard deviation.
[2] Based on judgments on 55–65 frankfurters. (W) indicates wetting was necessary.
[3] Plus signs indicate relative frequency.
[4] E = excellent; G = good; P = poor.
[5] Any means within the same series with different letters following are significantly different from each other at the 5% level.
[6] Oil added in three portions as was necessary to avoid severe separation of fat.

ing from easy peeling (excellent) to difficult peeling (poor) which was observed as adhesions between casings and frankfurters.

The mean specific gravity of frankfurters in each lot was calculated from values obtained by weighing the water displaced by each of three to five weighed frankfurters. The mean specific gravity of frankfurters prepared from vacuum treated emulsions was determined similarly.

An Instron TT-B floor model was used in determining the skin strength and elasticity of frankfurters. To determine the skin strength a load was applied at a rate of 0.5 in /min to the surface of the frankfurters by means of a 1/4-in diameter rod which produced a puncture. This procedure was applied to three or four surface areas on each of three samples from each lot.

The elasticity of three frankfurters from each lot was measured by placing a frankfurter in a compression cage. A load was applied which compressed the frankfurters at a rate of 0.2 in /min to produce compression of 0.3 in. Compression was reduced also at a rate of 0.2 in /min until zero load was attained. The areas were measured by an integrator. The data are reported as a ratio between the energy recovered after deformation and the energy expended in compression.

RESULTS & DISCUSSION

FORMULAS used in preparing frankfurters containing either 25% or 35% beef fat, pork fat, or cottonseed oil and results of analyses of finished products are shown in Table 1. Results show there were only small differences in content of fat, protein and moisture of frankfurters formulated so as to be of comparable composition. Percentages of added moisture (moisture, % – 4 x protein, %) ranged from 2.4–8.4, and were somewhat lower than those sought in commercial production.

Table 2 shows the rpm used in comminution, the maximum temperature attained by each lot of emulsion comprising each series, data on shrinkage, specific gravity, and results of examinations conducted to detect "fat-caps" and determine peeling scores. The discussions which follow refer to data in Table 2 with exceptions as noted.

The curves in Figure 1 show the time required for emulsions to attain temperatures ranging from 45°–85°F; the measurements were begun after ice, curing agents and spices, and fats, or oil, had been added. Examination of the curves shows that time for chopping was highly dependent upon the rpm used. Points on the curves, particularly those shown for chopping at 2500 or 5000 rpm, indicate temperature rise was similar in chopping emulsions containing either of the fats or the oil. Calculations based on the curves indicate that temperatures increased at maximum rates of approximately 1.7°, 4.1°, and 11.4°F/min in chopping at 1500, 2500, and 5000 rpm, respectively. An indication of the practical importance of high rpm is that the time required to attain 60°F, a typical temperature in commercial operations, was reduced by approximately 50% on increasing rpm to each next higher rate.

The average and range of viscosity values obtained with Brookfield equipment on emulsions comminuted to temperatures in the range studied are shown in Figure 2. Data on emulsions containing 25% or 35% of the fats, or the oil, and comminuted at different rpm are combined since varying fat level or rpm in chopping did not produce statistically significant differences; it is to be noted, however, that replications were limited and these data do not indicate that the variables did not affect viscosity to some extent. The results indicate that at 45°F emulsions containing beef or pork fat had higher viscosities than those containing cottonseed oil ($P < 0.05$), probably because the tissue fats were largely unmelted. At higher temperatures viscosities decreased as chopping and temperatures increased, the viscosities of emulsions prepared with beef fat or cottonseed oil being relatively high and approximately equal and those of emulsions prepared with pork fat somewhat lower. The tendency for viscosity to decrease during chopping contrasts with the increasing viscosity of meat protein stabilized model o/w emulsions as emulsification progresses (Swift et al., 1961); the explanation may be that any effect of fat emulsification was masked by the viscosity of lean portions swollen and viscous after the action of curing agents and water (Hamm, 1960), and that the decreasing viscosity resulted from a continued mincing of the lean portion. A similar masking may also have been responsible for the fact that the viscosity values were not closely related to emulsion stability. Lack of a direct relationship is shown by the fact that the viscosity of emulsions prepared with beef fat and those prepared with cottonseed oil and chopped to 55°F were relatively high and approximately equal; in the smokehouse, "fat-caps" formed in those

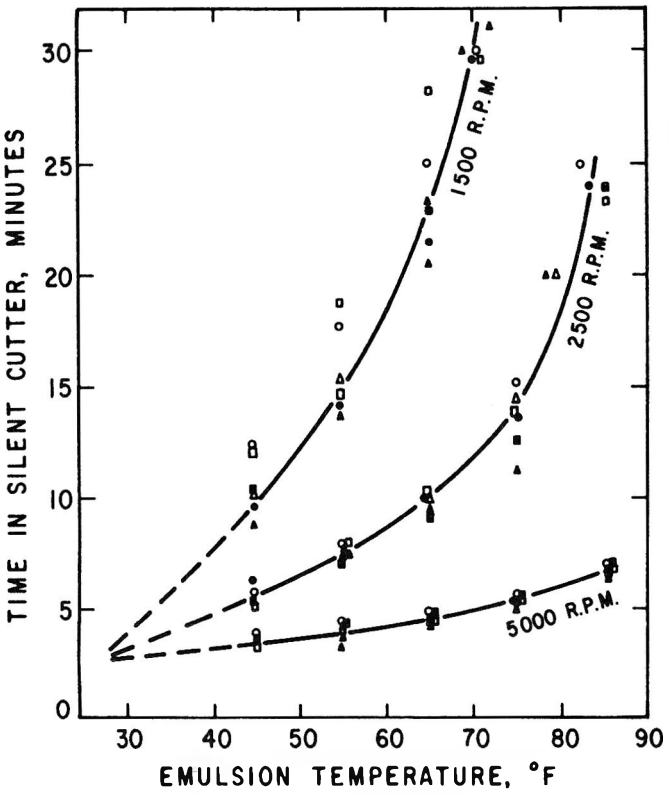

Fig. 1—Time required to attain temperatures from 45°–85°F in comminuting at 1500, 2500, or 5000 rpm. 25% beef fat (○); 35% beef fat (●); 25% pork fat (△); 35% pork fat (▲); 25% cottonseed oil (□); 35% cottonseed oil (■).

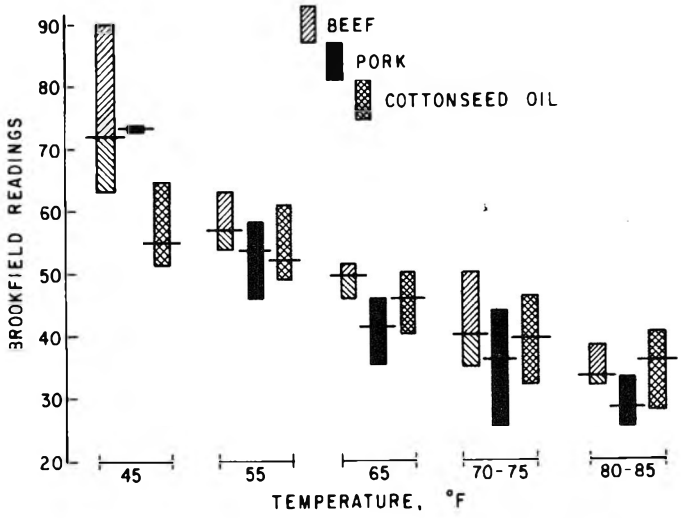

Fig. 2—Range and average of Brookfield readings on emulsions prepared with beef or pork fats, or cottonseed oil at temperatures attained during comminution.

prepared with beef fat but not in those prepared with cottonseed oil (Table 2). Other evidence is that little variation existed among the low viscosities of emulsions prepared with beef fat and chopped to 80°–85°F, although "fat-caps" formed on two of the four in the smokehouse. Based on these results, determination or control of emulsion viscosity cannot substitute for, or be a valuable addition to, control of time and/or temperature in assuring emulsion stability.

The mean percentage of shrinkage in cooking and smoking each series is shown. Results indicate that shrinkage was affected by the level and type of fat in the frankfurters, but not the rpm used in chopping emulsions. Increasing shrinkage correlated with decreasing content of fat in frankfurters prepared with beef or pork fat ($r = -0.83$, $P < 0.01$) and those prepared with cottonseed oil ($r = -0.96$, $P < 0.01$); the shrinkage of the latter frankfurters was the largest.

Data are given on the incidence of fat separation in the form of "fat-caps" among the lots of frankfurters. "Fat-caps" principally formed on frankfurters containing beef fat and, most frequently, those prepared from underchopped emulsions. Otherwise, only the appearance of "fat-caps" on frankfurters prepared from emulsions chopped to 45°F at 1500 rpm containing 35% pork fat, or 35% cottonseed oil (if not added in portions), was an indication of underchopping. The results suggest that the dispersion of beef fat was slowed by the relatively high temperature required to produce sufficient melting. The data show that emulsions chopped slowly (25 min at 1500 rpm) were stable on chopping only to 65°F, while, in chopping at 2500 or 5000 rpm, 75°F was required to attain stability. This indication that optimum temperature increased with increased rpm of chopping probably signifies that a combination of time and temperature affected melting of fat and, as a consequence, its dispersion. The existence of a relationship of time and temperature has been previously recognized, presumably as the result of observations in commercial sausage making (MacKenzie, 1966).

Table 3—Relation of skin strength and maximum temperature attained during comminution.

Series No.	Fat Type[1]	Fat Content %	Emulsification Rpm	Number of samples	Regression of skin strength on temp (°F)	Correlation coefficient	F test[2]
1	BF	25	1500	38	2.235–0.0121T	0.70	36.2**
2	BF	35	1500	37	2.313–0.0166T	0.72	39.8**
3	BF	25	2500	38	2.103–0.0066T	0.35	5.3*
4	BF	35	2500	38	1.454–0.0007T	0.04	0.08[N.S.]
5	BF	25	5000	38	2.009–0.0081T	0.59	19.5**
6	BF	35	5000	38	2.150–0.0144T	0.88	125.0**
7	PF	25	1500	38	2.134–0.0148T	0.71	39.4**
8	PF	35	1500	28	2.753–0.0223T	0.72	29.9**
9	PF	25	2500	28	1.673–0.0043T	0.33	3.4[N.S.]
10	PF	35	2500	36	1.457–0.0065T	0.66	27.0**
11	PF	25	5000	38	1.445–0.0058T	0.46	10.0**
12	PF	35	5000	38	1.333–0.0057T	0.68	33.1**
13	CO	25	1500	38	2.454–0.0170T	0.80	66.9**
14	CO	35	1500	38	1.683–0.0035T	0.22	2.0[N.S.]
15	CO	25	2500	38	1.973–0.0085T	0.52	13.9**
16	CO	35	2500	38	1.445–0.0045T	0.36	5.5*
17	CO	25	5000	48	1.643–0.0020T	0.18	1.5[N.S.]
18	CO	35	5000	48	1.268–0.0005T	0.05	0.1[N.S.]

[1] Beef fat, pork fat, and cottonseed oil are indicated by BF, PF, and CO, respectively.
[2] N.S. – Not significant at 5% level of probability.
*$P < 0.05$; **$P < 0.01$.

Overchopping was produced only by chopping emulsions containing beef fat for long periods to high temperatures. The lengthy comminution (over 30 min) required to attain 71°F did not affect the stability of emulsions comminuted at 1500 rpm, nor did rapid comminution (ca. 7 min) at 5000 rpm to produce temperatures of 85°F. "Fat-caps" formed, however, on frankfurters prepared from emulsions comminuted 24 or 25 min at 2500 rpm to temperatures of 82° or 83°F. These results indicate that in overchopping a limit involving a time-temperature relationship was exceeded. In addition, since emulsions containing pork fat or cottonseed oil chopped under the same conditions were stable, the results indicate that one or more characteristics of beef fat produced problems in emulsification.

The relative ease with which casings could be removed from the lots of frankfurters is reported. Peeling was scored as poor in removing casings from five lots of frankfurters, two each among frankfurters containing the different fats and one containing the cottonseed oil. All had been prepared from emulsions chopped to 78°F or above. "Fat-caps" had formed on only one. Although peeling scores on other lots which had been chopped to high temperatures were rated as good, the results suggest that chopping to high temperatures was responsible for poor peeling when it occurred, possibly owing to a lack of the surface greasiness which has been reported to be a factor (Saffle et al., 1964).

Results of specific gravity determinations of frankfurters from all lots are shown in Table 2. The data indicate that the specific gravity became 1.000 or higher in frankfurters prepared from emulsions chopped to the highest temperatures attained. This required a reduction of approximately one-half the air present in frankfurters prepared from emulsions chopped to 45° or 55°F (frankfurters prepared from vaccum-treated emulsions ranged from 1.04–1.05). It is reasonable to assume that, as temperature increased, air became less soluble.

The effect of skin strength (puncture test) of chopping emulsions to increasing temperatures is shown in Table 3 by regression equations. Equations for each series indicate that increasing the time-temperature of chopping tended to decrease skin strength: 13 of 18 relationships being significant at the 95%, or higher, level of confidence. It has been reported that skin strength is developed by a migration of protein to the surface of frankfurters and subsequent denaturation during smoking (Saffle et al., 1964). Assuming this mechanism to be the source of skin strength, migration in frankfurters prepared from emulsions chopped to 45° or 55°F, including some lots on which "fat-caps" formed, was greater than in frankfurters prepared from emulsions chopped at higher temperatures. A possible explanation of decreased protein migration could be that proteins were increasingly utilized in membrane formation as chopping proceeded to higher temperatures and were insoluble.

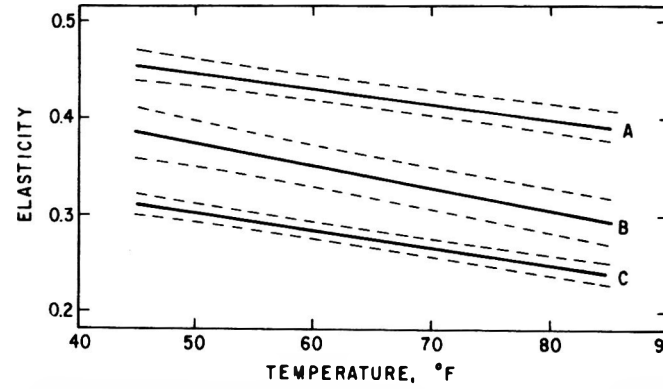

Fig. 3–Elasticity of frankfurters prepared from emulsions containing cottonseed oil (A), pork fat (B), or beef fat (C) and chopped to different temperatures. Values indicate recovered/expended energy applied during compression. 95% confidence limits calculated on means are shown.

Regression lines of mean values of elasticity on temperature are shown in Figure 3. Analyses showed that differences in the elasticity of frankfurters prepared with 25% or 35% of the fats, or the oil, and chopped at different rpm were not statistically significant and the data were pooled. The results show that the elasticity of frankfurters decreased in the order cottonseed oil, pork fat and beef fat. In informal tasting, frankfurters prepared with cottonseed oil were found to have a fine, unfamiliar and poor texture. Its relatively high elasticity can be equated to rubberiness, since a perfectly elastic system would have an elasticity of one on the scale shown in Figure 3. Increasing time and temperature of chopping decreased elasticity to a degree, but elasticity primarily depended on the lipid present. Differences in the dispersion of the fats, or the oil, during chopping could account for the observed differences. A second paper will consider such evidence.

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Mention of commercial names does not imply endorsement by the U.S. Department of Agriculture.

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EFFECTS OF TYPES OF FAT AND OF RATES AND TEMPERATURES OF COMMINUTION ON DISPERSION OF LIPIDS IN FRANKFURTERS

SUMMARY—The effect of time, temperature and rpm of comminution of emulsions was determined on the dispersion of approximately 25% of beef fat, pork fat or cottonseed oil in frankfurters. The numbers of lipid particles 5 μ or less in diameter increased in frankfurters containing either beef or pork fat as comminution was continued to higher temperatures, with pork fat dispersed more thoroughly. Fat tended to separate from frankfurters containing beef fat in particles 200 μ or more in diameter. In contrast, no specific degree of dispersion of particles 5 μ or less in diameter consistently indicated emulsion stability, or its lack. Increased rpm during comminution produced an increased dispersion of beef or pork fat. Under the same conditions pork fat was dispersed more finely than beef fat. Dispersion of cottonseed oil produced finely dispersed particles beyond the resolution of light microscopy, as was confirmed by electron microscopy which showed a substantial number of particles to be less than 1 μ in diameter.

INTRODUCTION

THIS ARTICLE is the second reporting results of a study of the effects of varying types of fat and of rates and temperatures of comminution on the processing and properties of frankfurters. Data on the viscosity of emulsions and on the processing, specific gravity, skin strength and elasticity of the frankfurters have been reported (Townsend et al., 1971). The results of a histological study of the dispersion of lipids in those frankfurters containing approximately 25% fat are reported here. Histological studies concerned with the formation of membranes which enclose fat have been relatively thorough (Hansen, 1960; Swift et al., 1961; Helmer and Saffle, 1963; Borchert et al., 1967); however, only limited data exist on the size and dispersion of fat particles (Saffle, 1968). The results reported deal with the effects of the processing parameters on dispersions and any relation of variations of these dispersions to emulsion stability.

EXPERIMENTAL

Samples

The samples were obtained from lots of frankfurters containing approximately 25% beef fat, pork fat or cottonseed oil: preparation involved comminuting emulsions at 1,500, 2,500 or 5,000 rpm to temperatures ranging from 45 to 85°F, stuffing, and cooking and smoking (Townsend et al., 1971). After preparation, those reserved for histological examination were stored under vacuum in polyester pouches at 0°F.

Histological preparation

The samples were allowed to thaw and were placed in buffered 10% neutral formalin for 2 wk. Sections removed from the center of these were cooled to −4°F and 16-μ sections were cut. These were mounted on chilled slides wetted with chrome glycerine jelly. They were then sprayed with a freshly prepared dilute solution of egg albumen, after which the slides were inverted over formalin for 1 min. The sections were stained 5 min in 0.5% solution of Sudan Black-B in 70% ethanol, washed in water, counterstained 5 min in 0.1% aqueous solution of nuclear fast red (Kernechtrot), washed in water and mounted in chrome glycerine jelly.

Estimation of size and number of fat particles

Measurements were made with a Bausch & Lomb Dynazoom microscope. A linearly scaled eyepiece micrometer disc was used in measuring size and a squared grid in counting numbers. The discs were calibrated for use at magnifications of 100 and 400×. Particles having diameters of 200 μ, or larger, were counted in 0.5-mm² areas of five sections prepared from 1–2 frankfurters at a 100× magnification. Particles having diameters of 5 μ, or less, were counted in three 625-μ² areas of sections prepared from a frankfurter in each treatment lot at a 400× magnification: the results are reported as particles per 0.5 mm².

Photomicrography

Photomicrographs were taken with a Zeiss microscope equipped with a 25/0.63 planoapochromat lens using Kodak High Contrast copy film.

Electron microscopy

Small pieces (¼-sq in.) were cut from the center of the frankfurters. The samples were prepared by prefixing in 5% glutaraldehyde in 0.1M phosphate buffer at pH 7.45 for 2 hr (Borchert et al., 1967). The samples were removed from the glutaraldehyde and cut into small blocks (~1 mm²) and washed overnight in 0.2M sucrose buffered with 0.1M phosphate at pH 7.45. The small blocks were fixed in 1% OsO_4 in 0.1M phosphate buffer for 4 hr. Embedding in epoxy resin was performed (Luft, 1961). Gold to silver sections were cut on an L.K.B. Ultratome III, stained with uranyl acetate and lead citrate and examined with an RCA EMU-3G electron microscope at 50 k.V. accelerating voltage.

RESULTS & DISCUSSION

TABLE 1 shows the average numbers of particles 5 μ or less in diameter in 0.5-mm² sections of frankfurters in which beef or pork fat was the principal lipid. A comparison of the data on frankfurters from which fat separated during heat processing (underlined) with those of the remaining frankfurters shows that no given dispersion consistently indicated emulsion stability or its lack. In general, the numbers of small particles increased as comminution was continued to higher temperatures. Results of statistical analyses applied to the linear regressions (Chow, 1960) indicate that increased dispersion was obtained with increased rpm during comminution; i.e., comminution of beef fat produced dispersion in the decreasing order 5,000 (C), 2,500 (B) and 1,500 rpm (A), and comminution of pork fat in the decreasing order 5,000 (F) and 2,500 (E). Results also indicate that pork fat was more finely dispersed than beef fat comminuted at each of the rates; i.e., at 5,000 rpm, F > C; at 2,500 rpm, E > B and at 1,500 rpm, D > A. On applying the histological technique and microscopic examinations to frankfurters containing cottonseed oil, the lipid particles counted were approximately one-half the numbers reported in Table 1. As will be further discussed, photomicrographs of these samples showed a larger number of small closely packed particles than were formed with the other lipids.

Table 2 shows the results of determinations of lipid particles having diameters 200 μ, or larger. The data indicate that in frankfurters from which fat separated during processing (underlined) larger numbers of particles of this size were observed. The results also indicate that numbers of large particles were considerably smaller in frankfurters in which pork fat was the principal lipid. Frankfurters containing cottonseed oil as the principal lipid were free of large particles; fat had not separated from them or those prepared with pork fat during heat processing. As would be expected, the decrease of large particles shown in Table 2 coincides with the increase of small particles shown in Table 1. As compared with counting small particles, the ease and significance of measuring the size of large particles recommends the measurement for use in process and product development.

Photomicrographs a to e inclusive (Fig.

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Fig. 1–Photomicrographs (400×) of emulsions containing 25% beef fat comminuted at 2,500 rpm to (a) 45°F, (b) 55°F, (c) 65°F, (d) 75°F and (e) 82°F, (f) at 1,500 rpm to 71°F, (g) at 5,000 rpm to 85°F, (h) emulsion containing pork fat comminuted at 5,000 rpm to 85°F, (i) emulsion containing cottonseed oil comminuted at 5,000 rpm to 85°F, (j) electron micrograph of emulsion containing cottonseed oil comminuted at 5,000 rpm to 85° (10,400×). (Continued next page.)

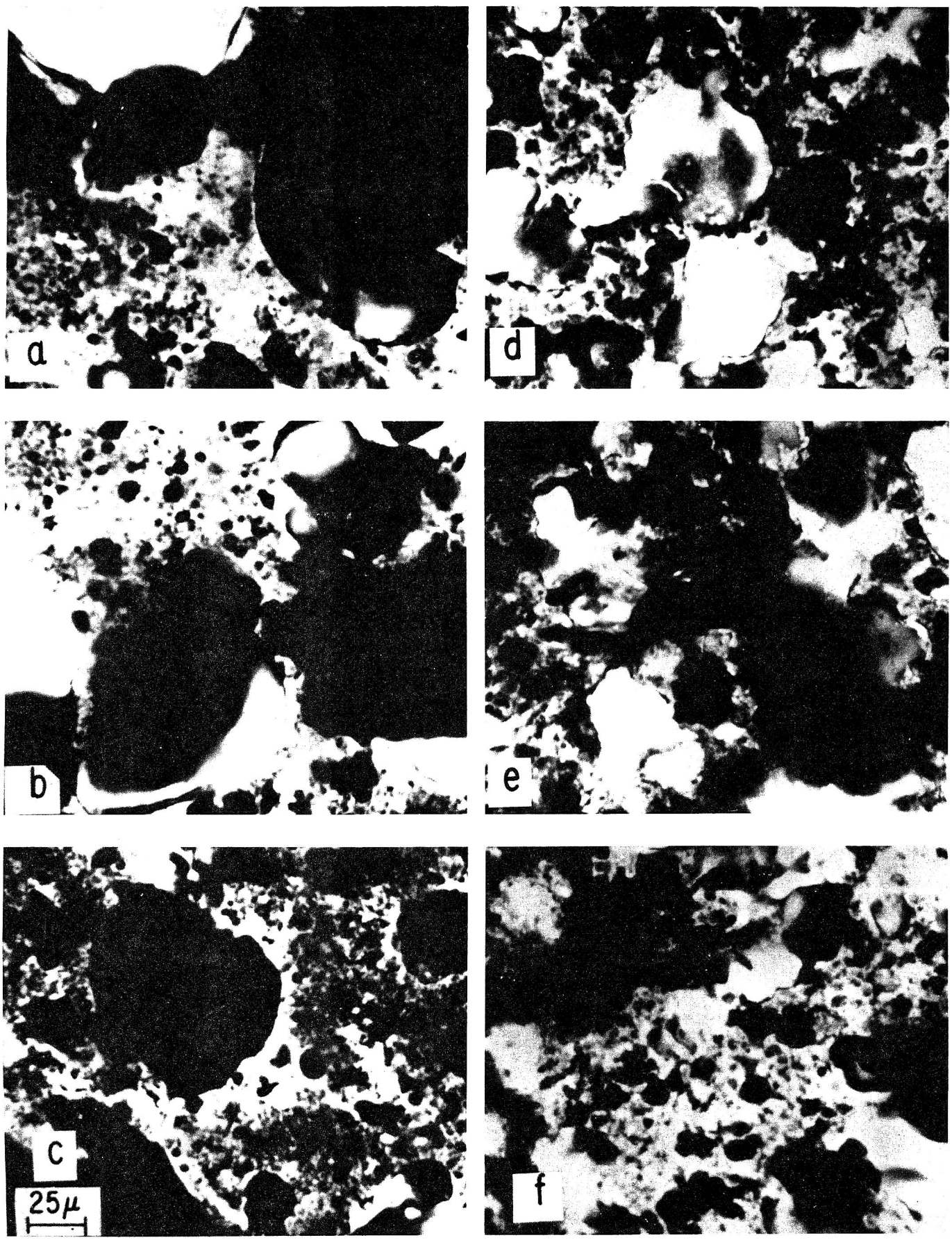

Fig. 1–Concluded

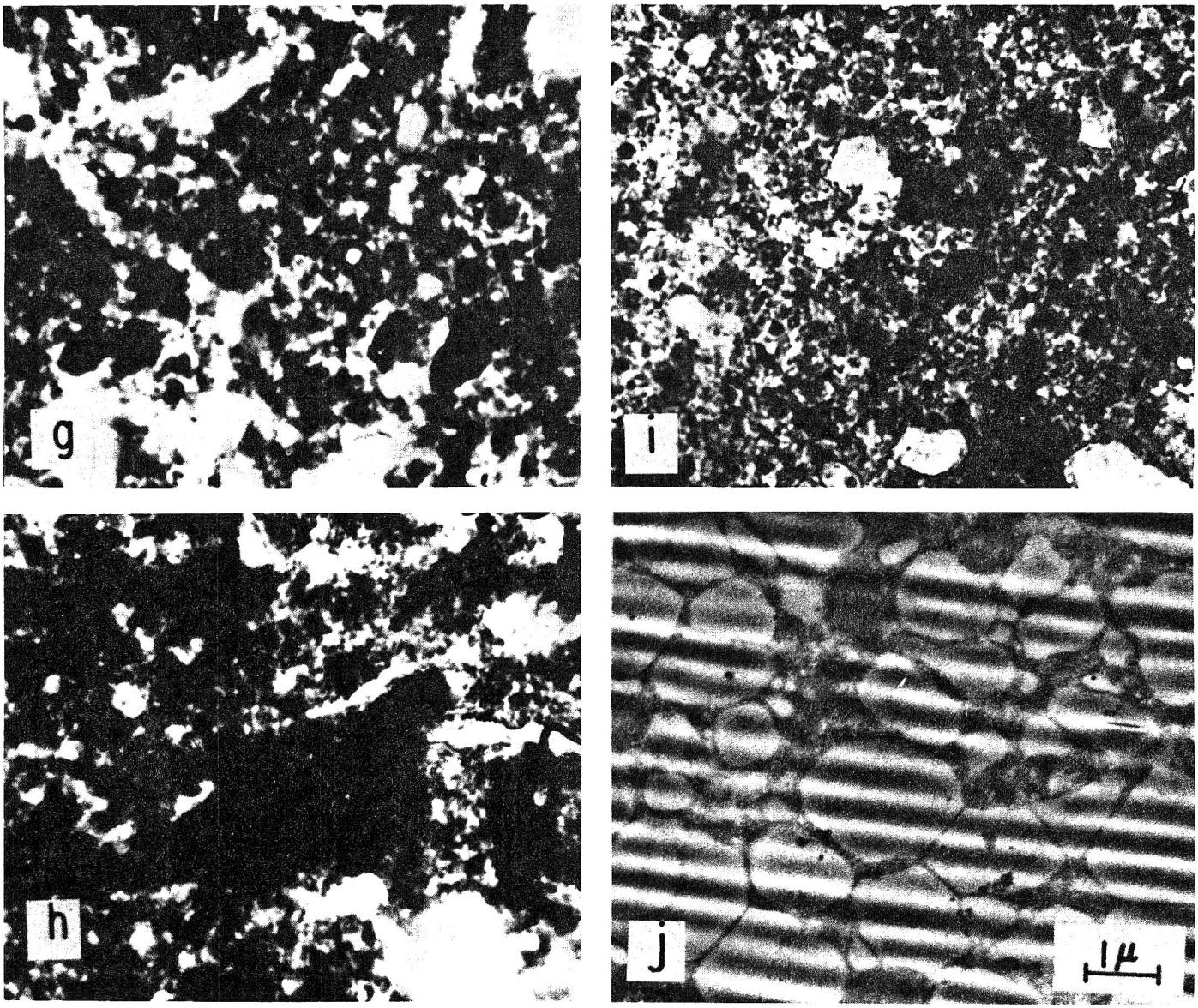

Table 1–Average numbers of small lipid particles in 0.5 mm² sections of frankfurters.

Series	Type fat	Emulsification (rpm)	Particles 5μ diameter or less per 0.5 mm² / 10² [a] Emulsification temperature (°F)							
			45	55	65	69	71	75	79	82	85
A	Beef	1,500	2550 ± 175 [b]	3340 ± 465	3960 ± 560	—	4090 ± 165	—	—	—	—
B		2,500	3135 ± 375	4140 ± 350	4090 ± 255	—	—	4720 ± 330	—	5470 ± 270	—
C		5,000	3690 ± 355	4475 ± 280	5025 ± 195	—	—	5690 ± 90	—	—	5500 ± 410
D	Pork	1,500	3570 ± 260	4080 ± 320	5235 ± 725	4760 ± 725	—	—	—	—	—
E		2,500	—	3950 ± 330	4230 ± 195	—	—	5440 ± 465	5965 ± 315	—	—
F		5,000	—	5160 ± 505	5490 ± 530	—	—	6340 ± 630	—	—	7230 ± 990

[a] Results of tests of equality between sets of coefficients of pairs of linear regressions (Chow, 1960).

B > A, P < 0.025
C > B, P < 0.01
C > A, P < 0.01

D = E, n.s.

F > E, P < 0.01
F > D, P < 0.10
D > A, P < 0.01
E > B, P < 0.05
F > C, P < 0.01

[b] Underlines indicate fat separation occurred in cooking these lots (Townsend et al., 1971).

Table 2–Particles 200 μ or more in diameter in sections of frankfurters prepared with beef fat or pork fat.[a]

			Emulsification								
			Temperature (°F)								
	Rpm	Fat	45	55	65	69	71	75	79	82	85
A	1,500	Beef	4.2[b]	4.0	2.45	—	0.65	—	—	—	—
		Pork		0.8	0.8	0.4	—	—	—	—	—
B	2,500	Beef	3.6	4.6	3.4	—	—	0.45	—	2.0	—
		Pork	—	2.4	1.2	—	—	0.5	0.8	—	—
C	5,000	Beef	3.2	4.8	3.4	—	—	1.65	—	—	0.65
		Pork		1.8	0.8	—	—	0.2	—	—	0

[a]Average of five replications on 0.5-mm^2 sections.
[b]Underlines indicate fat separation occurred in cooking these lots (Townsend et al., 1971).

1) show as black areas the dispersion in frankfurters prepared with 25% beef fat from emulsions comminuted to temperatures ranging from 45–82°F at 2,500 rpm. Comminuting at 45 (a), 55 (b) or 65°F (c) failed to produce adequate dispersion as shown by the separation of fat from the frankfurters in the smokehouse (Townsend et al., 1971). Comminuting to 75°F (d) produced the relatively fine dispersion shown in frankfurters from which fat did not separate. The poorer dispersion obtained on extended chopping to 82°F (e) illustrates the effects of overchopping which produces coalesced fat during lengthy chopping to relatively high temperatures (Townsend et al., 1971).

Photomicrographs f, d and g show as black areas the dispersion of beef fat frankfurters prepared from beef fat emulsions chopped at 1,500 rpm to 71°F (35 min), 2,500 rpm to 75°F (15.3 min) or 5,000 rpm to 85°F (6.8 min), respectively. The finest of the three dispersions is that shown in photomicrograph g. The results, as well as those shown in Table 1, indicate that chopping at 5,000 rpm not only produced a finer dispersion at a given temperature, but that achieving the equivalent was not possible by an extension of chopping at 1,500 or 2,500 rpm, since 71 and 75°F were the highest attainable temperatures yielding stable emulsions.

Photomicrographs g, h and i show as black areas the dispersion obtained in frankfurters containing beef fat, pork fat or cottonseed oil, respectively, as the principal lipid prepared from emulsions comminuted at 5,000 rpm to 85°F. They show the dispersion of lipids to be in the increasing order beef fat, pork fat and cottonseed oil. Photomicrograph j prepared using an electron microscope shows as light areas the dispersion of cottonseed oil obtained on comminuting at 5,000 rpm to 85°F. The appreciable portion of small particles, some approximately 0.1 μ as described by Borchert et al. (1967), accounts for the problem encountered in counting particles in studying dispersions of this fat using light microscopy.

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Mention of commercial names does not imply endorsement by the U.S. Department of Agriculture.

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EFFECT OF NEAR INFRARED ENERGY ON RATE OF FREEZE DRYING OF BEEF
1. Chamber Pressure and Radiation Intensity

SUMMARY—The effects of vacuum chamber pressure and intensity of near infrared energy on freeze drying rate for 1-in.-thick slices of eye round were investigated. The radiation source was a 500-w quartz iodine lamp. Decreasing the chamber pressure increased the freeze drying rate, especially during the early stages of the drying cycle corresponding to the initial and constant rate periods. The critical moisture content seemed to be about 43%. Different energy intensities of the same radiation characteristics were obtained by varying the distance between infrared heater and product. For distances of 9, 13.5 and 18 in., the inverse-square law was not followed; the drying rate was faster than predicted and appeared to vary linearly with distance.

INTRODUCTION

FREEZE DRYING is an expensive process. To achieve a moisture content below 4% in a beef cube or steak without harm to its quality, a drying time of about 12 hr must be employed. If this time could be safely shortened, the cost could be almost proportionally reduced. This would require an increase in either the rate of mass transfer or the rate of heat transfer, whichever is the limiting factor.

Mass transfer is facilitated by a large vapor pressure differential between the ice interface in the food product and the atmosphere in the drying chamber (Harper and Tappel, 1957). Therefore, there is general agreement that the ice interface in the food should be at the highest temperature that does not harm product quality. However, reports on chamber pressure show varied results.

Dyer (1965) concluded that a faster drying rate can be obtained by decreasing either the total gas pressure or the partial pressure of water above the interface. Yao et al. (1956) obtained rapid drying rates for chicken meat when pressures at or below 1 mm Hg were employed. Peck et al. (1960) made the recommendation that a freeze dryer should be operated at a pressure of about 3/4-mm Hg.

Saravacos (1965) and Lusk et al. (1965) observed no significant differences in the over-all drying rates when chamber pressures were in the range of 0.05–1.5 mm Hg. However, Lusk et al. (1965) noted a faster initial drying rate at lower pressures.

Ginnette et al. (1958) noted an increase in drying rate of carrot dice with an increase in total pressure. Harper (1962) postulated that the thermal conductivity of the porous dry material is a combination of the thermal conductivity of the dry matrix and that of the gas filling the pores; since the thermal conductivity of this gas increases with increase in pressure, the drying rate should also increase.

Levinson and Oppenheimer (1948) observed that infrared radiation reduced the drying time by more than half when compared to conduction freeze drying. Similar results were obtained by Zamzow and Marshall (1952). Tappel et al. (1955) used infrared radiation in the 2–8-μ wavelength range for the freeze drying of 1-in.-thick pieces of biceps femoris muscle and reported drying times comparable to conventional conduction heating.

Larson et al. (1967) varied the wavelength of peak emission from a 500-w quartz lamp between 0.96 and 1.10 μ. He found that the drying rate increased with decreasing wavelength but relatively severe product charring occurred after 1 hr. By absorbing longer wavelengths with a filter, charring initiation time was delayed by 30 min. Best results were obtained using intermittent radiant heating with high initial intensity which was progressively reduced.

Thus, the general objective was to evaluate near infrared radiation as the energy source for freeze drying of beef. Specifically, the effect of two variables on the drying rate was determined: chamber pressure between 0.70 and 4.5 mm Hg and radiation intensity which was varied by adjusting source to product distance.

MATERIALS & METHODS

The dehydration apparatus

Manufactured by Vacudyne Corporation (Chicago Heights, Ill.), the drying chamber was a 5-ft cube. A 6-in.-diameter evacuation port, located in the center of the rear side, was connected to a single-stage vacuum pump (Model KDH-130, Kinney Vacuum Pump Co., Boston, Mass.), which could reduce the pressure to about 1 mm Hg in 10 min. Total pressure in the chamber was measured with a thermocouple vacuum gauge (Model VT-4B, Hastings-Raydist, Inc., Hampton, Virginia).

The chamber was fitted with two condenser coils. One, installed along the rear wall inside the chamber, was constructed of 17 turns, each 4 ft long of 1 1/8-in. o.d. copper tubing and maintained at −20°F. The second, in the form of a U-shaped cold-finger located between the chamber and the vacuum pump, was 15 ft long of 5/8-in. o.d. copper tubing and maintained at −40°F.

The radiation source was a 500-w quartz iodine lamp (Hodge, 1961), 500 T-3Q/CL (Large Lamp Department, General Electric Co., Nela Park, Cleveland, Ohio), with a 3-in.-long heated tungsten filament. When operated at its rated voltage, 120 v, it burned at a color temperature of 3000°K, had a peak emission wavelength of slightly less than 1 μ and followed the normal tungsten energy distribution curve.

The sample was weighed at frequent intervals during drying without breaking the vacuum. The meat slice was held in a vertical position on one pan of a specially constructed double-pan beam balance and a chain counterweight in the other pan. To make a weighing after some drying, a constant-speed motor in the chamber was activated to remove sufficient chain to re-establish balance. The time of motor operation, calibrated in terms of weight removal, was recorded.

Raw material

Raw beef eye round, U.S. Good Grade, was used. Surface fat and connective tissue were trimmed, the muscle tied in a circular shape, wrapped in freezer paper and frozen for 2 days at 0°F. 1-in.-thick slices were sawed at right angles to the meat fibers. End slices were discarded, care being taken to avoid any thawing of the meat and the slices returned to 0°F storage. Three holes, 1.5 in. deep and 3/32-in. diameter, were drilled into the side of each slice for insertion of 28-gauge copper-constantan thermocouples; two just below each cut surface and one in the center between them. After de-

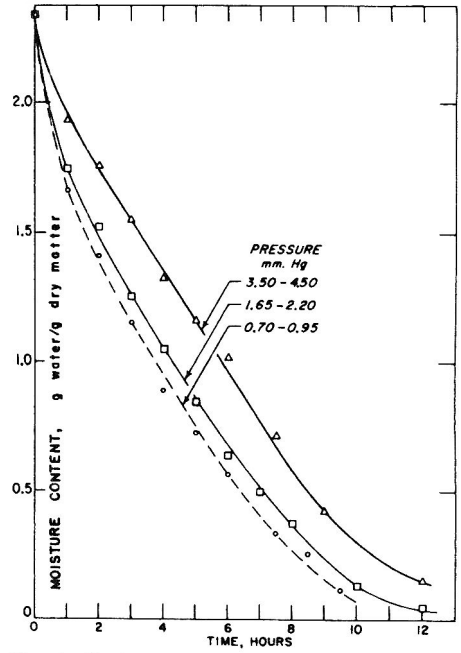

Fig. 1—Drying curves at three chamber pressures for thick slices of raw eye round with high-intensity infrared radiation as heat source.

hydration, the samples were vacuum packed and held at −40°F. Moisture content of the whole dehydrated slice was determined by the magnesium perchlorate desiccator method (Salwin, 1963).

Expression of results

Each slice was weighed on a Mettler balance to 0.2g before and after dehydration. The moisture content at any time during drying was calculated from the initial weight, initial moisture content and the weight loss to that time (as shown by chain removal from the balance pan) in terms of T value, g water/g dry matter. A drying curve of T vs. time was plotted. The drying rate was determined as change in T per hour at a given moisture content and time by taking the slope of the drying curve at this point. The rate could then be plotted against either parameter.

RESULTS & DISCUSSION

Chamber pressure

Total pressure in the vacuum chamber was controlled at three levels; high, 3.5–4.5 mm Hg; medium, 1.65–2.20 mm Hg and low, 0.70–0.95 mm Hg. The high and medium pressures were obtained by bleeding air into the drying chamber. Only one infrared heater, placed 12 in. from one cut surface of the meat, was employed. After drying in this position for 6 hr, the chamber was momentarily opened and the sample turned so the other cut surface faced the heater, until drying was terminated.

The drying curves in Figure 1 show that moisture content at any time was lowest with the lowest chamber pressure and almost as low with the medium pressure; the moisture was much higher at the high pressure. For instance, at 10 hr the T values were 0.08, 0.13 and 0.32, respectively. Extrapolations of these curves indicate that a T value of 0.03 would have been reached in about 11, 12.5 and 16 hr at low, medium and high pressure, respectively.

Figure 2 shows the variation in rate of drying with moisture content derived from the drying curves in Figure 1. Three different regions can be discerned: a high but rapidly falling initial rate, then a relatively constant rate and, finally, a falling rate. The high initial rate at all three pressures can be attributed to evaporation from the surface where dry-layer resistance to heat and mass transfer is still negligible. At a T value of 1.9, the rate under the high-pressure condition had fallen to 0.2, whereas rates for the medium- and low-pressure conditions were still 0.50 and 0.63, respectively, thus illustrating the beneficial effect of low pressure on this initial rate.

The constant-rate region is well defined for the high-pressure condition (Fig. 2); the rate remained at 0.18 from a T value slightly above 1.8 to about 0.8. In fact, at all three pressures the falling rate region seemed to be initiated at the same T value, 0.8, and the rates were quite similar at any given moisture content in this region. This suggests that both the critical moisture content and the rate in the falling rate region are independent of chamber pressure.

Some additional evidence for this observation can be derived from a plot of temperature at the center of the sample against moisture content (Fig. 3). It can be observed that for all three pressures a faster rise in center temperature started after a T value of about 0.75 was reached. As the moisture content decreased below this point, the increase in center temperature was similar. Also, the initial center temperature at a T value of 2.4 (Fig. 3) varied directly with pressure; for high, medium and low pressure it was +3.5, −7 and −13°F, respectively. At these pressures the equilibrium sublimation temperature for ice is about 29, 14 and −2°F, respectively.

The discrepancy between meat and ice temperatures has already been partially explained by Dyer (1965), who showed that the solutes suspended in the aqueous layer of the meat depress the equilibrium vapor pressure of beef by approximately 20% when compared to pure ice at the same temperature. The freezing point depression in the present experiment exceeded the values reported by Dyer. This is probably because continuous sublimation during actual freeze drying creates a dynamic rather than equilibrium situation.

It was concluded from these experiments that low chamber pressures decrease drying time because they increase drying rate in both the initial and constant-rate stages of drying.

Radiation intensity

According to the inverse-square law, when the radiant energy from a small surface is intercepted by a larger one, the energy per unit area of receiving surface is inversely proportional to the square of the distance between the surfaces. Thus, if a piece of meat is moved twice as far from the radiant heat source, it will receive 1/4 as much energy. The question arises as to whether the drying rate will also be reduced to 1/4.

Three slices of meat were obtained from the same muscle. Two infrared heaters applied radiant energy simultaneously to both sides of each slice. A 9-in. heater to sample distance was employed first. Whenever the surface of the meat

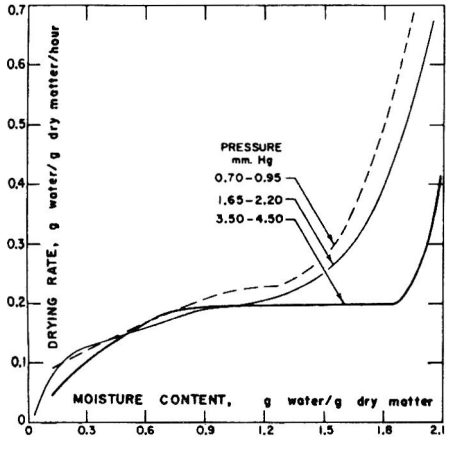

Fig. 2—Drying rate curves at three chamber pressures for thick slices of raw eye round with high-intensity infrared radiation as heat source.

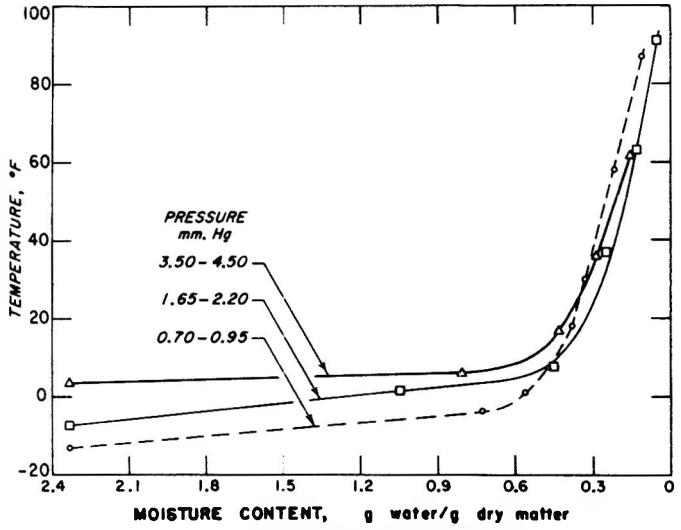

Fig. 3—Change in center temperatures with moisture content at three pressures during freeze drying of thick slices of raw eye round with high-intensity infrared radiation as heat source.

reached 130°F, the voltage was reduced to prevent surface overheating; the time at each voltage was recorded. The same combination of voltage and time used with the 9-in. distance was then applied to experiments at 13.5 and 18 in., even though the surface temperature did not rise to 130°F. Thus, the total radiant energy emitted by the heaters during drying was the same for all three distances.

Drying rate comparisons were based on the moisture lost by each sample during a given drying time. A Moisture Loss Ratio (M.L.R.) was calculated as the decrease in T value at the longer distance divided by the decrease in T value during the same time at the shorter distance, multiplied by 100. The moisture contents at 2-hr intervals and the M.L.R. calculated from them are shown in Table 1.

Only at the 9-in. distance was the moisture content reduced to below 5% in 8 hr, at 13.5 in. it was still 31% and at 18 in., 45%. Thus, distance did have a large effect on drying rate. However, as shown by the high M.L.R. in Table 1, in all cases the drying rate at the longer distance was higher than that predicted by the inverse-square law from the rate at the shorter distance. The M.L.R. increased linearly with drying time in each comparison, probably because at any given time the sample at the shorter distance was drier, so that its rate of moisture loss decreased faster in relation to that of the sample at the longer distance.

It is of interest to note that the M.L.R. for the 13.5/9 and the 18/13.5 half-distance comparisons were practically the same (Table 1) and the product of these two M.L.R. is equal to that experimentally found for the 18/9, or full-distance comparison. These findings indicate a linear relationship between drying rate and heater-to-product distance.

Two reasons may account for this discrepancy of the observed results with the inverse-square law. First, Moon (1936) pointed out that in applying the inverse-square law without significant error, the distance between the source and the object must be at least five times the greatest dimension of the source. In this experiment only the 18-in. distance exceeded this limit. Secondly, the radiation emitted by the heater was not propagated in all directions, but rather was in "parallel" rays aimed in the direction of the sample; therefore, the intensity impinging on the sample was high.

The encouraging result from this experiment is that the drying rate as a function of the heater-to-sample distance was actually higher than theory would predict. This means that in an actual drying situation the infrared heater-to-product distance can be varied by a few inches without greatly affecting drying rate.

Table 1—Effect of heater-to-sample distance on moisture content and on moisture loss ratio with time of freeze drying of beef by infrared radiation. Expected values based on inverse-square law.

Drying time	Distance (in.)			Distances compared		
(hr)	9	13.5	18	13.5/9	18/13.5	18/9
	T value (g water/g solids)			Moisture loss ratio (%)		
0	2.84	2.84	2.84	—	—	—
2	1.37	1.71	2.00	78	75	58
4	0.71	1.14	1.48	80	80	64
6	0.29	0.75	1.10	82	83	68
8	0.05	0.46	0.81	85	85	73
Expected	—	—	—	44	44	25

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EFFECT OF NEAR INFRARED ENERGY ON RATE OF FREEZE DRYING OF BEEF
2. Spectral Distribution

SUMMARY—Infrared radiation was extensively investigated as a heat source for freeze drying 1-in.-thick slices of beef. Two approaches were used to study the effect of spectral regions on the drying rate. First, filters which transmitted definite wavebands in the near infrared were interposed between the heaters and the product. Secondly, different spectral distributions were obtained by varying the voltage applied to the heaters while keeping the total radiating power constant. From the work with filters it was concluded that the short wavelengths, 1 μ or less, gave the most rapid drying. Similarly, the work with voltage variation showed that drying rate was improved by increasing intensity and decreasing wavelength to about 0.95 μ. The shortest complete drying cycle using infrared heating was 7.0 hr as compared to 11 hr for the conventional control. Samples were evaluated for surface appearance, rehydration characteristics and organoleptic quality of cooked meat. The quality of samples produced with infrared radiation of short wavelengths predominantly at about 1 μ was judged to be similar to that obtained with conventional heating.

INTRODUCTION

IN FREEZE DRYING of thick sections such as steak, radiation should have two characteristics: good penetration through the dry outer layer and high-energy intensity to provide the latent heat for sublimation. The most important restriction is that this radiation must not harm the product. There are a number of radiation sources available. These differ not only in intensity but in spectral distribution, characterized by the wavelengths of peak energy emission.

Asselbergs et al. (1960) compared the relative efficiency of quartz lamps, quartz tubes and resistance elements for peeling apples. The depth of penetration of the radiation into apple tissue corresponded to the energy between wavelengths of 1.4 and 5.0 μ. Best penetration was obtained with the quartz tube. Tappel et al. (1955) used infrared radiation in the 2–8 μ range to dry 1-in.-thick pieces of biceps femoris muscle. The drying times were comparable to those for conventional conduction heating. Forrest (1959), using quartz tubes, reported that the freeze drying rate for 16-mm-thick fish sticks was comparable to that achieved with contact plate heating with expanded metal inserts. Maguire (1965) noted that radiant heating permits a higher platen temperature during the first hours of sublimation, without detectable product damage. Larson (1962) regulated the voltage applied to a 500-w quartz lamp to vary the peak wavelength between 0.96 and 1.10 μ. He found that the drying rate increased with decreasing wavelength, but relatively severe product charring occurred after 1 hr. By using a Pyrex filter which absorbs longer wavelengths, charring initiation time was delayed by 30 min.

The purpose of this work was to study the effect of spectral distribution of radiant energy in the near infrared region on freeze drying rate and quality of beef eye round. It was desired to find a waveband which is preferentially absorbed by the ice and water in the meat so that a high intensity can be applied without burning the surface.

MATERIALS & METHODS

THE DRYING chamber, meat samples and general experimental procedures were as described in Part 1. The various experiments in this study were replicated two or three times. In cases where the reproducibility was low, generally due to faulty surface temperature measurements, the experiment was repeated a fourth or fifth time until reliable data were obtained.

Energy sources

The infrared heater, described in Part 1, was mounted on a ring stand with two reflector surfaces of aluminum foil above and below. When operated at its rated voltage, 120 v, it burned at a color temperature of 3000°K, at which the wavelength of peak emission was slightly less than 1 mμ.

To vary this wavelength, the heater was operated at several voltages; for each voltage, the color temperature as specified by the manufacturer was substituted into Wien's displacement law, to obtain the peak wavelength.

Energy of radiation reaching the sample changed with voltage and distance from the lamp. This was measured with a bismuth-silver 12 junction surface thermopile, sensitivity 0.07 μv per μw sq cm. (The Epply Thermopile, Inc., Newport, R.I.) and a microvoltammeter (Model 150-A, Keithey Instruments, Cleveland, Ohio).

For control experiments simulating conventional freeze drying at low temperature, two black, coated aluminum electric heating plates, 6 by 6 by 1 in. thick, were supported on each side of the product. The distance between the plates and the product was adjusted to 2 in. Each plate was controlled by a separate powerstat. The following drying cycle, obtained from a chart (Ziemba, 1963) showing the optimum time-temperature conditions for meat, was used with these plates:

Drying time (hr)	Heating plate surface temperature (°F)
0–3/4	100–225
3/4–1 1/4	225–275
1 1/4–3 3/4	275
3 3/4–4 3/4	250
4 3/4–6	225
6–9	175

Radiation filters

In pursuing an alternate method of wavelength selection, four filters were investigated: 1-69, 7-69, 7-56 (Corning Glass Works, Corning, N.Y.) and 2-44 (Infrared Industries, Inc., Waltham, Mass.).

Evaluation of surface appearance

The dehydrated meat slices were presented to several experienced judges who examined both surfaces of the sample and rated the appearance on a 9-point hedonic scale. A sample

Table 1—Comparison of drying time in the high-moisture range with the relative amount of radiant energy at different peak wavelengths impinging on the samples.

Filter No.	1-69	7-69	7-56	2-44
Total transmitted waveband (μ)	0.40–0.98	0.75–1.10	0.84–4.50	2.45–5.55
Wavelength of maximum transmission (μ)	0.54	0.83	2.00	2.75
Percent of source energy impinging on sample	4.6	8.6	62.6	16.4
Drying time (T value 2.46–0.75)	8.6 hr	8.2 hr	7.5 hr	9.4 hr
Relative efficiency of incident radiation for drying	2.53	1.37	0.21	0.65
Surface appearance (9 is perfect)	5.5	8.5	6.5	7.0

having a bright pink color, with no darkened, grayish or faded areas was given a score of 9 and all other samples were compared to it; 5 was barely acceptable and 1 very poor.

Rehydration tests

The weighed freeze-dried meat slices were rehydrated for 5 min in 350 ml distilled water at room temperature. The samples were then placed on a screen for 1 min to drain off excess water, and reweighed. The moisture content after rehydration and percent of original weight regained were calculated.

Organoleptic evaluation

Samples were rehydrated as above, broiled and judged on the usual 9-point hedonic scale (9—excellent, 5—barely acceptable) by a panel of 10 experienced judges for color, aroma, flavor, off-flavor and texture.

RESULTS & DISCUSSION

TWO APPROACHES were taken to obtain different spectral distributions. First, filters isolating waveband regions corresponding to the water absorption bands in the near infrared region were interposed between the heater and the sample. Secondly, voltage applied to the heater was varied.

Waveband selection with filters

Inspection of the transmission curve for water in the near infrared wavelength between 0.7 and 3.2 μ (Anderson Physical Laboratories, Champaign, Ill.) revealed four absorption bands. The weakest absorption band has a peak at about 0.96 μ, a stronger absorption follows with a peak at 1.45 μ, a yet stronger absorption occurs at a peak of 1.94 μ and the strongest and broadest absorption occurs between 2.55 and 2.90 μ with a maximum at 2.76 μ. Four filters with wavebands of maximum transmission corresponding to these four absorption bands were chosen. The filters were placed 5 in. in front of the samples, completely shading them. Total distance between the heater and the sample was 12 in. The heater was operated at 115 v. Surface temperature was controlled not to exceed 130°F. After one side of the meat was exposed to the heater for 6 hr, the sample was turned around.

The drying curves obtained with the four filters are shown in Figure 1. Drying rate curves (not shown) indicated that differences occurred mainly during the initial drying period. The drying time between T values of 2.46 and 0.75g water per g dry matter with each filter is shown in Table 1. The spectral distribution characteristics of each filter are also shown in Table 1. These drying times were fairly close together at about 8 hr. However, the filters gave greatly different energy transmissions (fourth line, Table 1). To compensate for these energy differences, a relative efficiency factor was calculated by taking the reciprocal of the product of fraction of energy (fourth line) and drying time (fifth line). These are shown in the sixth line of Table 1.

The efficiency was highest for the filter with a peak transmission at 0.54 μ. It fell to almost half for the filter transmitting at a peak wavelength of 0.83 μ and to less than one-fourth for the filters at the longer wavelengths. Based on the moisture range covered and the energy actually reaching the sample, it was concluded that the short or very near infrared wavelengths, 1 μ and slightly less, were more advantageous to freeze drying.

Waveband selection of voltage variation

In the preceding work with filters, the effect of wavelength was confounded with intensity; in this work, therefore, the intensity was maintained constant. No filters were used. Instead, voltage to the heater was varied; this changed the temperature of the filament and consequently the wavelength of peak radiation. The wavelength distribution of this radiation would not be so discrete as that obtained through an interposed filter. At each voltage, intensity measurements were made with a thermopile at a number of distances from the heater. The intensities obtained were plotted against distance. The distance on the curve for each voltage giving 100 and 180 Mw/cm² was used to position the heaters in relation to the product, so that energy impinging on the sample remained the same as voltage was changed.

The peak wavelengths (obtained from the manufacturers' literature) at each voltage was as follows: 120 v—0.95 μ; 90 v—1.05 μ; 60 v—1.26 μ; 42 v—1.44 μ.

At high moisture content. The first study was done in the moisture range from the initial T value, about 2.7g water per g dry matter, to a T value of 0.75, the critical moisture content. One heater was employed. First, the intensity was maintained at 180 Mw/cm² and three voltage-distance combinations were applied: 120 v—10.6 in.; 90 v—8.5 in.; 60 v—6.0 in. In a second series the intensity was maintained at 100 Mw/cm² and the following four voltage-distance combinations were applied: 120 v—14.6 in.; 90 v—11.6 in.; 60 v—8.5 in.; 42 v—6.0 in.

Results of these experiments may be expressed in terms of time required to dehydrate the samples from T values of 2.7—0.9. At 180 Mw/cm² the times were 5.1, 5.3 and 5.9 hr for 120, 90 and 60 v, respectively; at 100 Mw/cm² they were 6.6, 6.5, 7.2 and 6.8 hr for 120, 90, 60 and 42 v, respectively. Thus, within each intensity, the shorter wavelengths gave faster drying. However, these differences were small, about ½ hr. In comparison, intensity had a larger effect; increasing intensity from 100—180 Mw/cm² decreased drying time in this range by 1.5 hr at the shortest wavelength. It is, therefore, advisable to employ in the high-moisture region, high-intensity radiant energy with predominant wavelengths in the very near infrared.

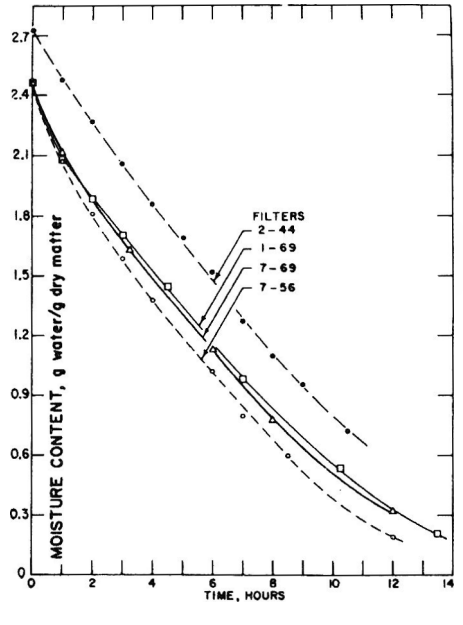

Fig. 1—Drying curves obtained with four filters of different wavelength transmission characteristics.

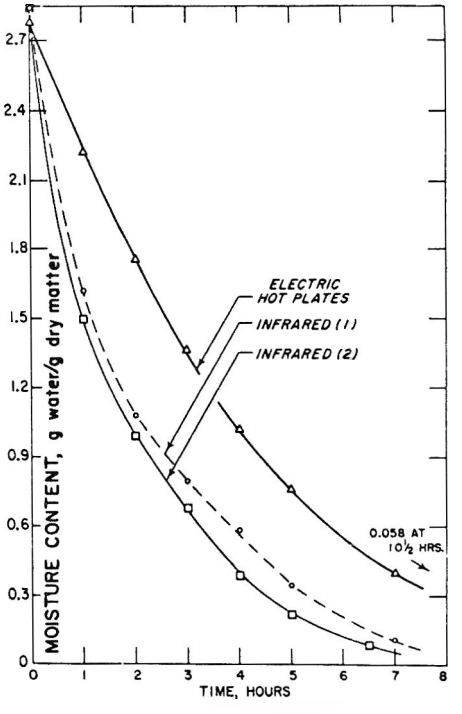

Fig. 2—Drying curves obtained for complete drying cycles while heating from both sides with infrared heaters and electric hot plates.

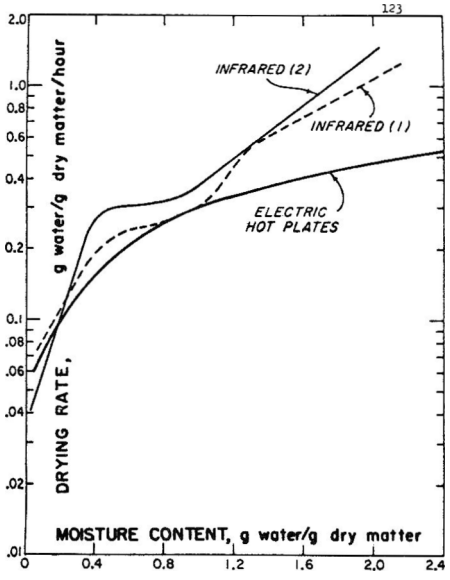

Fig. 3—Drying rate curves obtained for complete drying cycles while heating from both sides with infrared heaters and electric hot plates.

At low moisture content. It was of special interest to observe whether the near infrared heating method will give a high drying rate at T values below 0.9 without charring the product surface. Several meat slices from the same muscle were freeze dried without the application of heat for 15–20 hr to a T value of about 0.8. These samples were used as the starting material for the studies at low final moisture levels. Three voltages were applied: 120, 90 and 60 v, at a constant intensity of 100 Mw/cm^2. Two heaters were used, one on each side of the sample. Meat surface temperature was controlled not to exceed 130°F; once this temperature was reached, the heaters were turned off and the surface allowed to cool to 100°F before the heaters were turned on again. As a comparison or control to the above, two electric hot plates, one on each side of the product, were used. These were controlled at 250°F for 0.5 hr, at 225°F for 1 hr and at 175°F for the rest of the time.

Results of this experiment are expressed on two time bases: first, total time in the dryer and secondly, time the heaters were on. The second was based on the reasoning that drying took place mainly while the heaters were on. This basis should give a better evaluation of the effect of spectral distribution.

Times required to dry from T value 0.66–0.025 at each voltage were compared. When total time basis was used, only a minute difference in drying time was obtained; the average was about 5.0 hr. Using the second basis, the times for 120 and 90 v were about the same, 3.3 hr, while for 60 v it was 3.8 hr. These results show an advantage for the very short wavelengths. On the total time basis, the hot plates, which were on all the time, gave only a slightly longer drying time, 5.4 hr, than the infrared heaters. Using the second time basis, this was about 50% longer.

Shortest complete drying cycle

For purposes of comparison with other heating methods, it was desirable to determine the shortest complete drying cycle that could be obtained without surface burning. In these experiments the pressure was kept below 1 mm Hg and the surface temperature was not allowed to exceed 130°F. Two heaters were employed to heat the sample from both sides simultaneously.

Both voltage and distance between heater and product were varied so as to apply the maximum radiant energy. Two slightly different experiments were made. In the first, during the first hour the distance was 6 in.; for the next 2.5 hr, the distance was increased to 9 in. and for the final period the distance was 12 in. At the beginning and each time the distance was changed, the voltage at the start was set at 115 v (0.97 μ). At each distance-time combination, as the meat surface temperature reached 130°F the voltage was successively reduced to 90 v (1.05 μ), 80 v (1.14 μ) and, finally, 60 v (1.3 μ).

Results of this experiment (Infrared 1) and one using two hot plates are shown in Figure 2. The time to dry from an initial T value of 2.83–0.05 was 7.7 hr for the infrared and 11 hr for the hot plate heating methods. Thus, this infrared method took only about 70% of the time taken with conventional heating.

In the second experiment, rather than decrease the voltage below 115 v as soon as the surface temperature reached 130°F, the heaters were turned off and the sample allowed to cool for about 5 min. The heaters were then turned on again at 115 v and the cycle repeated several times before reducing voltage to the next step. This on-off cycling was followed at each voltage setting. The over-all effect of this second procedure was that the sample was exposed to more high-intensity very near infrared radiant energy.

These results (Fig. 2, Infrared 2) show that it took only 7.0 hr for the same T value differential as above. Thus, this technique gave another 10% reduction in drying time.

The drying rate curves for these data are shown in Figure 3. The curves show that infrared heating increased the drying rate over conventional heating at all moisture contents, especially in the initial and constant rate regions.

Quality evaluation

The freeze-dried samples produced during this work were evaluated for appearance of the dry surface, rehydration and organoleptic quality of the cooked meat.

Surface appearance. Two factors influenced the rating: fading of the pink color and general darkening or incipient burning. Samples dehydrated with the use of filters were evaluated in one group; the average hedonic scores are shown at the bottom of Table 1. Samples dehydrated with filter 7-69 (0.83 μ) had the best surface appearance, those with filters 7-56 (2.0 μ) and 2-44 (2.75 μ) were intermediate and those with filter 1-69 (0.54 μ) were considered barely acceptable. These results suggest that radiation in the near infrared (just above the visible range) preserved the appearance very well. In sharp contrast, radiation primarily in the visible range did cause some undesirable darkening.

All samples dehydrated with different voltages at constant intensity (100 Mw/cm^2) were evaluated in another group. The hot plate controls were included. The same average score of 7.2 was obtained for the control, the 120 v (0.95 μ) samples and the 90 v (1.05 μ) samples; the 60 v (1.26 μ) score was 6.8 and the 42 v (1.44 μ) score was 6.3. It was concluded that the very near infrared produced a better surface appearance than the middle infrared radiation. This corroborates the above findings with filters. The important point is that samples dehydrated with very near infrared were not worse in appearance than were the control samples.

Rehydration evaluation. 26 samples representing all infrared dehydration conditions were rehydrated. The percent of original moisture regained ranged between 66.5 and 80.9% with an average of 74.9%. The observed variation can be accounted for by differences in fat marbling. No relationship was evident between rehydration and the dehydration conditions used. Samples dehydrated with the electric hot plate averaged 72.8%. It was concluded that the near infrared heaters did not have an adverse effect on rehydration capacity.

Organoleptic quality. 11 samples representing the different variables investigated in this study were rehydrated, broiled and evaluated. All of the samples were satisfactory (above a score of 5) in every category; most of the over-all organoleptic averages were about 7.2. No great differences existed among them. The most interesting observation was that the sample dehydrated completely by high-intensity near infrared radiation (Infrared 2, above) received the highest over-all organoleptic score, 7.5, as compared to 7.3 for the hot plate control. It was concluded that heating by near infrared radiation did not harm organoleptic quality of the dehydrated product.

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HEAT PASTEURIZATION OF CRAB AND SHRIMP FROM THE PACIFIC COAST OF THE UNITED STATES: PUBLIC HEALTH ASPECTS

SUMMARY—A study of the potential public health hazard presented by coagulase-positive staphylococci, salmonellae and Clostridium botulinum *in the meat of Dungeness crab and Pacific Coast shrimp pasteurized in flexible plastic containers revealed potentially toxinogenic staphylococci on the commercial nonpasteurized product, in 15% of the shrimp and 9% of the crab samples. No salmonellae or* Cl. botulinum *were isolated from, respectively, 26 and 54 samples of shrimp or 74 samples of crab. Pasteurization for 1 min at 180°F destroyed large inocula (10^7 and 10^8 cells) of staphylococci and salmonellae introduced into packages of the products, but processing for 5 min at 180°F allowed some members of an inoculum containing 10^3 spores of* Cl. botulinum *type E to survive. While storage at 40°F prevented the growth on crab and shrimp meat of all staphylococci and salmonellae tested, it permitted growth and toxin formation by* Cl. botulinum *type E after 30–40 days. No toxin could be detected in packages inoculated with type A and proteolytic B spores and held at 50°F or lower. A 0.1% dip of sodium benzoate, with or without fumaric acid, did not prevent growth and toxinogenesis by* Cl. botulinum *types A, proteolytic B or E. It was concluded that for complete safety a holding temperature of 36°F or lower at all times would be required, but that it could not be expected to be maintained in commercial channels.*

INTRODUCTION

RENEWED commercial interest in the heat pasteurization of Pacific Coast shrimp and crab meat prompted us to undertake a feasibility study of the procedure. Because metal cans are usually associated with sterilized foods that can safely be kept at room temperature, we used instead flexible, heat-sealable plastic pouches, which have the added advantage of allowing faster heat penetration into the flat packages, thus minimizing the adverse effects of heat on the product. Although results were encouraging with respect to extension of storage life, this report deals only with safety aspects of the procedure and presents the results obtained with the three bacterial types we considered most important; namely, coagulase-positive staphylococci, salmonellae and *Clostridium botulinum.* Of these, *Cl. botulinum* has three attributes that make it by far the greatest potential hazard: It produces a lethal toxin, forms heat-resistant spores and has been found, among other places, along the Pacific Coast of the United States, Craig et al. (1968). Furthermore, the nonproteolytic types of this organism can grow and produce toxin at refrigerator temperatures and leave relatively unaltered, though toxic, the protein food in which they have grown; type E is the most common and has been isolated on the Pacific Coast from Dungeness crab, Craig et al. (1968); Eklund and Poysky (1965), and from a variety of fish, Craig et al. (1968). These considerations and the paucity of available information about how these organisms behave on cooked crab and shrimp meat under various conditions prompted us to focus most of our attention on *Cl. botulinum.*

In this report we have attempted to assess the potential hazards on the basis of: 1) the incidence of the potential pathogens on shrimp and crab meat commercially prepared in the San Francisco area, 2) the effect of pasteurization on these bacteria, 3) the ability of crab and shrimp substrates to support the growth of these organisms and 4) the storage temperature required to prevent bacterial multiplication in the event of survival or postpasteurization contamination under otherwise favorable conditions for growth.

EXPERIMENTAL

THE WORK was carried out on Pacific Coast crab (*Cancer magister*), also known as Dungeness, and on the coastal species of shrimp (*Pandalus jordani*).

Pasteurization

We used 6 oz of meat in 6 by 8-in. Mylar-polyethylene pouches, no more than 1 in. thick when filled. After most air was squeezed out, pouches were heat-sealed, placed in wire-mesh baskets and immersed in water at 185°F for 1, 5 or 10 min, after the center temperature, as measured by means of thermocouples placed at the centers of some packages, had reached 180°F. Heating for more than 5 min adversely affected product quality. We, therefore, report results for only the two shorter heating times.

Bacteriological media

In addition to the standard media, we used the following special ones based on crab and shrimp meat: homogenates, prepared by blending one part of meat with one part of 2% NaCl solution (commercial crab and shrimp meat was found to average 2% salt content) and autoclaving, and broths, prepared by boiling 500g of meat for 30 min in 1 liter of 2% NaCl solution, filtering and autoclaving.

Isolation procedures

Coagulase-positive Staphylococcus aureus. We estimated these by the MPN method. After enrichment in Trypticase Soy Broth, 10% NaCl, for 48 hr at 37°C, samples were plated out on Staphylococcus No. 110 Medium containing 2% egg yolk, Carter (1960), and incubated at 45°C, Raj (1966); suspect colonies were confirmed by the coagulase tube test using Difco dehydrated plasma.

Salmonellae. To establish the presence or absence of these organisms, 1-ml aliquots of the initial 1:10 dilution of the food were inoculated in triplicate into Mannitol Broth according to the method of Gerichter and Sechter (1966). After incubation for 24 hr at 37°C, 0.2 ml from the positive tubes was inoculated into Selenite F Broth and incubated at 37°C for 24 hr more. All tubes were then plated out on MacConkey's and on Brilliant Green agar. Sus-

Table 1—Effect of commercially processed crab and shrimp meat with its microbial flora on the detection of two strains of Clostridium botulinum type E.

Average no. spores inoculated	Crab and shrimp meat added	Toxic jars/Total inoculated	
		Saratoga	Beluga
1	No	1/3	1/3
10	No	3/3	3/3
100	No	3/3	3/3
1	Yes	2/3	1/3
10	Yes	3/3	3/3
100	Yes	3/3	3/3
1000	Yes	3/3	3/3

Table 2—Recovery rate (%) of Clostridium botulinum type E spores from packages of shrimp heated 5 min at 180°F.

Strain	No. of spores inoculated			
	10^3	10^4	10^5	10^6
Alaska strain	0	20	40	100
Saratoga strain, crop 2	25	80	100	100

pect colonies were identified according to the scheme of Johnson et al. (1966).

Cl. botulinum. To isolate type E, we used the cooked meat medium of Dolman (1957) as modified by Eklund et al. (1967a). We distributed it in 200-ml amounts into 8-oz screw-capped glass jars, overlaid it with Vaspar and introduced approximately 20g of inoculum per jar. We incubated the jars at 24°C and on the fifth day after gas appeared tested for the presence of toxin.

To estimate the sensitivity of the isolation procedure and to assess the danger of interference from other organisms, we inoculated various numbers of type E spores into jars with or without the usual amount of crab or shrimp meat and tested for toxin as previously described. The results, shown in Table 1, suggest that the method is sensitive and that the organisms present on processed crab and shrimp meat do not interfere with toxinogenesis. For types A and proteolytic B we used stratified beef heart broth with meat particles and incubated at 37°C.

Detection of toxin

In enrichment cultures for types A and proteolytic B, we added 2 ml of 0.1M pH 6.5 phosphate buffer to 1 ml of the broth, centrifuged the mixture and injected 0.4 ml intraperitoneally into mice. Toxicity was verified, when necessary, by means of specific antisera. For type E we used the same procedure, except that we incorporated 0.2% trypsin (Difco 1:250) into the added buffer and incubated the mixture for 1 hr at 37°C before centrifuging it. This enhanced toxicity by a factor of 35.

To detect toxin in the packages we added not more than 5 ml of pH 6.5 buffer to each, thoroughly mixed its contents, took the drained wash liquid as our sample and proceeded as for detection of toxin in enrichment cultures. Preliminary tests showed that under our conditions this procedure was as sensitive as that involving homogenization of the material.

RESULTS

Incidence of bacteria of public health significance

Coagulase-positive staphylococci were isolated from only four of 26 samples of shrimp and six of 70 samples of crab meat. The contamination levels were usually about 10 cells per gram.

Salmonella. We found no *Salmonella* in 26 samples of unpasteurized shrimp and 74 of crab meat. In two instances, paracolon organisms of the Providence group were isolated.

Cl. botulinum. We failed to isolate *Cl. botulinum* from the 74 samples of crab and 54 of shrimp meat tested. The samples, though few, were collected during at least a 6-months' period for each product.

Because *Cl. botulinum* had already been found on Pacific Coast marine food species, we did not try to show again the presence of the organism on the raw material. That we found none on the processed product could probably be ascribed to the cooking. Another 130 attempts to isolate *Cl. botulinum* from pasteurized and control crab and shrimp meat stored under various conditions of refrigeration not surprisingly failed.

Effect of pasteurization

In our attempts to assess the effect of pasteurization on heavy contaminations with the organisms under study, we usually used the seafoods themselves as media to approximate presumed actual conditions.

Staphylococcus aureus. We used a mixture of three coagulase-positive isolates from crab meat. 1 ml (containing approximately 10^8 cells) of a 24-hr broth culture was introduced into the centers of each of six crab and six shrimp meat pouches. Each bag was sealed and heated for 1 or 5 min at 180°F. No staphylococci were recovered.

Salmonella. In preliminary heat-resistance tests of several species of *Salmonella* (*enteritidis, typhimurium, pullorum, panama, newport* and *senftenberg* strains 775W and S8), heating tubes of nutrient, crab or shrimp broth containing 10^7 cells of the inoculum for 3 min at 154°F destroyed all except *S. senftenberg*, which survived 3 but not 6 min at that temperature. Heating for 1 min at 180°F destroyed 10^8-cell-inocula of *S. senftenberg* 775W introduced into packages of shrimp and crab meat.

Cl. botulinum type E. In spite of the apparent absence of this organism on processed crab and shrimp meat, it could be introduced into the picking room. Its less strict anaerobic requirements might then allow it to become established in some forgotten particle of meat and cause heavy contamination of the product. To test the effect of pasteurization under these conditions, we inoculated groups of 5–9 pouches of meat with various numbers of type E spores, then heated the packages for 5 min at 180°F. The results, shown in Table 2, indicate that a heat pasteurization schedule that would maintain the quality of the meat would not assure the destruction of accidentally introduced spores of *Cl. botulinum*, even of the relatively heat-sensitive type E.

Effect of temperature and medium on the growth of potential pathogens

In this series of experiments, the results with *Cl. botulinum* so overshadowed the data for the other bacteria that we report the latter in abbreviated form: After inoculating approximately 5×10^4 cells each of five species of *Salmonella* and five isolates of coagulase-positive *Staphylococcus* into tubes of crab and shrimp broths and homogenates as well as into nutrient broth, we incubated them at various temperatures. Growth was followed by viable counts; when it occurred, it proceeded equally well in all media tested, indicating that crab and shrimp are good growth substrates for these bacteria. Growth was markedly slowed but not stopped at 10°C (50°F), whereas at both 4.4°C (40°F) and 1.1°C (34°F)

Table 3—Effect of incubation temperature on the formation of toxin by various strains of Clostridium botulinum in crab and shrimp homogenates.

Storage temp (°F)	Medium	Cl. botulinum type inoculated[a]			
		E, Beluga[b]	E, Saratoga[b]	A	B
98	Shrimp	*	*	12–14[c]	24–26
	Crab	*	*	8–10	8–10
75	Shrimp	6–8	4–6	20–26	20–26
	Crab	4–6	2–4	17–21	10–12
50	Shrimp	6–8	6–8	> 75	> 75
	Crab	6–8	6–8	> 75	> 75
40	Shrimp	> 75	> 75	> 75	> 75
	Crab	> 75	> 75	> 75	> 75

*Not incubated at this storage temperature.
[a] 5×10^4 spores inoculated into each tube.
[b] All type-E-inoculated samples were trypsinized.
[c] First figure indicates last day sample was nontoxic, and the second, the day toxicity was first detected.

Table 4—Effect of incubation temperature on the production of toxin by various strains of Clostridium botulinum in previously heat-pasteurized shrimp and crab meat in air-impermeable plastic pouches.

Storage temp (°F)	Product	Cl. botulinum type inoculated[a]			
		E, Beluga[b]	E, Saratoga[b]	A	B
75	Shrimp	11–13[c]	11–13	8–10	3–5
	Crab	5–7	5–7	2–3	5–7
50	Shrimp	> 75	> 75	> 75	> 75
	Crab	16–20	26–30	> 70	> 70
40	Shrimp	> 75	> 75	> 75	> 75
	Crab	30–40	> 70	> 70	> 70

[a] 5×10^4 spores inoculated into each tube.
[b] All type-E-inoculated samples were trypsinized.
[c] First figure indicates last day sample was nontoxic, and the second, the day toxicity was first detected.

the number of viable bacterial cells actually declined.

To study the ability of *Cl. botulinum* to grow and produce toxin in shellfish meat at different temperatures, we used tubes of shrimp and crab meat homogenates inoculated with approximately 10^4 spores of each of types A, B and E. The final pH of the shrimp medium was 7.6 and of the crab, 6.8. Results in Table 3 show that all four organisms tested produced toxin in crab and shrimp. Storage at 50°F prevented toxinogenesis by types A and proteolytic B but not by the E types. At 40°F none of the tubes became toxic within the experimental storage period, possibly because of the relative small inoculum used.

In several other experiments, approximately 50,000 botulinum spores were introduced into each pasteurized package, which was then kneaded, stored and tested for toxin—those at 75°F every other day, at 50°F every fourth day, and at 40°F every tenth day.

Table 4 presents composite results from these experiments. The important point is the appearance of toxin in crab meat after 30–40 days at 40°F, although in only one of the three experiments conducted under apparently identical conditions but with different lots of crab meat. Five of eight remaining samples of this group were toxic on day 45. When toxicity was first detected, the meat had only a slightly sour odor and could conceivably have been eaten. On day 45 the sourish odor was only slightly stronger, underscoring the peculiar hazard represented by *Cl. botulinum* type E in this case.

Effect of sodium benzoate on growth and toxinogenesis of Cl. botulinum

As the accepted commercial practice is to dip the shrimp and crab meat in a 0.05% solution of sodium benzoate, we examined the effect of this chemical on *Cl. botulinum*. In one experiment we aseptically added benzoic and fumaric acids, singly and in combinations, to tubes of cooked meat broth. The concentrations of the chemicals ranged from 0.01–0.05%. All inocula of various types of *Cl. botulinum* grew well. Dipping shellfish meat into a 0.1% solution of these chemicals, followed by packaging, pasteurization and inoculation with *Cl. botulinum*, did not prevent or delay toxinogenesis when compared with non-dipped controls.

Finally, to find out whether benzoate enhances the sporicidal effect of heat, we introduced type E (Alaska) spores into packages of shrimp dipped into a solution containing 0.1% each of sodium benzoate and fumaric acid. After pasteurization for 5 min at 82.2°C (180°F), cooling and subculturing into recovery broth, we recovered the inoculated organism from five of six packages inoculated with 10^5 spores and six of six packages that had received 10^6 spores. In undipped control packages, recovery rates were respectively two of five and five of five.

DISCUSSION

OUR DATA and information available in the literature enable us to assess the microbiological hazards connected with pasteurization. First, regardless of the low numbers of staphylococci in processed shrimp and crab meat, or of the apparent absence of salmonellae and of *Cl. botulinum*, we must assume that spores of the latter will be present on the product at one time or another. Furthermore, the encouraging effect of pasteurization on staphylococci and salmonellae should be of academic interest only, when considered in the light of our *Cl. botulinum* data.

To be potentially dangerous, a food must: 1) come in contact with a food-poisoning organism, 2) provide a suitable substrate for its growth (and, in the case of toxin producers, toxinogenesis) and 3) be subjected to other favorable conditions, such as temperature.

In Dungeness crab meat and Pacific Coast shrimp these three conditions may very well be present simultaneously. Both products are suitable substrates, as we have shown, and the danger of contamination is real: *Cl. botulinum* spores are found in the marine environment of the Pacific Coast, and keeping them away from the meat-picking room depends on several tenuous and poorly controlled factors. Furthermore, if *Cl. botulinum* spores do find their way on to the meats of these shellfish, it is only a matter of adequate time and temperature before toxin is produced. In this respect unpasteurized packages are safer than pasteurized ones, as the normal spoilage flora of shrimp and crab meat renders the product unusable before toxicity develops. For instance, if we assume that detectable toxin was present in unpasteurized crab meat the day after the last negative finding and before the first positive one, the product stored at 50°F would have spoiled 14 days before it became toxic, and at 40°F, 27 days before; however, by killing off the normal spoilage flora, pasteurization may render the product more dangerous. Proteolytic types of *Cl. botulinum* would, under favorable conditions, produce putrid odors that would prevent consumption of the food, but a nonproteolytic type would produce no such warning odors. Other bacteria that survive pasteurization are not active spoilers, and they render the product inedible only long after toxin has been produced by *Cl. botulinum* type E.

Thus we must conclude that commercially processed Dungeness crab and Pacific Coast shrimp might well become contaminated with *Cl. botulinum* spores, and that a heat pasteurization process that leaves the quality of the product undamaged does not guarantee destruction of contaminants. A food thus contaminated must depend for its safety on a solid controlling factor such as pH or salt. In the shellfish under consideration, however, we must rely solely on temperature, and this brings up the third condition mentioned earlier. We have shown that type E toxin can be produced in crab meat at 40°F, and others have shown that *Cl. botulinum* nonproteolytic types F and B (which we did not study), both present in the marine environment off the Pacific Coast, can grow and produce toxin at 38°F, Eklund et al. (1967a; 1967b). Moreover, Schmidt et al. (1961) found that *Cl. botulinum* type E will grow and produce toxin at 38°F but not at 36°F. Thus for complete safety, crab and shrimp pasteurized under our conditions would have to be stored at 36°F or lower at all times. Because such a low temperature is not consistently maintained in commercial channels or in the home, the chance for danger exists, and with *Cl. botulinum* chances are best not taken.

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COMPUTER-AIDED PREDICTIONS OF FOOD STORAGE STABILITY: OXIDATIVE DETERIORATION OF A SHRIMP PRODUCT

SUMMARY—A study was conducted on stability of a freeze-dried shrimp bar packaged in various materials. The purpose of this study was to develop a method for predicting the storage stability of dry foods stored in semipermeable containers and deteriorating through oxidation of lipids. In the shrimp bar, organoleptic deterioration was correlated with absorption of oxygen and with loss of carotenoid pigment; these indices of oxidation were used for the study. Rates of oxidation were studied as a function of oxygen concentration and permeability characteristics of materials used to package the bars determined. A mathematical model for prediction of storage life of the product was formulated using the kinetic data for shrimp oxidation and the permeability data. An iteration procedure utilizing a high-speed computer was used to solve the pertinent equations and to predict storage life. Predictions were compared with results of actual storage tests; those based upon pigment loss were better than those based upon changes in flavor score.

INTRODUCTION

FEW attempts have been made to predict a priori the storage life of foods stored in flexible packages. The need for these predictions is evident, especially for space flight where weight and size limitations on the food supply require optimization and economy. This paper is a report of a method developed for predicting the stability of foods which deteriorate through an oxidative mechanism, and the application of the method to evaluate the loss of carotenoid pigments in a freeze-dried shrimp product.

Past studies on measurement of food storage life in packages have been limited to packaging the foods in systems allowing large differences in oxygen and water transport through the package. The foods were stored at different external conditions and tested periodically either for chemical deterioration or for organoleptic acceptability.

Daoud and Luh (1967) studied the stability of dehydrated red bell peppers stored at several temperatures in packages made either of an aluminum laminate or of a mylar-saran-polyethylene laminate. Quality of the peppers was evaluated by measurement of the carotenoid pigment content, which was correlated with visual rating of color. They found that the aluminum laminate which had a lower oxygen permeance than the all-plastic laminate, protected the peppers from deterioration more effectively. However, the authors made no attempt to correlate rates of deterioration with oxygen permeability, except on a qualitative basis. A similar approach was used in a study on freeze-dried green asparagus (Syn and Luh, 1965).

Hu et al. (1968) showed that aluminum laminates gave the same level of protection against oxidative deterioration as tin cans, for both dried potatoes and freeze-dried pork. These were held for 6 months at various external conditions of temperature and humidity. A qualitative attempt was made to show the effect of increased permeability to oxygen due to pinholes in terms of storage stability.

Jurin and Karel (1963), Karel and Go (1964) and other investigators have studied the control of respiring fresh fruits and vegetables by storage in films of different CO_2 and O_2 permeabilities. Mathematical models were developed to enable selection of the packaging material needed for a given storage life. However, these techniques have not been applied to dehydrated foods undergoing oxidation.

A major problem in predicting the extent of deterioration of an oxidizable food in a package is that the oxygen level is continuously changing due to permeation through the package and reaction with the food. It does not reach a steady-state level as is often the case for respiring fruits and vegetables. A further complication is that the rate of oxidation of carotenoid pigments as well as that of other lipids is a function of relative humidity as well as oxygen level. Martinez and Labuza (1968) showed that the rate of loss of carotenoids in freeze-dried salmon decreased when held at relative humidities above those corresponding to the monolayer value for water adsorption as calculated by the usual methods (Labuza, 1968).

Marcuse and Fredrikson (1968) have shown the effect of oxygen level on the rate of oxidation of lipids in model systems. The rate falls off sharply below 5% O_2, a condition that would exist initially for a packaged food. Tuomy et al. (1968) showed similar results for freeze-dried beef and chicken stews. Chen and Gutmanis (1968) evaluated the kinetics of loss of color in chili pepper as a function of storage under conditions of different moisture contents and in the presence of either oxygen or nitrogen. As would be expected, an increased moisture content protected the chili from color loss, as did the absence of oxygen.

Thus, many factors must be considered in the prediction of storage life of an oxidizable food in a flexible film package. This study is an attempt to combine these factors into a useful mathematical model, designed for a specific storage problem but capable of adaptation to other similar problems.

Table 1—Oxygen permeance of packaging materials.

Material	Manufacturer	Thickness (mil)	k/x[a] Literature @ 77°F	k/x[a] Experimental 37°C, < 0.1% RH
Scotchpak 5	Minnesota Mining & Manufacturing Co.	2.0	108.5	351
Scotchpak 48	Minnesota Mining & Manufacturing Co.	4.5	77.5	203
Scotchpak 104	Minnesota Mining & Manufacturing Co.	2.7	15.5	105
Laminate S (mylar-foil-polyethylene)	Standard Packaging Corp.	2.9	<1	[b]

[a] cc(STP)/m² · day · atm.
[b] Too low to measure accurately.

EXPERIMENTAL

Food

The food used in this study was a specially prepared freeze-dried shrimp cocktail bar obtained from Swift & Co., Chicago, Ill. This was designated "Shrimp Bar/Sauce" (Guide No. 14B) and was prepared according to the U.S. Army Natick Laboratories Space Food Proto-

type Production Guide for Manned Orbital Laboratory Feeding System Assembly RFP-FO4695-67-R-0076. The material was received immediately after production, packaged under nitrogen with 6 bars per can. Each bar weighed approximately 30–35g, had an initial moisture content of 0.87g H_2O/100g solids and a fat content of 11%. The bars were stored in the cans at –40°F until needed.

Food preparation

Cans were removed from the freezer and thawed at room temperature. The cans were opened in a glove box through which dry air was circulated. Samples were weighed into test flasks or into packages as required and then placed in desiccators at a relative humidity of $<0.1\%$ RH.

Static stability tests

Oxygen absorption. Samples of 2–3g were weighed in triplicate into 30-ml Warburg flasks connected to manometers, and were placed in a constant temperature water bath at 37°C. Oxygen absorption was calculated by standard techniques (Umbreit et al., 1961). Apiezon B oil was used as the manometric fluid to give a high sensitivity.

Carotenoid pigment. Samples of 4–5g dry weight were weighed into 250-ml Erlenmeyer flasks while in the glove box. After being transferred into various desiccators, the desiccators were evacuated for 15 min and the vacuum then broken with mixtures of oxygen and nitrogen with oxygen concentrations of 3.0–3.5, 7, 7.8 and 21%. The samples in these desiccators were then maintained at 37°C for up to 6 weeks. At weekly intervals, samples were removed and carotenoid pigments measured. The remaining flasks in the desiccators were re-equilibrated to the test conditions.

To measure the carotenoids, the lipid fraction of the sample was extracted by adding 80 ml of 3:1 (v/v) $CHCl_3$:MeOH into the sample flask which was then shaken for 30 min after flushing the flask with nitrogen. The solution was then filtered using a vacuum Büchner funnel (50 mm) and the filtrate evaporated on a rotary evaporator. The residue was then dissolved in 20 ml MeOH:40 ml $CHCl_3$:20 ml H_2O and transferred to a 250-ml separatory funnel. Then 20 ml H_2O and 20 ml $CHCl_3$ were added, the mixture shaken and left to separate (usually 1–2 hr, during which time the separatory funnel was covered with aluminum foil). The bottom layer was then drawn off and 1 ml made up to 5 ml with $CHCl_3$. The optical density was then measured at 475 and 390 mμ (Lusk et al., 1964). Results are reported as the ratio of the optical densities at 475 and 390 mμ, since this ratio is related to unoxidized pigment content.

$$\frac{OD_{475}}{OD_{390}} = A_S = \text{Color Index}$$

Organoleptic evaluation. Samples were stored at various oxygen levels over a desiccant for up to 6 weeks. Each week a sample was removed and reconstituted in water (90 cc/16 g solids) at 80°F for 15 min with occasional stirring. The food was judged by 8 tasters on a 9-point hedonic scale. A rating of 3 constituted unacceptability. An identified control was simultaneously presented for comparison. This was usually rated 6–7 (fair – good quality) by the panel. The average score was computed from the panel's results and is presented as the flavor score.

Package stability studies

Oxygen permeability of packaging materials. The manufacturer's data for the films used in this study did not specify the oxygen permeability at the temperature of this study (37°C). Oxygen permeability was measured by the concentration increase method of Karel et al. (1963). Since the packages were stored at $<0.1\%$ RH, it was assumed that water vapor did not affect O_2 permeance. It was also assumed that the presence of nitrogen and of other gases does not affect the permeability of the package to oxygen, since these gases do not interact significantly with the packaging materials. This assumption is confirmed by many studies in the literature (Stannett and Szwarc, 1962; Michaels and Bixler, 1968). The permeability data are shown in Table 1. The variation between the manufacturer's and our experimental values may be partly due to different test temperature and partly to differences in other test conditions.

Stability of packaged foods. To test the mathematical models developed for prediction of storage stability, the shrimp bars were packaged in flexible films and stored at 37°C in dry air. At weekly intervals, the loss of carotenoid pigment and the flavor score were determined. All packages were sealed on a FlexVac Model 69 sealer (Standard Packaging Corporation, Clifton, N. J.). No attempt was made to determine the internal oxygen pressure in the package with time.

Porosity. Porosity of the shrimp bar was measured with a Beckman Model 390 Air Comparison Pyncnometer.

RESULTS & DISCUSSION

FOR A FOOD that deteriorates due to oxidative reactions, the rate of deterioration is often a function of the oxygen partial pressure. Packaging the food under vacuum in a flexible container results in a rate of deterioration limited by the rate of oxygen permeation into the package. It is desirable to be able to predict the type of film necessary to give protection for the desired commercial storage life. Protection in excess of this minimum may result in increased cost and below the minimum would cause deterioration before the required shelf life expires.

Prediction of the stability of the food in a package under actual storage conditions requires a knowledge of the oxygen partial pressure in the package with time. Since actual determination of this internal partial pressure is difficult, several assumptions were made, to predict the oxygen concentration with some degree of confidence. These assumptions include:

1. In the vacuum-packed food all of the volume of gas inside the package is due to the internal pore volume of the food. This is true if the flexible package is tightly sealed around the surface of the food material.

2. Permeation of other gases and vapors into the pore volume does not affect the changes in partial pressure of oxygen. Since nitrogen permeates at about 1/3 the rate of oxygen and the partial pressures of the gases are independent, nitrogen does not affect the oxygen pressure until the total internal pressure exceeds the external pressure. This would not normally occur before the end of the required shelf life. Vacuum-sealed foods held for over 2 years in materials similar to the ones used in this study never showed total loss of vacuum, justifying our assumption, especially for short-term storage.

3. The area for gas penetration is assumed to be only the area of contact of the package with the food, i.e., the seal areas are assumed to have no effect.

Given these assumptions, the rate of oxygen input into the package containing the food is:

$$\left[\frac{dVO_2}{dt}\right]\left[\frac{1}{W_S}\right] = \left[\frac{k}{x}\right]\left[\frac{A}{W_S}\right]\left[P_{O_2(out)} - P_{O_2(in)}\right] \qquad [1]$$

$\frac{dV_{O_2}}{dt}$ = rate of oxygen penetration into package – [cc(STP)/day]

W_S = weight of solids contained in package (g)

k = permeability of packaging material $\frac{cc(STP) \cdot mil}{m^2 \cdot day \cdot atm}$

x = thickness of package (mil; 0.001 in.)

A = area of contact of package (m^2)

$P_{O_2(out)}$ = external oxygen partial pressure (atm)

$P_{O_2(in)}$ = internal oxygen partial pressure (atm)

also:

$\frac{k}{x}$ = permeance of packaging material $\frac{cc(STP)}{m^2 \cdot day \cdot atm}$

The permeability-related characteristics of the package and its contents may be combined into a parameter ϕ. We call it the "packaging parameter" and it is defined by Equation [2]:

$$\phi = \left[\frac{k}{x}\right]\left[\frac{A}{W_S}\right] \qquad [2]$$

For any given packaging condition, everything in Equation [1] except the internal oxygen partial pressure is known if the external conditions are kept constant. The internal partial pressure of oxygen changes with time as O_2 pene-

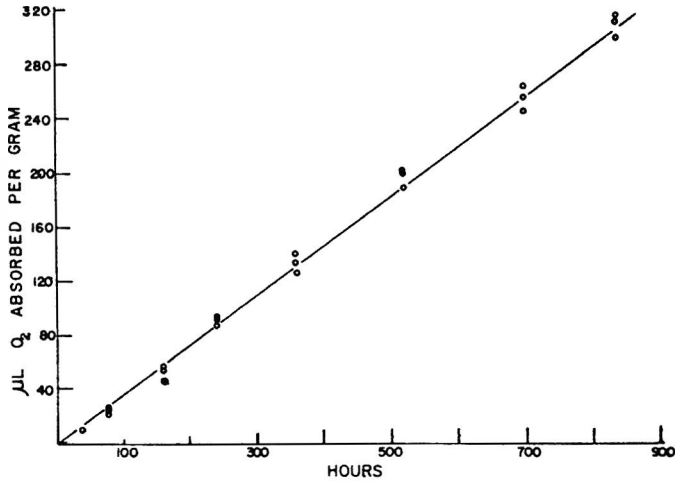

Fig. 1—Oxygen absorption by shrimp in air.

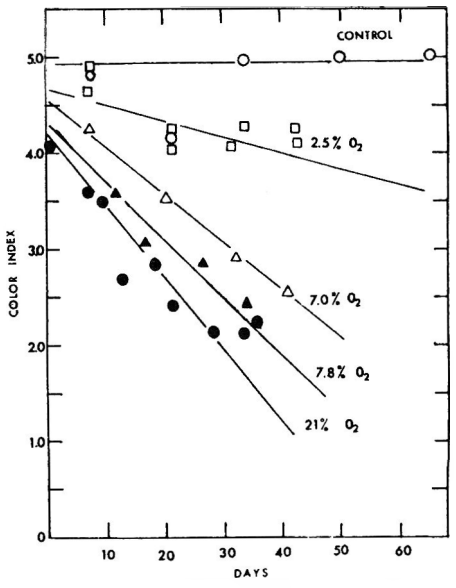

Fig. 2—Carotenoid pigment loss as a function of O_2 concentration.

trates into the package, but it is also reacting with the food, thus creating an unsteady state condition. The rate of oxidation of fatty acids is given by Bolland, 1949:

$$\frac{dV'_{O_2}}{dt} = k_1 [RH] [ROOH]^{1/2} \cdot \left[\frac{P_{O_2(in)}}{k_2 + P_{O_2(in)}} \right] \quad [3]$$

where:

$\frac{dV'_{O_2}}{dt}$ = rate of oxygen consumption by the food – [cc(STP)/day]

k_1, k_2 = constants

[RH] = oxidizable lipid content

[ROOH] = peroxide content

$P_{O_2(in)}$ = internal oxygen partial pressure

Karel (1960) has shown for model systems that rate of oxidation can be represented by Equation [4]:

$$\frac{dV'_{O_2}}{dt} = \frac{1}{C + D/P_{O_2(in)}} \quad [4]$$

where:

C and D = constants

We have also applied Equation [4] to other data, including the results of Tuomy et al. (1968) for freeze-dried beef and chicken stew, and found it to be applicable. Marcuse and Fredrikson (1968) have found a similar relationship for fat emulsions.

Given the above equations, the rate of accumulation of oxygen in the package with time is:

$$\left[\frac{dV'_{O_2}}{dt}\right]_{acc.} = \frac{dV_{O_2}}{dt} - \frac{dV'_{O_2}}{dt} \quad [5]$$

Karel (1960) has shown that combining the above equations into an analytical function results in an equation difficult to integrate. However, if Equation [5] can be solved, then the internal oxygen partial pressure is:

$$P_{O_2(in)} = \frac{V_{O_2(acc.)}}{V_T} \quad [6]$$

Table 2—Correlation of oxidation data.

Oxygen partial pressure $P_{O_2(atm)}$	dA_s/dt[a]	Rate of oxygen absorption dO_2/dt (μliters/g/day)
0.21	0.073	8.60
0.078	0.044	5.20
0.07	0.037	4.30
0.025	0.017	2.00

[a] *Rate of carotenoid pigment loss (carotenoid color index units/day).*

Table 3—Conditions for storage study.

1. Food: Shrimp bar
   Porosity: 0.48

2. Packaging materials:

Material	k/x	$\phi \times 10^2$ Run 1	$\phi \times 10^2$ Run 2
Scotchpak 5	350	19.2	–
Scotchpak 48	200	–	9.4
Scotchpak 104	100	5.5	4.7
Laminate S	10	0.55	0.47

3. Weight of solids and area per package:

Run 1 W_s = 10 g: $A = 0.005$ m²; $\frac{A}{W_s} = 5.5 \times 10^{-4}$

Run 2 W_s = 16 g: $A = 0.0075$ m²; $\frac{A}{W_s} = 4.7 \times 10^{-4}$

4. Storage conditions:

Temperature: 37°C
Atmosphere: Dry (< 0.1% RH) air (21% O_2)

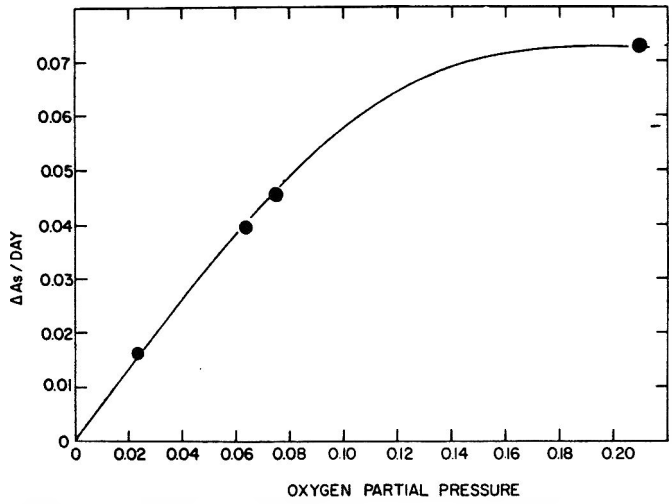

Fig. 3–Rate of carotenoid loss as a function of O_2 partial pressure.

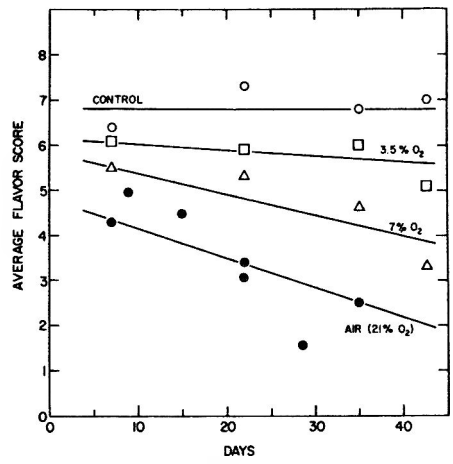

Fig. 4–Deterioration of organoleptic flavor score as a function of O_2 partial pressure.

where:

V_T = pore volume of food

$V_{O_2\ (acc.)}$ = oxygen concentration in pores of food

To solve these equations for the shrimp bar, the rate of oxidation as a function of time was measured by Warburg techniques. Figure 1 shows the consumption of oxygen for the product held in air at 37°C, for which a straight line results. Unfortunately, it is quite difficult to measure rates of oxidation at lower partial pressures with standard Warburg methods (Marcuse, 1967). For this reason, and also because of direct correlation with color quality of the product, the present study used loss of carotenoid pigments as an index of deterioration. Loss of pigment was determined as a function of time at several oxygen concentrations and results shown in Figure 2. The assumption was then made that the proportionality between oxygen absorption and pigment loss, observed for tests conducted in air, would also hold for lower oxygen concentrations. The validity of this assumption seemed reasonable on theoretical grounds, was confirmed by some spot checks and is made more likely by the fact that the rate dependence of pigment loss follows the curve typical for autoxidative processes, as shown in Figure 3. It was recognized, however, that this assumption was not actually proven prior to its application, and its validity would become tested in the experimental test of the overall predictions of this study.

Given the above assumption of proportionality between oxygen absorption and pigment loss, an extrapolation of rates of oxygen absorption at different oxygen concentrations was obtained (Table 2). Using these data, Equation [4] may be written as:

$$\frac{dV'_{O_2}}{dt} = \frac{10^2}{6.44 + 1.16/P_{O_2\ (in)}} \qquad [7]$$

with $\frac{dV'_{O_2}}{dt}$ expressed in μliters O_2/g/day.

To predict shelf life, correlation must also be made between the degree of oxidation or of pigment loss and acceptability. In particular, the degree of deterioration at which the food becomes unacceptable is needed. Figure 4 shows results of organoleptic evaluation of shrimp bars stored at various oxygen concentrations at 37°C and $< 0.1\%$ RH. At a flavor score of 3, the shrimp becomes unacceptable. It can be seen that in air the shrimp becomes unacceptable in 22–28 days, at 7% O_2 it is stable for over 50–55 days, and at 3.5% O_2 the stability is in excess of several months. Using the time to reach unacceptability in air, it is found from Figure 1 that at that time the bar has absorbed about 200 μliters O_2/g, and from Figure 2 that the color index (A_S) has dropped to a value of 2.2–2.5.

Similar comparisons made at 7% oxygen showed that the same values of oxygen absorption and carotenoid color index were reached at 50 days, the time at which organoleptic unacceptability was reached. These results were quite encouraging, since they confirmed the assumption of a "cut-off" value of acceptability, which depends on the extent of the oxidative reactions but not on the rate at which this extent is reached. It was assumed, therefore, that 200 μliters O_2/g and A_S = 2.2–2.5 constituted such cut-off values.

The above assumptions combined with the given equations were used to calculate shelf life of the shrimp bar. The prediction was made using an iteration procedure in which conditions in the package and extent of deterioration were determined by following the indicated steps for time intervals of 6 hr and a total time of 100 days. An iteration procedure was chosen because functions combining Equations [1], [6] and [7] cannot be integrated readily, whereas iteration procedures are readily accomplished with the help of a digital computer. An IBM 360/65 digital computer at the MIT Computation Center was used.

The first step was to calculate the amount of oxygen that would permeate into the package in this short time interval using Equation [1] for a given packaging parameter ϕ, external oxygen partial pressure $P_{O_2(out)}$ and assuming that at the beginning $P_{O_2(in)} = 0$. From this, the partial pressure of O_2 in the package $[P_{O_2(in)}]_t$ was found from Equation [5]. If oxygen is already in the package $[V_{O_2(in)}]_{t-1}$, this is added to the incremental value before obtaining the internal partial pressure. From the value of the partial pressure now in the package, using Equation [7], the amount absorbed by the food in this short time interval is calculated $V_{O_2\ abs.}$ and added to the amount previously absorbed

$$[V_{O_2\ abs.}]_{t-1}$$

Also the amount of pigment lost in this time period can be calculated by writing the equation for pigment loss in the same manner as for oxidation:

$$-\frac{d(A_S)}{dt} = \frac{1}{7.60 + 1.28/P_{O_2}} \qquad [8]$$

where:

$\frac{d(A_S)}{dt}$ is expressed in carotenoid color index units per day.

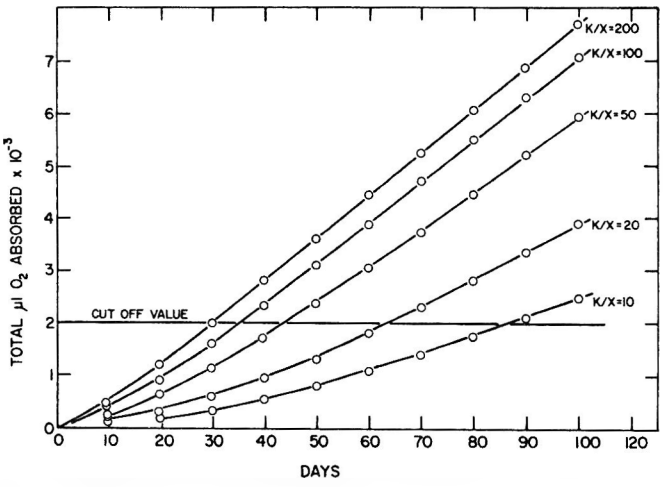

Fig. 5—Predicted O_2 absorbed for shrimp with sauce stored in air for different permeances to O_2 ($A/W_s = 5.5 \times 10^{-4}$ m^2/g).

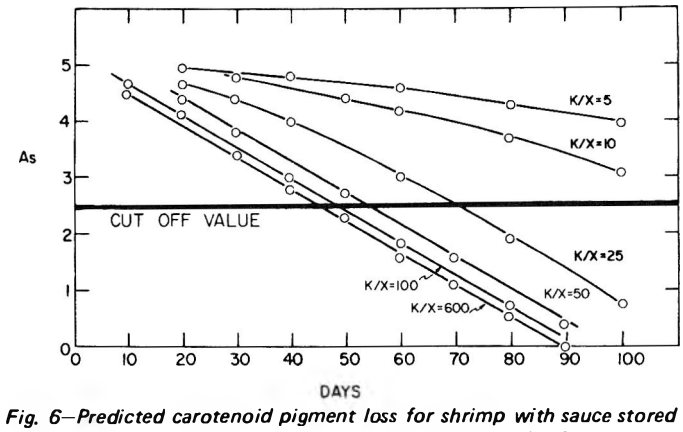

Fig. 6—Predicted carotenoid pigment loss for shrimp with sauce stored in air for different permeances to O_2 ($A/W_s = 5.5 \times 10^{-4}$ m^2/g).

Since the oxygen in the package is now reduced by the amount absorbed, the new partial pressure of oxygen in the package $\left[P_{O_2\,(in)}\right]_t^*$ was calculated based on the new volume of oxygen $\left[V_{O_2\,(in)}\right]_t^*$.

The next amount of oxygen permeating into the package was then calculated for the next time increment based on the new internal partial pressure. This process was repeated for 100 days for different package permeances (k/x) and different area to weight of solids ratios (A/W_S). The steps in equation form are:

Step 1.

$$\Delta V_{O_2\,(in)} = \frac{kA}{x}\left\{\left[P_{O_2\,(out)}\right] - \left[P_{O_2(in)}\right]_{t-1}^{*}\right\}\Delta t$$

Step 2.

$$\left[V_{O_2\,(in)}\right]_t = \left[V_{O_2\,(in)}\right]_{t-1}^{*} + \Delta V_{O_2(in)}$$

Step 3.

$$\left[P_{O_2\,(in)}\right]_t = \frac{\left[V_{O_2\,(in)}\right]_t}{V_T}$$

Step 4.

$$\Delta V_{O_2 abs.} = \frac{1}{C + D/\left[P_{O_2(in)}\right]_t}\Delta t$$

Step 5.

$$\left[V_{O_2 abs.}\right]_t = \left[V_{O_2 abs.}\right]_{t-1} + \Delta V_{O_2 abs.}$$

Step 6.

$$\left[V_{O_2(in)}\right]_t^* = \left[V_{O_2(in)}\right]_t - \Delta V_{O_2 abs.}$$

Step 7.

$$\left[P_{O_2(in)}\right]_t^* = \left[\frac{V_{O_2(in)}}{V_T}\right]_t^*$$

Step 8.

$$\Delta A_S = \frac{1}{k_4 + k_5/\left[P_{O_2(in)}\right]_t}\Delta t$$

Step 9.

$$(A_S)_t = (A_S)_{t-1} - \Delta A_S$$

Step 10.

$$t + 1 = t + \Delta t$$

Step 11. Repeat steps 1—10 until total time desired.

A high-speed digital computer was used to solve the 11 steps. The output of this procedure gives oxygen absorbed per gram of product and the carotenoid color index (A_S) as functions of time. From a plot of these deterioration parameters, the time to reach the cut-off values can be found directly. To facilitate the use of this procedure for any condition, the above iteration procedure was performed for 3 different area-to-weight ratios and for a large series of oxygen permeances. Figure 5 shows curves of calculated oxygen absorbed for 1 area-to-weight ratio and Figure 6 shows (A_S) as a function of time for the same conditions. Using the calculated times to reach the cut-off values, a plot of the packaging parameter ϕ vs. maximum permissible days of storage was constructed in Figure 7 based on oxygen absorbed and in Figure 8 based on carotenoid pigment loss.

Times to reach loss of acceptability

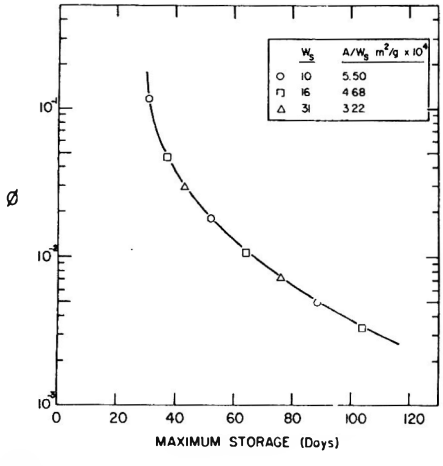

Fig. 7—Predicted maximum shelf life for shrimp with sauce as a function of the packaging parameter ϕ, based on oxygen absorption.

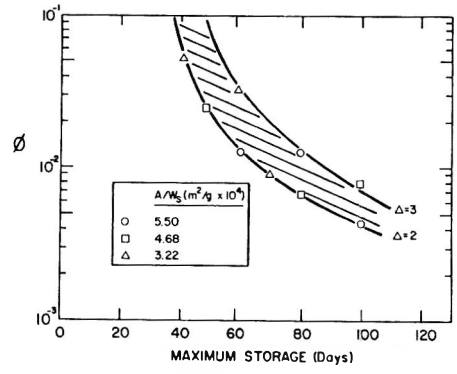

Fig. 8—Predicted maximum shelf life for shrimp with sauce as a function of the packaging parameter ϕ, based on carotenoid pigment loss.

Table 4—Comparison of actual with predicted storage stability shrimp bar.

Package	Days to reach unacceptability			
	Actual		Predicted	
	Flavor score	Color index	Oxygen absorbed	Color index
Run 1				
Scotchpak 104	> 60	>60	35	40–50
Scotchpak 5	50–60	42–48	30	42–55
Laminate S	>100	>80	>100	90–120
Run 2				
Scotchpak 48	50–60	30–42	30	40–50
Scotchpak 104	80–90	55	38	44–58
Laminate S	>100	–	95	95–130

based on color index (Fig. 8) represent a range of values rather than a single curve, because of variation in initial values of the color index. A value of $\Delta = 3$, for instance, indicates that a drop in color index numerically equal to 3 would result in unacceptability, given the initial color index value.

To test the validity of the mathematical model for prediction of storage stability, 2 actual storage studies were made under test conditions shown in Table 3. Results of organoleptic evaluation and the measured values of the color index are shown for Run 2 in Figures 9 and 10.

From the results of the actual storage test, the time to reach organoleptic unacceptability and a color index cut-off were compared to the times predicted in Figures 7 and 8 by the mathematical model. Results of this comparison are shown in Table 4.

The results show that the predictions are reasonably accurate. The predictions based on pigment loss in most cases bracket the actual time to unacceptability. The predictions based on oxygen absorption show shorter storage life than actually observed, especially in Run 1. However, it is felt that these results are sufficiently accurate to confirm the basis for a new method of obtaining packaging requirements.

The observed discrepancies between predicted and observed results are probably due primarily to deviations from the assumed constant ratio, applicable at all partial pressures of oxygen, between rates of pigment loss and of oxygen absorption. It seems that this assumed relationship overestimates the rates of oxygen absorption.

In addition, errors may have arisen from internal resistances to oxygen diffusion within the shrimp bars, and from deviation in actual package permeances from the measured permeabilities.

SUMMARY & CONCLUSIONS

A MATHEMATICAL model for prediction of the packaging requirements of dehydrated oxidizable foods was developed. Standard equations for oxygen penetration into the package and for oxidation rate as a function of oxygen partial pressure were combined with the assumption that the internal oxygen fills the food pore volume. Equations were solved by an iteration process using a high-speed digital computer. From tests at static conditions of oxygen concentration combined with organoleptic tests, cut-off values were obtained which represented maximum permissible oxidative deterioration. Times under specified packaging conditions to reach these cut-off values were calculated and presented as plots of a "packaging parameter" ϕ vs. time. Plots of this type can be used to predict maximum safe storage time under given storage conditions, or to determine the required package properties for a given required storage time.

An actual storage test of shrimp bars packaged in films of different permeabilities proved that the prediction curves gave good results. This study shows, therefore, the feasibility of developing prediction plots for required packaging protection for any dehydrated food deteriorating by oxidative mechanisms. The present study was conducted at constant low humidity, thus holding one of the important variables constant. The more complex problem of simultaneous changes in moisture and oxygen is under investigation in our laboratory.

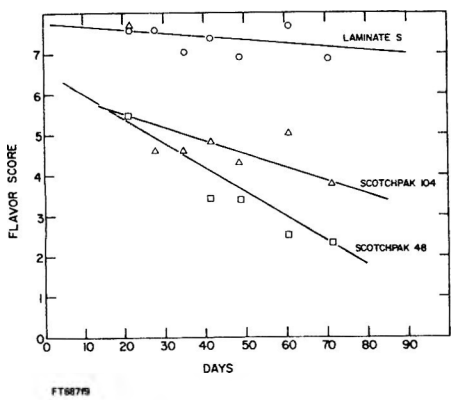

Fig. 9—Organoleptic evaluation of shrimp with sauce stored in air in specified packages.

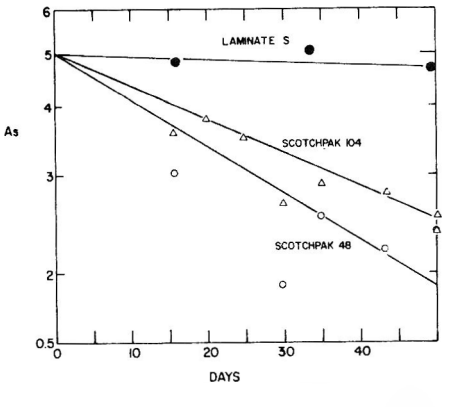

Fig. 10—Carotenoid pigment loss of shrimp with sauce stored in air in specified packages.

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STORAGE TEMPERATURE EFFECTS ON THE PROTEOLYTIC ACTIVITY OF RADIATION-SURVIVING BACTERIA IN OYSTERS

SUMMARY—The activity of 2 radiation-surviving and strongly proteolytic strains of Pseudomonas *and* Achromobacter *were compared to the activity of 2 lesser active strains of* Neisseria *and* Bacillus *in fresh oysters during iced (32°F) and refrigerated (40°F) storage for 15 days. Radiation doses used for the oysters were 100 and 800 krad. The activity of the former bacteria was higher than that of the latter 2 at both temperatures and radiation doses. Neither the nonirradiated nor the irradiated uninoculated oysters displayed significant increases in proteolytic activity when they were ice-stored for 15 days, but storage at 40°F for the same period resulted in significant activity increases in the nonirradiated. This emphasizes irradiation and storage temperature as related factors. A slight decrease in pH at 15 days in both nonirradiated and 100 krad-irradiated oysters corresponded to the increase in bacterial numbers.*

INTRODUCTION

10 SPECIES of bacteria which survived low-dose gamma radiation were isolated from Gulf oysters and identified by Liuzzo et al. (1968). They observed that irradiation at 200–300 krad reduced considerably the proteolytic activity of the bacteria. Their studies were conducted under laboratory conditions to obtain basic fundamental reasons for the activity behavior of the bacteria subjected to radiation.

The study herein reported was conducted to ascertain the combined effects of radiation and low-temperature storage of oysters on the proteolytic activity of 4 of the radiation-surviving organisms. The experiments were conducted under more practical conditions than those by Liuzzo et al. (1968).

2 radiation-surviving bacteria shown to be highly proteolytic by Liuzzo et al. (1968) were *Pseudomonas erythra* and *Achromobacter butyri*. In this study, activity of these bacteria was compared to that of *Neisseria flavescene* and *Bacillus laterospores*, 2 organisms displaying less proteolysis than the former 2.

EXPERIMENTAL

THIS experiment was conducted on oysters packed in pint-size cans, to simulate practical conditions. In this manner, a more applicable study was designed which more closely showed the effects of storage temperature and radiation on proteolysis under commercial conditions than that conducted by Liuzzo et al. (1968).

Oysters used in this study were obtained raw and were processed by methods described by Liuzzo et al. (1968). The pint cans (350 g of oysters per pint) were divided into 5 lots, each consisting of 6 cans (Table 1). 1 lot served as an uninoculated control whereas lots 1, 2, 3 and 4 were inoculated with *P. erythra*, *A. butyri*, *N. flavescene* and *B. laterospores*, respectively. The inocula consisted of a 1-ml Difco Bacto nutrient broth suspension of each bacterium containing 3.5×10^8 cells. The density of the inocula was 10^6 cells per gram of oysters. Concentration of cells in a 1-ml suspension of nutrient broth was determined by McFarland's nephelometer method (Manual of Clinical Laboratory Methods, 1958). These inocula were added to the oyster pints aseptically.

After inoculation, the 6 cans in each lot were divided into 3 radiation treatments: nonirradiated and irradiated at 100 and 800 krad. The irradiated procedure employed was described by Liuzzo et al. (1968). After inoculation and irradiation, ½ of the samples from each treatment was analyzed immediately; the other half was stored in ice (32°F) and in a refrigerator (40°F) for 15 days, at which time the proteolytic activity and pH values were determined. Proteolysis was measured by Sørensen's formal titration procedure (Levy, 1933; 1934). Bacterial counts were determined on each sample by the standard pour-plate technique (Difco Manual, 1966).

RESULTS & DISCUSSION

OYSTER tissue autolysis was not caused by gamma irradiation (Table 2). Neither the nonirradiated nor the irradiated oysters displayed significant increases in proteolytic activity when they were ice-stored for 15 days. Therefore, any evident activity is assumed to be bacteriologically induced. Results in Table 2 indicated that storage in ice of nonirradiated oysters held the bacterial activity to a minimum, whereas storage at 40°F for 15 days resulted in significant increases in proteolytic activity ($P < 0.01$).

Irradiated oysters (100 and 800 krad) displayed smaller proteolytic increases than nonirradiated oysters after 15 days' storage at both temperatures. It is assumed that the destruction of bacteria by irradiation was responsible for this phenomenon. Lack of autolytic activity due to irradiation is further emphasized by the reduced activity observed in oysters irradiated at the higher level.

Table 3 shows that the pH of the nonirradiated and irradiated oysters was 6.5 prior to iced storage. The slight decrease in pH after 15 days' storage of both nonirradiated and irradiated oysters (100 krad) corresponded to the increase in numbers of bacteria. A lack of decrease in pH of the 800 krad-irradiated oysters was probably due to the large reduction of bacterial numbers.

Spoilage of oysters can be characterized by a gradual and continuous decrease in the pH value (Hunter and Linden, 1923; Baldwin et al., 1941; Piskur, 1947). The general appearance and odor of each sample after 15 days' storage in both ice and refrigeration temperatures were recorded. In general, all samples irradiated at 100 krad remained "sea fresh" until after the 15th day, whereas the same state of freshness for the nonirradiated samples was lost by the 7th to 10th day. The samples irradiated at 800 krad had a characteristic odor which can best be described as "spoiled oyster." In addition to this, the latter sample had a brownish color on the surface and in its fluid. This was probably due to irradiation burn. Novak et al. (1966) reported that levels of radiation beyond 200 krad resulted in the production of a light-yellow exudate which lowered the oyster's acceptability for appearance.

Table 1—Experimental design for oyster pints.

Lot	Organism	Radiation levels[a] (krad)		
Control	Uninoculated	0	100	800
1	*Pseudomonas erythra*	0	100	800
2	*Achromobacter butyri*	0	100	800
3	*Neisseria flavescene*	0	100	800
4	*Bacillus laterospores*	0	100	800

[a]After inoculation and irradiation, ½ of all treatment samples was analyzed immediately, the other half stored for 15 days at 32° and 40°F prior to analyses.

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Table 2—Effects of gamma irradiation on the proteolytic activity of oyster tissues (autolysis).

Dose (krad)	Storage (days)	Ml of 0.1 N sodium hydroxide[a] Iced storage (32°F)	Refrigerated storage (40°F)
0	0	2.50	2.50
	15	3.40	5.44[b]
100	0	2.60	2.60
	15	3.40	3.60
800	0	2.45	2.45
	15	3.25	2.50

[a]Amount used to titrate free amino acids to pH 9.1–9.2.
[b]Significant increase over 0 days ($P < 0.01$).

Table 3—pH values and bacterial counts of oysters stored in ice.

Storage (days)	Radiation level (krad) 0 pH	0 Count[a]	100 pH	100 Count[a]	800 pH	800 Count[a]
0	6.5	6×10^5	6.5	2×10^5	6.5	2×10^4
15	6.1	1×10^6	6.3	8×10^5	6.5	3×10^4

[a]Bacteria/g of oysters.

Table 4—Proteolytic activity of radiation-surviving bacteria in oysters after 15 days of storage.

Radiation dose (krad)	Organism	ml of 0.1 N Sodium hydroxide[a] Storage at 32°F	pH	Storage at 40°F	pH
Control[b]	–	3.30	6.5	5.55	6.2
0	*Pseudomonas erythra*	7.50	5.8	10.20	5.6
100		6.10	6.0	8.52	5.7
800		3.40	6.5	3.85	6.4
0	*Achromobacter butyri*	10.22	5.5	13.52	4.2
100		9.11	5.7	12.00	5.2
800		5.25	6.4	7.35	5.8
0	*Neisseria flavescene*	5.22	6.4	9.35	5.7
100		4.83	6.4	8.55	5.8
800		3.88	6.6	4.35	6.2
0	*Bacillus laterospores*	5.83	6.3	10.00	5.6
100		5.33	6.3	8.38	5.8
800		4.12	6.4	5.00	6.3

[a]Amount used to titrate free amino acids to pH 9.1–9.2.
[b]Control is uninoculated and nonirradiated oysters.

Table 4 shows that the pH of the nonirradiated inoculated samples and the samples irradiated after inoculation with *N. flavescene* and *B. laterospores* remained virtually unchanged when stored at 32°F. However, changes were observed when these samples were stored at 40°F. Major decreases in pH were evident in these treatment samples when they were inoculated with *P. erythra* and *A. butyri*. The pH's were lower in those pints stored at 40 than at 32°F. However, only slight decreases in pH were noted in samples uninoculated or inoculated with the organisms which survived the 800-krad dose.

These observations coincide with those reported by Liuzzo et al. (1968), who reported an inverse relationship between irradiation and bacterial numbers and subsequent metabolic activity. When the oysters were irradiated at 800 krad, a larger number of the inoculated organisms was destroyed, thereby reducing the activity. This relationship between radiation dose, bacterial numbers and pH is also seen in Table 3.

Results in Table 4 also show that the proteolytic activity was kept lowest in all treatments, which included the 800-krad dose after inoculation. In some instances the activity was lower than the uninoculated control.

Proteolytic activity was less in the samples stored at 32° than those stored at 40°F when the oysters were irradiated at 100 and 800 krad after inoculation. This emphasizes the importance of storage temperature as a related factor.

The proteolytic activity of *P. erythra* and *A. butyri* was higher than that of *N. flavescene* and *B. laterospores* at both 32 and 40°F. This indicated that members of the former genera are an influencing factor in causing oyster spoilage during iced and refrigerated storage. Chung (1963) reported that *Pseudomonas* and *Achromobacter* were highly proteolytic bacteria and caused spoilage of seafood generally during storage. It was obvious in the case of *Neisseria* and *Bacillus* that storage of irradiated oysters at 32°F was more effective in keeping the proteolysis lower than storage at 40°F.

Results from this study not only demonstrate the advantages of low-dose radiation in preserving oysters, as was also shown by Novak et al. (1966), but they further emphasize the role of iced-storage in combination with radiation as a means of retarding bacterial growth and metabolism and, subsequently, spoilage.

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A SYSTEM FOR CONTINUOUS THERMAL PROCESSING OF FOOD POUCHES USING MICROWAVE ENERGY

SUMMARY—A system for continuous microwave sterilization of foods packaged in plastic pouches has been designed, constructed and tested. Air pressure was used to prevent pouch rupture. Pouches containing food for use in military rations were sealed and introduced through an air lock on to a conveyor inside a plastic pipe within a microwave cavity. Microwave energy was supplied up to 10 kw at 2,450 MHz. Conveyor speed and power were regulated to provide the process time and temperature. Cooling was achieved in a cooling bath. Pouches were overpackaged in a foil laminate for additional protection. Several food products were processed. Total process times of 9–14 min were achieved.

INTRODUCTION

ALTHOUGH the application of microwave energy to the heating of food materials is a relatively new field, there is a surprisingly small body of open literature available on the concept of sterilization of foods in hermetically sealed containers on a continuous basis using this form of heat input. This may be explained in part by the fact that while work may be under way in this field, much of it may be of a proprietary nature. Certainly, the bulk of the literature has appeared as patents.

Pasteurization of liquids and surface inhibition of microorganisms have been more adequately described. Decareau (1968) recently summarized the present state of the art in both sterilization and pasteurization.

True sterilization in containers either prior to or after hermetic sealing was discussed by Jackson (1947), who also summarized earlier work. Most studies were conducted at relatively low frequencies compared to present standards. The work of Jackson and others was done in glass containers of various types, including baby-food jars, and was primarily in the form of batch experiments. Jackson discussed problems encountered, including local heating, arcing and burning of containers, and suggested the use of higher frequencies than those previously used.

Landy (1965) discussed a batch process in which food material could be sterilized in both glass and plastic tubes in a batch process with microwave energy. Mention was made of the use of a rigid form in some cases to maintain constant volume of the flexible containers used. Thus, a counterbalance to internal pressure is necessary to attain practical sterilization temperatures.

Long et al. (1966) discussed a method by which food products could be sterilized by microwave energy on a continuous basis in plastic pouches. In this case the pouch was open to the atmosphere during the heat treatment and subsequently sealed. A labyrinth-type opening in the pouch was designed to preclude the entry of microorganisms during the process. This method was limited to those products which could be sterilized at 212°F.

Jeppson and Harper (1967) discussed a continuous method of sterilization of foods in which a hydrostatic pressure system was used to maintain the hydrostatic head (and, hence, over-pressure for materials in flexible plastic pouches), and a heating section filled with mineral oil to provide a non-lossy medium for the product.

Jeppson (1964) further discussed continuous microwave processing of skim milk (in 8-oz glass jars) and heat-sensitive fruits, using conveyorized ovens.

A number of present and planned Army operational rations are being designed around the concept of food packaged and sterilized in flat, rectangular flat seal design, plastic-laminate pouches as a replacement for the conventionally canned items.

Figure 1 is a photograph of three pouches showing typical products now being investigated for use in these experimental operational rations. This photo shows the relative size and general configuration of the pouches. The pouches used in the continuous microwave system are similar in size and configuration, but do not contain the aluminum foil layer, because of the reflectivity of the foil to microwaves. These components are now processed principally in conventional steam-water retorts on a batch basis, for time periods of as much as 1 hr, depending on the thermal process requirements of the product.

The present work was undertaken in an attempt to design and test the feasibility of a rapid, continuous process which would result in improved product quality and achieve the economics of continuous processing.

Microwave energy was selected, since very rapid heating times can be obtained with direct coupling of the energy into the product rather than placing reliance on the transfer of heat energy through the container surface and subsequent conduction to the center of the mass.

EXPERIMENTAL

The process

The process involved passing food packaged in sealed plastic pouches through a microwave energy field on a continuous basis to achieve a sterilization temperature. This was followed by cooling of the pouches and a subsequent aseptic overpackaging with a suitable plastic-foil laminate to afford adequate storage and handling protection to the product.

Since temperatures of 250°F and higher

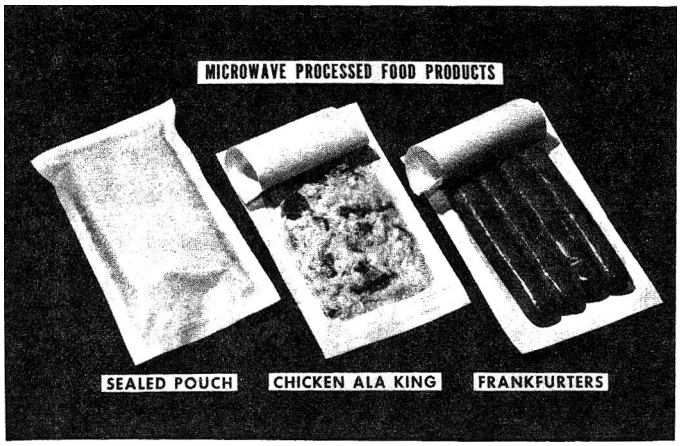

Fig. 1—Pouches containing typical food products showing relative size and general configuration of pouches.

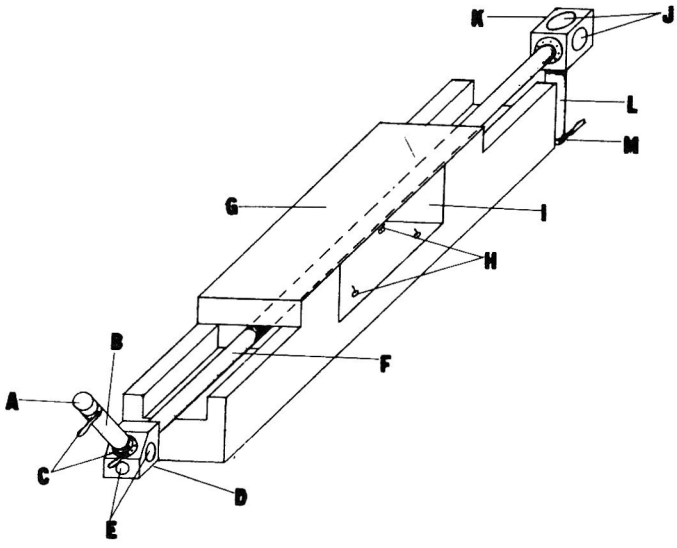

Fig. 2—Schematic drawing of microwave processing system.

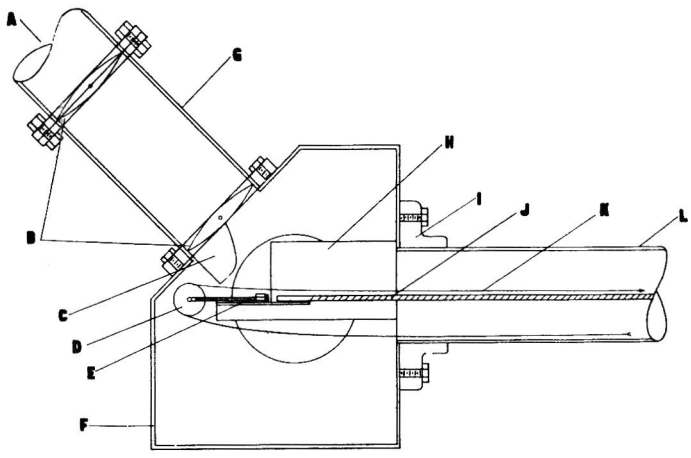

Fig. 3—Cross-sectional sketch of valve assembly at feed end of system.

are achieved in the sterilization of foods, internal pouch pressure will exceed 15 lb per sq in. This pressure will exceed the rupture point of known plastic film(s) laminates at these temperatures, particularly at the sealed areas. An external counter-balance in pressure must be provided for this purpose.

Thus, the equipment designed provided four essential components: 1) A pressurized system. 2) A means of passing individual pouches continuously in and out of the system. 3) A conveyor to carry the pouches through the system. 4) An applicator to apply the microwave energy.

The equipment to accomplish these objectives was designed to provide an experimental machine as inexpensively and as simply as possible, to establish the feasibility of the process and to study the quality of the resulting product. No attempt was made to refine the design or to provide sophisticated automation, although design features were selected which would permit future refinement.

Equipment

Chamber. The principal component of the system was a 25-ft-long cylindrical fiberglass-reinforced epoxy tube. A metal (aluminum) box at each end contained the drive and idler mechanisms of the conveyor system and other accessory devices. Fiberglass epoxy was chosen as a tube material because of its strength at elevated temperatures and its relative transparency to microwave energy. The dimensions of the tube were dictated by: a) The dimensions of the microwave cavity available. b) The pouch size (the standard pouches are approximately 4.75 by 7 by 0.5 in.). c) Availability of standard pipe. The commercially available pipe selected was Chemline (Dow Smith Inc., Little Rock, Arkansas) with the following characteristics:

Epoxy Fiberglass Tube Characteristics

Outside diameter	4.580 in.
Inside diameter	4.360 in.
Wall thickness	0.110 in.
Maximum pressure–temp rating	150 psi at 300°F

Standard flanges and sleeves were used with the pipe to permit attachment of the pipe to the end boxes and to assemble sections of pipe, to permit disassembly of the system for the microwave cavity if desired. Figure 2 is a sketch of the complete system. The letter F designates the fiberglass epoxy tube passing through the microwave oven unit G; letters K and D are the aluminum end boxes constructed of 3/8-in. aluminum plate with appropriate openings to accept the tube, the entrance valve system A-B-C and the receiver chamber L, with exit valve M; letters E and J are view and access ports to the end boxes. The microwave cavity I is shown with microwave antennas H.

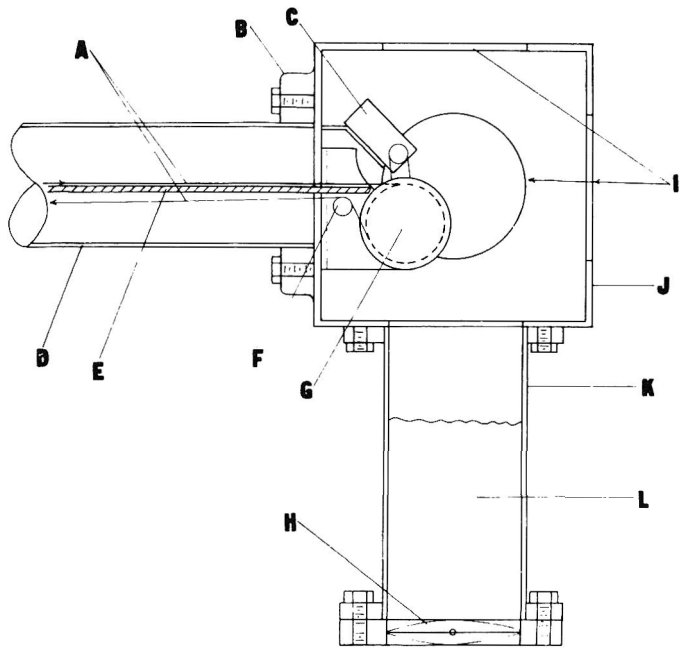

Fig. 4—Cross-sectional sketch of exit end of system with cooling receiver.

Table 1—Engineering characteristics of microwave processor.

Continuous processor	
Feed rate	1 pouch/min
Time in cavity	1–12 min
Cooling time	5 min
Pressurization time	1 min
Optimum load	2–5# (6–14 pouches)
Belt speed	0–7 ft/min
Power range	1.25–10 kw
Over pressure	0–40 lb/sq in
Dielectric temp rise	20–30°F
Belt length	24.5 ft
Cavity belt length	9.7 ft

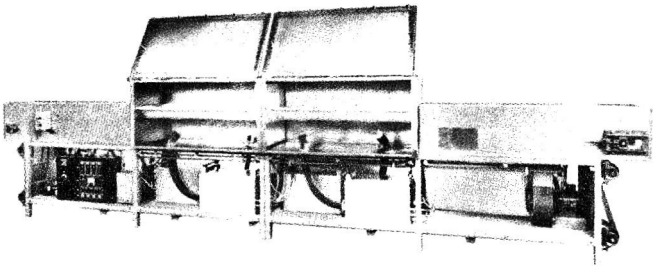

Fig. 5–Microwave applicator (Litton Model C-10S-2) Microwave Conveyor.*

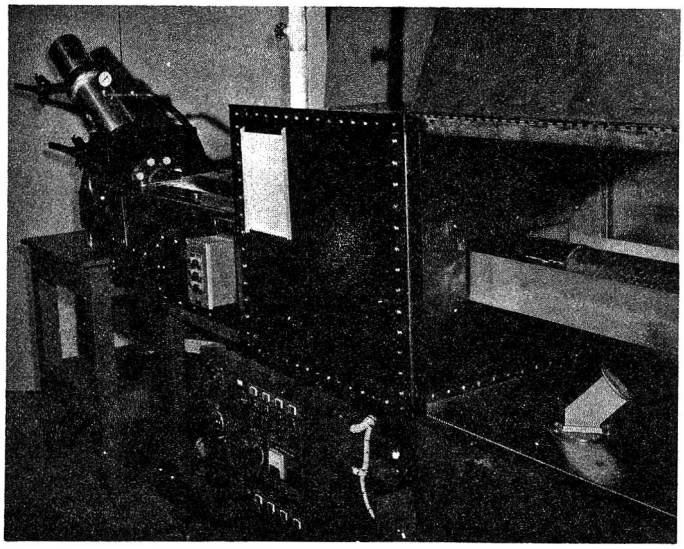

Fig. 6–View of feed end of microwave processor showing valve assembly and epoxy tube in cavity.

Valve system. To pass the pouches in and out of the pressure system, some type of valve arrangement was necessary. Various devices were considered including rotary valves, barometric legs and several special designs. An airlock arrangement was finally adopted which utilized a standard, commercially available butterfly valve (Rockwell Manufacturing Co., Pittsburgh, Pa.). A wafer-type, hand-lever-operated, 6-in. i.d. valve rated for 50 psi shut-off service was used. The entrance system utilized two of these valves to provide an airlock system. Figure 3 shows a cross-sectional sketch of the valve system with a section of tube.

The valve system is mounted at an angle to the horizontal of approximately 45 degrees into the feed box end of the pressure system, as shown. In this way the pouch slides by gravity through the airlock assembly G. During operation the lower section of the assembly (attached to the end box F) is under system pressure (15–18 psi) and the lower B valve is shut. The pouch is introduced into the upper section at A. The first valve B is opened and the pouch slides into the first chamber G. The first valve B is then closed and the chamber between the two valves is pressurized by compressed air. When the pressure on both sides of the second valve B is equalized, this valve is opened and the pouch slides into the pressure system and is guided on to the conveyor K by chute C and guide H. The second valve B is then closed, the first chamber is depressurized and the cycle is repeated. Letter D is the endless belt idler roller with screw take-up adjustment E. The polypropylene strip J serves to support the belt within the tube; letter I shows the flange attachment of the tube.

Refinements such as pouch-positioning sensors, pressure transducers and electric or hydraulic operation of the valves can easily be introduced to automate the system. In future redesign of the equipment it is planned to do this.

Conveyor system. To move the food pouches through the epoxy fiberglass tube and hence through the microwave field, a continuous belt conveyor system is provided within the main pressure system. 1/8-in.-thick strips of polypropylene were cut to a width slightly less than the internal diameter of the epoxy tube (approximately 4 in. wide and in lengths of approximately 4.5 ft). These strips were fastened together with flat-head nylon bolts and laid within the epoxy tube to serve as a bearing surface for the continuous belt movement. The belt passes over the top of the polypropylene surface and returns under it in the tube. The belt is a neoprene Typalon (chlorinated sulphonated polyethylene) MIL-C-13285 type I, class I coated polyester fabric material 3.5 in. wide and approximately 50 ft continuous length. Figure 4 is a cross-sectional sketch of the end box of the system. A geared motor C with suitable gear reduction drives a 4-in. drum G with an idler roller F providing approximately 80% wrap-around on the drum. The belt is pulled from the opposite end to the entrance feed box by the drive. The belt speed can be controlled from zero to approximately 7 ft per min by an appropriate speed-control device. The drive motor C is located within the end box, eliminating the need for external shafting and pressure-tight bearings. The 1/32-hp motor has a maximum operating temperature rating of approximately 200°F. Tension adjustment is provided for the belt to compensate for expansion and contraction under varying temperatures.

Microwave applicator. A model C-10S-2 (Litton Industries, Atherton Div., Minneapolis, Minn.) microwave conveyor unit was available in these Laboratories–Figure 5.

The main cavity is 9 ft 7 in. long by 27 in. wide by 26 in. high; however, the entrance and exit sections to the cavity are 5 in. high and 14 in. wide. It was not desired to reconstruct the unit; thus, the 5-in. dimension limited the outside diameter of the epoxy tube which passes through the main cavity. The belt of the unit and its drive were not used, of course, since the epoxy tube contained its own conveyor system.

The microwave unit operated at a frequency of 2,450 MHz and was powered by four water-cooled, modular, magnetron generators which provided 0–10 kw average power in eight steps of approximately 1.25 kw each. (Each module operates 1.25 kw each, or 2.5 kw.) This modular design offered flexibility of operation not obtainable with a single generator, giving advantages which have been discussed by Gerling (1968).

One of the principal advantages of a conveyor-type system which moves the product through a fairly long cavity is the evening-out of nonuniformities of electro-magnetic field strengths which always exist in a cavity applicator, no matter how skillful the design. The product in this situation is subjected to a minimum dose of energy during its travel through the cavity.

Another advantage of this microwave unit is the provision of water loads in the entrance and exit sections of the main cavity, which permit operation of the unit with small loads in the cavity, e.g., a few food pouches at a time, if desired, without damage to the generator tubes.

Cooling

After sterilization, the food pouches must be cooled to at least 212°F to reduce internal pressure prior to removal from the pressure system. In any production operation this can be accomplished by incorporating a cooling section within the pressure system in which the belt conveying the pouches passes over a cooling platen prior to discharge from the system. Experiments were conducted and preliminary design data have been collected to permit addition of a cooling section in the process system at a later date. For the present experiments (Fig. 4) a receiver chamber K utilizing cooling water L on an emptied batch basis is being em-

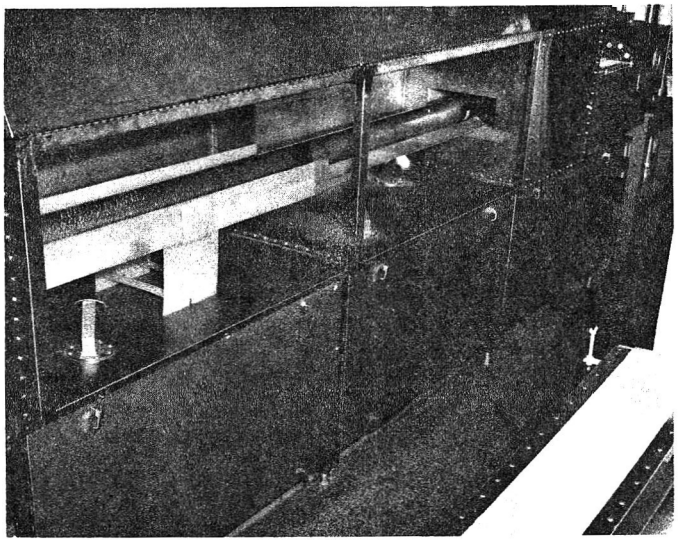

Fig. 7—View of epoxy-fiberglass tube in microwave cavity.

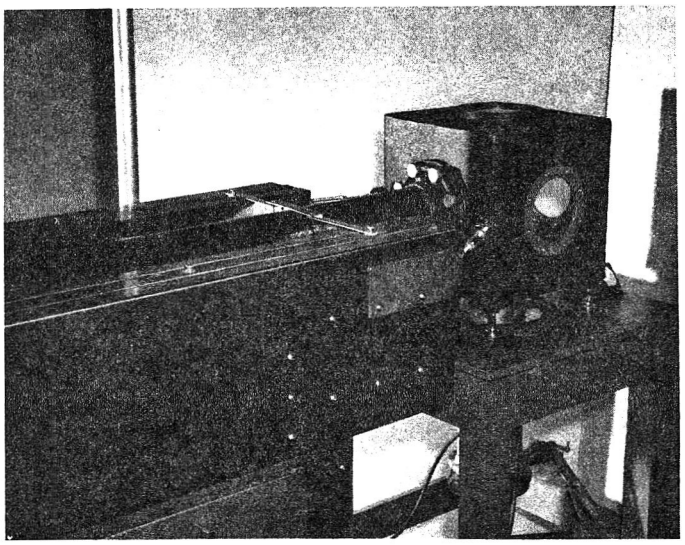

Fig. 8—View of exit end box.

ployed through valve H. In either case pouch temperatures can easily be reduced from the process temperature (250°F) down to 212°F in approximately 3–4 min.

Packaging

The process described is designed to sterilize solid or semisolid food products sealed in flexible plastic film laminate pouches. Investigation of various films and laminates most suitable for this purpose is continuing. To date, a 4.5 by 7 in. polyethylene (2 mil) mylar (polyester) (1 mil) laminate has been used which is readily heat sealable. In these experiments the pouches were heat sealed on three sides, filled with approximately 6–8 oz of product and the fourth side heat sealed.

Since no known solely plastic laminate will provide physical and microbiological protection to the food product subsequent to processing, particularly when subjected to the rigors of the military supply system, an overpack was considered to ensure physical protection and storage stability. The present experimental military foil laminate (3 mil polyolefin–0.35 mil aluminum foil–0.5 mil polyester) was considered suitable for this purpose.

Overpackaging, however, introduces the problem of possible bacterial contamination between the outer surface of the pouch and the inner surface of the overpack, resulting in possible contamination and subsequent spoilage of the product. To obviate this problem, sterilized pouches can be removed from the cooling receiver aseptically and overpacked with a sterile pouch in a "clean" environment (box or clean area). They could be passed directly into a sterile environment and overpacked with sterile pouches on a continuous basis.

Figure 6 is a photograph of the entrance valve assembly showing the epoxy tube in the microwave cavity. Figure 7 is a photograph of the microwave cavity with tube. Figure 8 shows the end exit box. Figure 9 shows the exit box with the cooling receiver attached below the box. Table 1 shows the performance characteristics of the continuous reactor.

The feed rate and other variables shown are based on continuous individual hand operation and are not intended to represent potential continuous production rates or capabilities, but rather to indicate the engineering characteristics of the pilot processor. Dielectric temperature rise refers to rise in temperature of epoxy fiberglass pipe in microwave cavity during operation.

Temperature measurement

To achieve sterility and consequent bacteriological stability in the food pouches, it is necessary to achieve temperatures of 250°F or greater in all portions of the food and to hold these temperatures for an equivalent of at least 3 min.

The measurement of temperature of the food results in two problems. The first is that of measuring the temperature of individual pouches in a continuous process, particularly in a closed pressure system. The second is measurement of temperature without a measuring device containing metal. Copson (1962) discussed the second problem and various approaches to low-loss thermometry. No satisfactory answer has been developed with respect to the first problem, although infra-red detection techniques hold promise for measuring at least surface temperature of the pouch.

In preliminary work, paper strip thermometry was used. Chemically treated paper strips alone or sealed into small glass tubing were employed. These are available (Paper Thermometer Co., 10 Stagg Dr., Natick, Mass. 01760) in 10°F increments and are essentially maximum and irreversible indicators which change from a light-gray to a jet-black color when their temperature rating is exceeded. Strips taped to the pouch surface tubes with the paper sealed inside or strips sealed in plastic and inserted into the food were used. These indicators have been useful and practical for the initial studies carried out.

RESULTS & DISCUSSION

LIMITED product studies have been conducted with the continuous microwave processor just described. Typical heating curves for water and two products—chicken a la king and frankfurters—are shown in Figure 10, which demonstrate the feasibility of the system design.

As would be expected, those products which were fluid in nature permitting convection within the pouches (e.g., chicken a la king vs. frankfurters) have a shorter heating time. Process times included a heating phase of 4–6 min as shown, approximately 3 min holding at process temperature (250°F or above) during which belt speed or microwave input may be regulated to avoid excess temperatures and resulting product damage, and a cooling period of 2–5 min for a total process of 9–14 min, close to the original design process contemplated. These times were obtained with an initial temperature of 75°F. Using a hot-fill (150–160°F), which would be normal practice, much shorter times to reach 250°F or above could be expected.

The project discussed in this publication represents the concept, design, fabrication and operation of a microwave system only. The next phase is that involving the food product itself, including acceptability, microbiological safety, stability and the economics of production. This work is now in progress and will be reported in a subsequent publication. The first concern with such a project was whether food products could be heated to the desired temperatures, held for the appropriate time, cooled and removed from system on a continuous basis—using the system described. It has been demonstrated that this can be done. Future work will include further temperature studies with additional foods, including extension of temperature measurements to include temperature distribution within the pouch. Once time-temperature-process parameters have been established, product quality studies will be

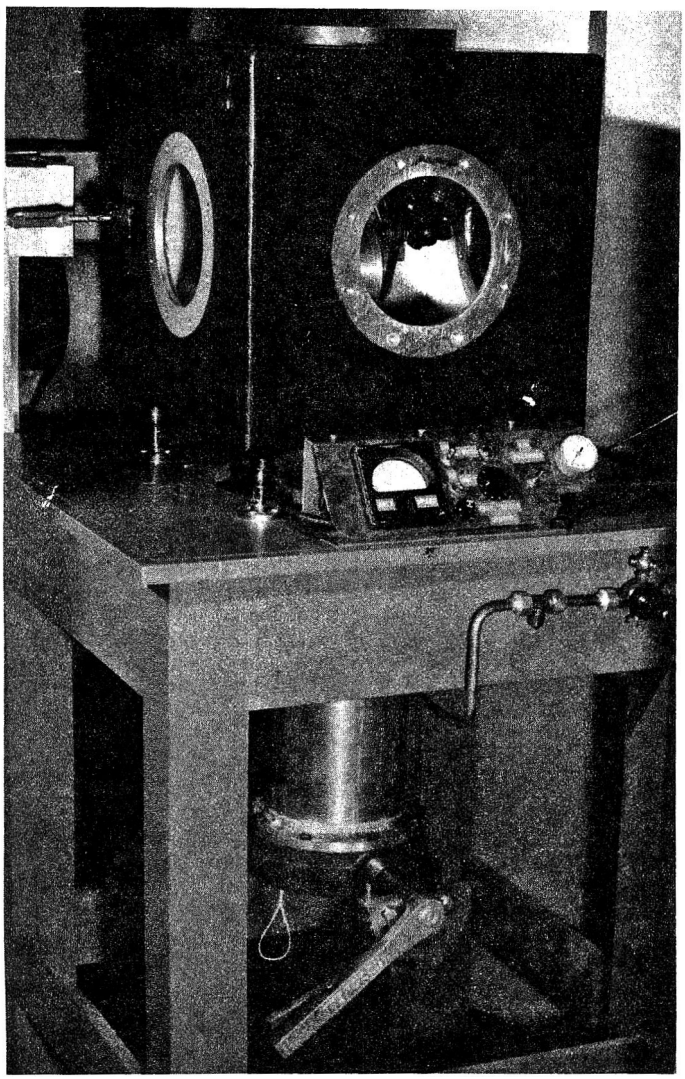

Fig. 9—View of exit end box with control panel and cooling receiver attached below.

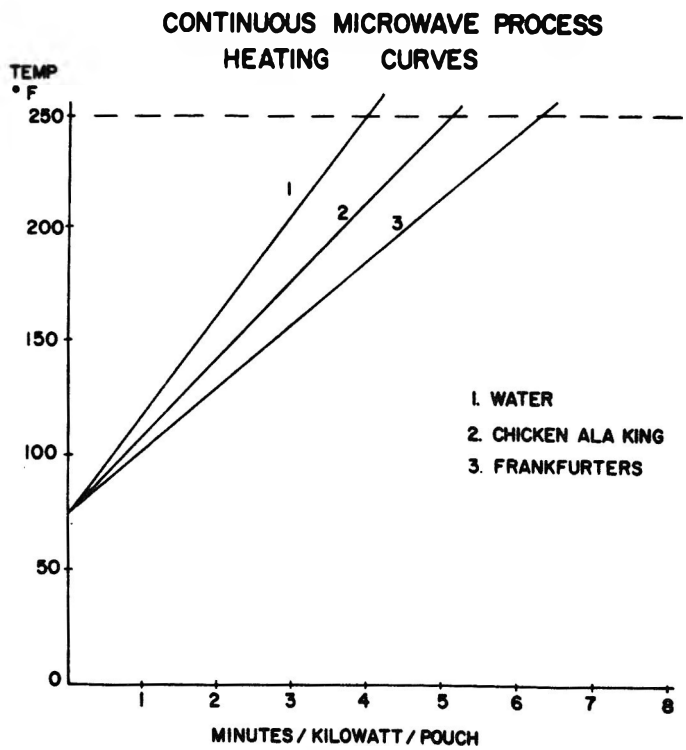

Fig. 10—Heating curves—continuous microwave processor.

required, including subjective panel acceptance comparing controls, conventionally processed and microwave-processed food components.

CONCLUSIONS

A CONTINUOUS microwave device was designed, constructed and tested. Initial trials with food products packaged in flexible plastic pouches demonstrated system feasibility and process times which approached the design parameter. More extensive product quality studies, microbiological criteria, economic evaluation and improved design features are currently in progress and will be reported subsequently.

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Mention of trade names in this report does not constitute an official indorsement of approval of the product by the Department of the Army.

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HEAT AND MASS TRANSFER IN A BATCH DRY RENDERING COOKER

SUMMARY—Data were obtained from a number of runs in which inedible offal, mainly from sheep, was processed in a full-scale batch dry rendering cooker at a commercial abattoir. Good accuracy of measurement of all variables, including weights of input and output materials, steam flows and temperatures was demonstrated by heat and mass balances. Overall heat transfer coefficients for jacket and shaft were calculated for a typical offal run and for a run in which water only was charged. For the offal run, coefficients declined rapidly from about 170 down to 70 Btu/ft²hr°F during the first hour, with the low value persisting for the remaining hour of the cycle. For the water run, there was a gradual decline in coefficient values from 170 to 130 Btu/ft²hr°F over a 2-hr period. Comparison of heat transfer behavior for these two cases shows that observed changes in the offal cook could not be explained by changes in heat transfer area caused by shrinkage of contents volume occurring during the cycle. As an alternative explanation, it is proposed that as evaporation of water proceeds, a phase inversion occurs from a tallow in water dispersion initially present in the cooker, to a water in tallow dispersion. A decline in the heat transfer coefficient sets in when tallow becomes the continuous phase, with a minimum value being reached when all water droplets have disappeared and remaining water is present only as "bound" water in the protein particles.

INTRODUCTION

A SURVEY of fat rendering in the Australian Meat Industry by Herbert and Wilson (1966) established that little is known about the chemical and/or chemical engineering aspects of batch dry rendering plants, even though this type of equipment is that most commonly used for rendering. Lack of basic knowledge makes difficult the evaluation and selection of new rendering processes; accurate plant design is impossible. Therefore, the Australian Meat Research Committee sponsored work within C.S.I.R.O., Division of Chemical Engineering, to investigate batch dry rendering processes with particular reference to heat transfer aspects.

After consideration of possible alternative experimental approaches such as laboratory or pilot plant studies of small models of batch cookers, it was decided to carry out the investigation on a full-size commercial cooker—one of three similar cookers forming the "inedible" by-products plant of a large Melbourne abattoir. This plant produces about 250,000 lb of inedible tallow and 430,000 lb of inedible meal from a typical weekly kill of 2,500 cattle and 35,000 sheep.

EXPERIMENTAL

Equipment

Figure 1 shows the main features of the cooker, including the 4 pairs of steam heated beaters which run within about ¾ in. of the inside wall of the steam heated jacket. The shaft supporting the beaters is driven at 30 rpm by a 35 h.p. electric motor. Jacket and shaft are supplied with steam from a main controlled at 80 psig; manually controlled stop valves are installed in each line. Jacket condensate discharge is via two 1 in. BSP thermostatic-type steam traps, and a non-condensable vent is supplied at the top of the jacket. Raw materials are charged through a short length of 18 in. dia pipe, which can be closed with a pressure tight lid. Steam evolved during evaporation of the contents vents through a 6 in. dia pipe leading out of the charging pipe, which reduces to 4 in. dia for most of its 200 ft run to a spray condenser. Internal pressure in the cooker can be adjusted by manipulation of a 6 in. dia gate valve in the vent pipe, and of a 2 in. dia valve in a line by-passing the 6 in. valve.

At the end of the batch cycle, the products are discharged into a percolator via a hinged door in the lower part of the unheated end plate. After the tallow has drained, the "cracklings" (cooked solid particles) are pressed in vertical hydraulic presses to remove most of the remaining tallow, and the press cake proceeds to milling and bagging as meat meal. The combined tallow from percolator and presses is filtered and stored.

Instrumentation normally fitted comprises indicating gauges for jacket steam pressure and internal pressure and an "end-point" controller which sounds an alarm when the electrical conductivity of the contents of the cooker becomes less than a preset value.

This type of cooker, internal dimensions 12 ft 6 in. long and 4 ft 6 in. dia, capacity 177 cu ft, is representative of many similar batch dry rendering plants installed in abattoirs in Australia and other countries.

Figure 1 and Table 1 give details of the posi-

Table 1—Description of test instruments

Label	Position	Type	Full scale reading	Recording equipment
F1	Vent-gas line	Differential press. transmitter	30 in W.G.	Foxboro M54 Consotrol Recorder
F2	Shaft steam line	Differential press. transmitter	50 in W.G.	Foxboro M54 Consotrol Recorder
F3	Jacket steam line	Differential press. transmitter	100 in W.G.	Foxboro M54 Consotrol Recorder
P1	Vent-gas line	Press. indicator	5 psig	
P2	Shaft condensate line	Press. indicator	100 psig	
P3	Jacket condensate line	Press. indicator	100 psig	
P4	Jacket condensate line	Press. indicator	100 psig	
P5	Charging pipe	Press. indicator -transmitter	100 psig	Foxboro M54 Consotrol Recorder
P6	Cooker jacket	Press. indicator -transmitter	100 psig	Foxboro M54 Consotrol Recorder
P7	Shaft steam line	Press. indicator -transmitter	100 psig	Foxboro M54 Consotrol Recorder
P8	Jacket steam line	Press. indicator -transmitter	100 psig	Foxboro M54 Consotrol Recorder
T2	Cooker contents	Thermocouple	150°C	Yew Temperature Recorder
T3	Cooker wall	Thermocouple	150°C	Yew Temperature Recorder
T4				
T5				
T6				

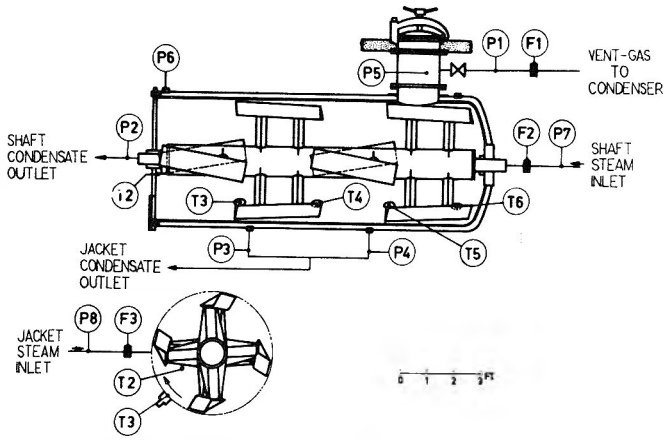

Fig. 1–Details of works cooker

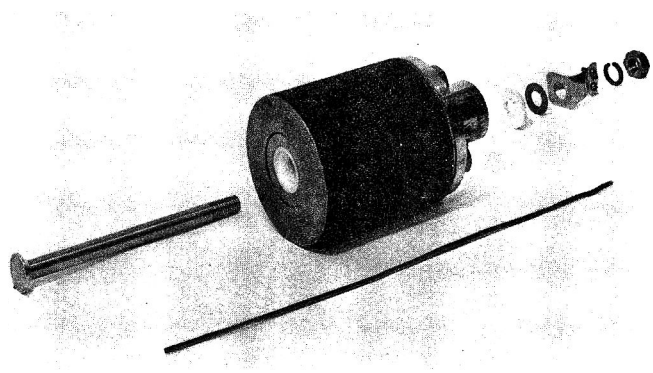

Fig. 2–Wall thermocouple assembly

tion and type of instruments installed for the experimental program. In addition, facilities were available for weighing input materials and products, comprising tallow from percolator and presses, and press cakes. Temperatures were measured by copper constantan thermocouples and recorded on a multi-point potentiometric recorder. The temperature of the contents (T2) was measured by a probe located in the lower part of the unheated end plate, and protruding about ½ in. inside the cooker. Other temperatures T3, T4, T5 and T6 were measured by probes (Fig. 2) installed flush with the inside wall of the cooker in the positions indicated in Figure 1.

Temperature probes T3, T4 and T5 were located along the length at places where the tips of adjacent pairs of beaters overlap, while the probe measuring T6 was installed at the end of the cooker, and so was influenced by only one pair of beaters.

Pneumatic pressure and differential pressure transducers and transmitters were employed to measure pressures and steam flows, the values of which were recorded continuously on strip chart instruments.

Table 2–Typical run data

Run No. 20 Date 22.10.68 — Batch time 132 min
Total jacket steam 3380 lb — Total shaft steam 2,064 lb
lb Steam/lb vent gas 1.22 — Total vent gases 4,459 lb
Charge weight 8,576 lb
Products weights – tallow: from percolator 360 lb; from press 1,176 lb
– total tallow 1,536 lb
– press cake 2,498 lb

Time from start	Jacket steam		Shaft steam		Vent gas	Contents		Wall	
-min.	psig	lb/hr	psig	lb/hr	lb/hr	psig	T2(°F)	T4(°F)	T6(°F)
6	23	2789	49	1805	354	0	168	177	190
12	48	2805	55	1645	1962	1	204	213	222
18	53	2772	57	1549	3698	3	222	231	237
24	58	2593	59	1516	3900	3	228	239	242
30	66	2473	61	1384	3690	3	226	240	246
36	72	2223	63	1295	3372	3	226	244	251
42	74	2003	65	1173	3076	2	224	248	255
48	76	1800	67	1109	2739	2	222	249	257
54	78	1671	68	1048	2552	2	222	253	264
60	76	1522	67	950	2380	2	222	257	267
66	76	1455	67	927	2257	2	221	257	269
72	77	1400	75	913	2194	2	221	258	269
78	79	1243	76	816	1230	7	228	266	275
84	80	905	79	560	0	28	262	280	287
90	81	798	79	473	1529	31	278	293	296
96	80	1000	79	518	1962	19	266	285	293
102	81	1045	79	518	1927	12	253	280	287
108	81	1050	80	521	1658	10	248	278	285
114	80	1045	72	408	1529	8	248	276	285
120	65	678	78	557	1285	6	249	278	287
126	17	0	81	524	935	2	244	264	269
132	25	527	81	428	360	0	239	253	255

Rendering cycle

The rendering cycle was essentially that used as normal procedure at the works, the important features of which are as follows: Between 8–9,000 lb of inedible raw materials are charged, comprising approximately equal quantities of crushed bones (heads and feet) and hashed gut materials, derived from a sheep-to-cattle slaughtering ratio of 14:1. Up to 400 lb of greasy solids from an effluent pit may also be included. Shaft steam is generally on but jacket steam is always off during the charging period which is of 10–15 min duration. On completion of charging, the charging lid is closed and jacket and shaft steam turned on fully. After initial preheating of the charge to the boiling point, evaporation of water commences at a high rate. Because of the resistance of the vent piping to gas flow, a pressure of up to 5 psig is built up initially inside the cooker, decreasing to below 1 psig as the vent gas flow decreases.

At a point in the cycle determined by measurement of the electrical conductivity of the contents, the end-point controller gives an alarm; the 6 in. valve in the vent pipe and the 2 in. by-pass valve are closed and internal pressure rises rapidly to 40 psig. The composition of the contents has, at this point, been reduced to about 20% water by weight from an original value of 50–60%. The pressure is then slowly reduced by gradual opening of the by-pass valve and finally reduced to atmospheric pressure by full opening of the main vent valve.

Another signal from the end-point controller indicates the completion of the cycle, but it is normal practice to examine a sample of cracklings to confirm that cooking has proceeded to a satisfactory point. Steam to jacket is shut off and the contents are discharged into the percolator.

The total cycle time, excluding charging and discharging times is 2–2½ hr, of which about 1½ hr is at slightly above atmospheric pressure and ¾ hr at pressures up to 40 psig.

Measurements were obtained from several runs in which plant control was left entirely to the works operators, who followed the above procedure fairly closely. However, for some runs, particularly those in which mass balances were required, some slight changes were considered justified. For example, shaft steam as well as jacket steam was turned off during the charging period, both being turned on simultaneously at the start of a run. It was also usually

Table 3–Leading mass balance data for 5 offal runs

	Run number				
	11	16	18	20	21
Charge weight (lb)	8411	8869	9118	8576	5157
Initial Water Content (%)	55.5	50.7	55.1	52.8	51.4
Total Vent gases (lb)	4528	4389	4798	4459	2575
Cycle time (min)	126	120	138	132	84
Time pressurization commenced (min)	82	70	93	74	67
Mass balance discrepancy (lb)	−157	−122	−228	−83	−109

necessary to restrict steam flow to the jacket for a few minutes in the initial stages of a run to keep the recording pen on scale. A major departure from normal practice was in Run 9 when water only was charged into the cooker and evaporation was continued at slightly above atmospheric pressure until all the water had gone.

Nomenclature

F = steam flow in lb/hr
L = latent heat of steam at flow meter pressure in Btu/lb
T_J = temperature of steam, saturated at jacket pressure in °F
Subscript J refers to jacket.
T_S = temperature of steam, saturated at average shaft pressure in °F
Subscript S refers to shaft.
T2 = temperature of contents in °F
U = overall heat transfer coefficient in Btu/ft²hr°F

RESULTS

FIVE MASS balance runs using offal materials and one using water only were included in 23 runs for which data were processed. The start of a run was taken as the time at which the jacket steam was turned on and the end of a run as that at which products were discharged. All relevant data for a run, including values of variables averaged over 6 min intervals from the start of the run, were punched onto cards and processed by PROGRAM FATREND on a CDC 3200 computer.

Data for one of the mass balance runs (Run 20) are given in detail in Table 2, and the corresponding mass balance is shown in Figure 3. Leading data from all five mass balance runs are tabulated in Table 3.

As assessment of the tallow, water and solids content of the heterogeneous feed materials is difficult, mass balances have been based on the weights and analyses of the products together with the total weight of vent gases produced during the run, as calculated by integration of the vent gas flow/time records. The accuracy of the mass balances can be checked by subtracting from the weight of input materials, the sum of weights of products and of vent gases. The discrepancy in mass balance is probably accounted for mainly by leakage from the cooker, appearing as LOSSES W 83 lb, leaving the cooker on Figure 3. Discrepancies varied from a maximum of −228 lb for Run 18 in which 9,118 lb was charged, to −109 lb for Run 21 in which 5,157 lb of offal was charged to the cooker. The low values of these discrepancies and the generally excellent agreement between heat and mass flow data at any given time interval in Table 2 indicate a high degree of measurement accuracy.

In a batch cycle, there will be a progressive reduction in the volume of the contents. However, in computing heat transfer coefficients, the heat transfer areas were taken as the total available for both jacket and shaft, i.e., jacket 178 ft² and shaft 101.5 ft², while heat losses were estimated as equivalent to 150 lb/hr steam, assumed all lost from jacket steam flow, F_J. On this basis, the coefficients were given by

$$U_J = (F_J - 150) \times L_J / \left[(T_J - T2) \times 178 \right] \quad (1)$$

$$U_S = F_S \times L_S / \left[(T_S - T2) \times 101.5 \right] \quad (2)$$

Values of U_J are plotted vs time in Figure 4 for a typical offal run (Run 20), together with values of U_J for Run 9 in which water only was charged to the cooker. Values of heat transfer coefficients have been tabulated in Table 4 for Runs 11, 18 and 20, in which charge and product weights, total vent gases and other run conditions were similar. Coefficient values at a given time are seen to be comparable; values from other runs, e.g., Run 21, Table 3 cannot be compared in this way since variations in run conditions (charge weight, water content, rate of heating etc.) resulted in substantial variations in cycle time. However, for all offal runs high initial values of jacket coefficient, around 170 Btu/ft²hr°F, decreased rapidly during the first half of the cycle to a value around 60 Btu/ft²hr°F and remained at the low value for the remainder of the cycle.

Table 4–Variation of heat transfer coefficients with time

Time	Jacket coefficients Btu/ft²hr°F			Shaft coefficients Btu/ft²hr°F		
	Run number			Run number		
min	11	18	20	11	18	20
18	160	162	170	190	178	154
24	152	154	160	204	178	158
30	146	148	134	202	174	138
36	136	132	112	198	164	126
42	114	112	96	170	142	110
48	90	94	82	132	120	100
54	78	80	76	112	106	94
60	64	70	68	100	92	86
66	58	64	64	90	86	82
72	60	60	62	86	83	80
78	60	56	56	88	80	76
84	60	52	60	84	76	80
90	60	48	68	88	74	92
96	60	43	72	124	72	78
102	60	43	62	108	74	64
108	64	46	58	102	90	60
114	60	60	58	100	78	50
120				92	64	66

DISCUSSION

OVER THE WHOLE cycle an average of 1.25 lb of heating steam at 80 psig were supplied to the shaft and jacket combined, in order to evaporate 1 lb of water from the contents. Heating steam, additional to that required for evaporation of water from the contents, was required to make up heat losses, to increase the sensible heat of plant and contents and to melt fat from the solid materials.

For runs in which shaft steam was left on during the charging period, 29% of heating steam supplied during each cycle was used for shaft heating, in contrast to 39% when shaft steam was turned off during the charging period. This difference was probably due to overheating and baking of protein materials on to the hot shaft, rotating in a small volume of charge materials. For a typical run, cycle time could be reduced by 15%, or approximately 20 min in a 2¼ hr cycle, if shaft steam was left off during charging.

From Table 2, it can be seen that wall temperature T4, which is substantially the same value as T3 and T5, was 9°F higher than T2, the temperature of the contents, at commencement of boiling (18 min from start) and 37°F higher after 72 min. The other wall temperature, T6, was 6°F higher than T4 after 18 min and 11°F higher than T4 after 72 min. The increasing differences between wall temperatures and contents temperatures reflect the less favorable heat transfer characteristics of the contents as evaporation proceeds and the higher value of T6 compared with the other wall tempera-

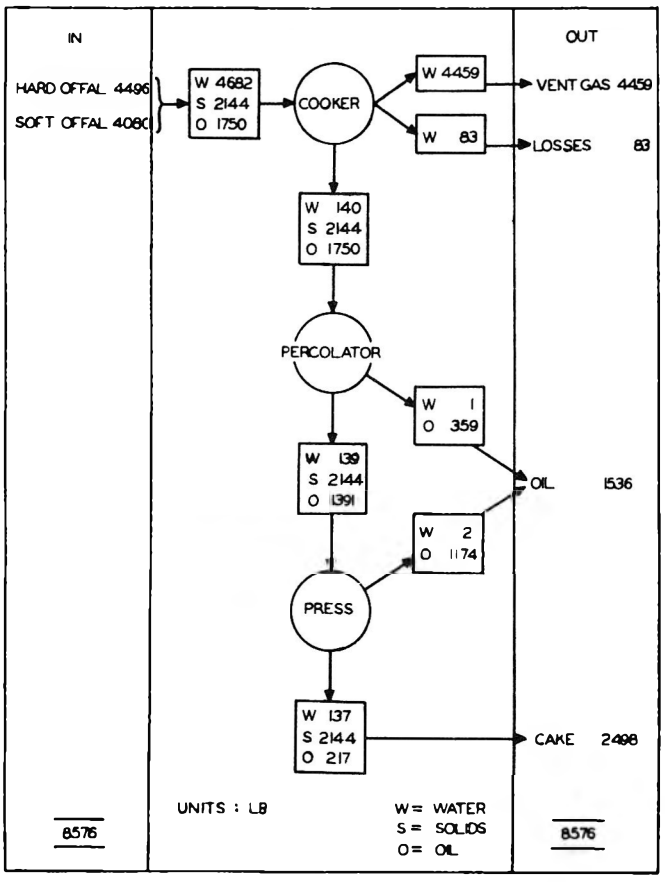

Fig. 3–Mass balance for Run 20.

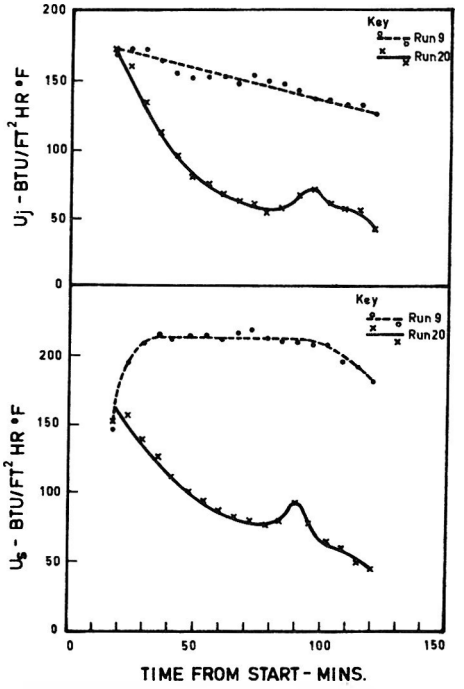

Fig. 4–Overall heat transfer coefficient vs time from start, for Runs 9 and 20.

tures may be explained by the difference in intensity of agitation at the wall near T6, compared with that at the other wall thermocouples. Increased agitation apparently results in improved heat transfer rates, which would be particularly desirable during the last hour of the cycle.

The plot of U_J vs time for Run 9 (Fig. 4) when water only was charged shows that the jacket overall heat transfer coefficient decreases linearly with time from an initial high value of 170 Btu/ft²hr°F to a minimum of 130 Btu/ft²hr°F at 120 min from the start. The corresponding coefficient for the shaft remains at a high value of around 210 Btu/ft²hr°F for about 70 min, then declines to 175 Btu/ft²hr°F at 120 min from the start.

With water only being evaporated, a substantially constant value of U_J might have been expected. The observed decrease is probably due to the assumption of constant heat transfer area in equations (1) and (2); in fact, a progressive reduction of area available for heat transfer must occur as the volume of the contents is decreased by evaporation from 77–11% of the cooker volume. A correction to make values of U_J constant throughout the cycle was not applied since it was found that it was not simply related to the contents volume, nor to contents volume plus an increment of volume to take into account splashing up the walls. The reduction in volume for an offal run was much less than for the water run (from 69%–35% for offal compared to 77%–11% for water over 120 min of cycle time).

The plot of U_J vs time for Run 20 (Fig. 4) is typical of the heat transfer variation during a batch run in which offal was cooked in accordance with normal works procedure. From a value of 170 Btu/ft²hr°F at time 18 min, U_J decreases to 68 Btu/ft²hr°F at time 60 min and only a slight further decrease occurs during the remainder of the cycle. There is an increase during the pressure period of the cycle, presumably due to an improvement in the heat transfer characteristics of the contents at elevated temperature. Values of U_S vs time follow a similar pattern, although the decrease is not as marked as that for U_J.

The decrease in heat transfer area due to volume shrinkage can only partly explain heat transfer behavior observed for an offal run, and the following explanation is proposed:

Following an initial preheat period, the contents comprise tallow and water phases, and a range of sizes of particles of bone and protein materials. Water forms a high proportion of the contents and is probably present as the continuous phase, and so has free access to heat transfer surfaces. Boiling commences at a value of coefficient similar to that obtained for the "water only" run.

Water is progressively evaporated and more tallow is liberated from the solid materials as the run proceeds until an inversion of the liquid phases occurs with water becoming the disperse and tallow the continuous phase. Heat transfer now relies in part on droplets of water contacting heated surfaces, due to agitation in the contents, but increasingly on an indirect mechanism, in which tallow is heated at the walls and, as it is mixed into the contents, transfers its heat to water droplets and solid particles. While water droplets remain, T2 does not increase substantially over the temperature of saturated steam corresponding to the pressure above the contents; however, wall temperatures T4 and T6 increase, as noted in Table 2.

The indirect mechanism would be expected to give a lower coefficient than the boiling water mechanism, with the heat transfer rate declining to a minimum value when the contents contain tallow only as the liquid phase, with the remaining water "bound" in the inner layers of the solid particles. Heat transfer would then be from heated surfaces to tallow and from hot tallow to the outside layers

of the solid particles, from which water would be evaporated by slow diffusional processes. A rise in T2 would be expected, but is presumably masked by the temperature changes caused by application of pressure to the contents; thus recent tests have indicated that, in the absence of pressure, T2 increases to about 250°F during the last 30–40 min of a run.

Electrical conductivity changes in the cooker contents have been shown to parallel heat transfer changes, giving substantial confirmation of the existence of a liquid phase inversion in the early stages of the cycle. Thus, in one run a wall conductivity probe (a slightly modified wall temperature probe) gave readings in excess of 4,000 micro mhos/cm for the first 25 min of the run. In the next 5 min there was a fall to below 500 micro mhos/cm; a value below 10 micro mhos/cm was reached 60 min from the start of the cycle and was maintained until the end of the 110 min cycle. Results from laboratory work by Herbert et al. (1970) on olive oil/water and tallow/water emulsions, confirm that inversion of oil and water phases occurs at well defined water contents in these emulsions.

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Data and results for runs not reported in this paper will be supplied on request to the authors.

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POPULATION DISTRIBUTION OF HEAT RISE CURVES AS A SIGNIFICANT VARIABLE IN HEAT STERILIZATION PROCESS CALCULATIONS

SUMMARY—Sterilization levels found in inoculated test packs are commonly in disagreement with predicted values. This study was made to determine if predicted sterilization values would be closer to actual test values if the population distribution of the slope indices from a sample of heat rise curves were used instead of the traditional slowest or mean single value of slope index in the sterilization calculations. A computer was programmed to calculate from the basic equations of thermal death times and heat penetration, the amount of sterilization achieved at designated time intervals in a population of food packages. Means and standard deviations of slope indices from both real and postulated heat penetration tests were fed into the computer together with specified processing conditions. Predicted spoilage levels were very close to those obtained from actual inoculated test packs. From input of postulated heat penetration values, it was demonstrated that the larger the standard deviation, the greater the error will be if only a single value of the slope index is used. Manual procedures are given for an accurate determination of the minimum process time required for sterilization. Methods are also given for data expansion to show a curve illustrating the complete relationship between process time and food sterility.

INTRODUCTION

MATHEMATICAL methods for determining the amount of sterility in a food process have been available since the 1920's when Ball (1923) and others developed the basic equations for thermal death times of bacterial spores and heat penetration rates in food containers. However, the accuracy of any process calculation obtained from use of the basic equations can be no better than the accuracy of any of the input variables (Hicks 1961). One such variable, the slope index of the heat rise curve, is a source of error in process calculations. The slope indices of heat penetration curves taken from a number of randomly selected food packages in a given population will vary.

Traditionally, the slope index of the slowest heating package of the test group is used in the process calculations; however use of this value introduces an inherent error in process determination because this slope index is certainly not the average for the total package population, nor is it necessarily the slowest heating package in the package population.

Process calculations using the traditional slowest slope index will only give a rough approximation of the process time to achieve commercial sterility and even then the addition of safety factors followed up by inoculated test pack studies may be necessary to determine an acceptable process. Attempts to predict levels of sterilities for process times over a range of inoculated test packs are usually far off the mark when predictions are based on the single slowest heat rise slope index value.

By examining a sample of slope indices of heat rise curves, it is possible—using accepted statistical methods—to obtain an estimate of the mean slope index and the standard deviation of the population of slope indices. From these estimates, the probability of individual slope indices varying from the mean by more than a specified amount can be computed. Thus, from sample heat penetration data, a relatively accurate prediction of the value and frequency of the heat rise slope indices over a package population can be determined.

This paper develops a method for using the predicted population distribution of the slope index values in the basic equations of process calculation. It will be shown that this method produces process predictions with high correlation to actual inoculated test pack results. While the general principles illustrated in this paper are applicable to both convection and conduction heating foods, the specific methods given are applicable only to convection foods. The addition of cooldown lethality into the computer program would enable this method to be used also for conduction foods.

PROCEDURES

General approach

Assuming that the slope indices of the heat rise curves of a population of food packages behave as a randomly occurring variable, the distribution of values of slope index about the mean will follow the normal bell shaped distribution curve. This distribution curve can be divided into segments such that for a given segment, encompassing a range of slope indices, the area under the curve will represent a percentage of the total population of packages under consideration. The slope index (f_s) selected for each segment is that value which divides the segment into two equal area portions.

For a specified process time and specified process condition, each of the segments of population will thus achieve a certain lethal value relating to the segmental slope index (f_s). Using the lethality so calculated for each population segment, the number of surviving spores and spoiled packages for that segment is calculated. The total calculated number of spoiled packages is accumulated for all the segments. When this is repeated over a range of process times, the minimum process time which accumulates zero spoiled packages is determined. Alternatively an accurate picture of process time-spoilage is obtained graphically when process time is plotted against percent spoilage.

Preparation of heat penetration data

A suitably large sample is drawn from a population of food packages and a determination for heat penetration is made on each package at the thermal center using conventional thermocouple methods. The resulting curves are then plotted on semi-logarithmic paper and in most cases a straight line portion is obtained (Fig. 1a).

To determine the mean slope index and standard deviation of these straight lines, the following procedure is used:

1. Determine the lag factor (j) for each heat rise line.

2. Determine the point in time ($t_{I=1}$), when the heat rise line crosses the abscissa line drawn through I=1.

3. Using equation (1), determine the mean lag factor ($\bar{j}$).

$$\bar{j} = \log^{-1}\left[\frac{\Sigma \log j}{n}\right] \quad [1]$$

4. Using equation (2), determine the mean initial temperature differential ($\bar{I}_o$).

$$\bar{I}_o = \frac{\Sigma I_o}{n} \quad [2]$$

5. Using equation (3), determine the common origin $\bar{I}_A$.

$$\bar{I}_A = (\bar{j})\,(\bar{I}_o) \quad [3]$$

6. On a new semi-logarithmic plot mark common origin ($\bar{I}_A$) and each of the $t_{I=1}$ points. Draw a straight line between $\bar{I}_A$ and each of the $t_{I=1}$ points (Fig. 1b).

7. Determine the slope index (f) values for each of the standardized heat rise lines and using equations (4) and (5), calculate the mean slope index ($\bar{f}$) and standard deviation (σ_f).

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$$\bar{f} = \frac{\Sigma f}{n} \qquad [4]$$

$$\sigma_f = \sqrt{\frac{\Sigma (f - \bar{f})^2}{n-1}} = \sqrt{\frac{1}{n-1}\left[\Sigma f^2 - \frac{(\Sigma f)^2}{n}\right]} \qquad [5]$$

Preparation of population segments

A bell shaped normal population distribution curve (Gauss, 1880) was divided arbitrarily into segments (Fig. 2). The area in each segment divided by the total area under the curve represents the percentage of population in that segment. Using a table of area-standard deviation relationships (Gauss, 1880) the number of standard deviations from the mean slope index at the mid area point was determined for each of the 20 population segments (Fig. 2). From the experimentally determined mean slope index ($\bar{f}$) and slope index standard deviation (σ_f), the segment slope index (f_s) is easily calculated.

Calculations

Determination of lethal process for a given range of process times under specified process conditions for the 20 population segments can be made using the following procedures:

Using a simple transposition of the hypothetical process time equation of Herndon et al. (1968), a theoretical process time (t′) is calculated for a given process time (B) for each of the 20 population segments represented by a previously determined slope index (f_s).

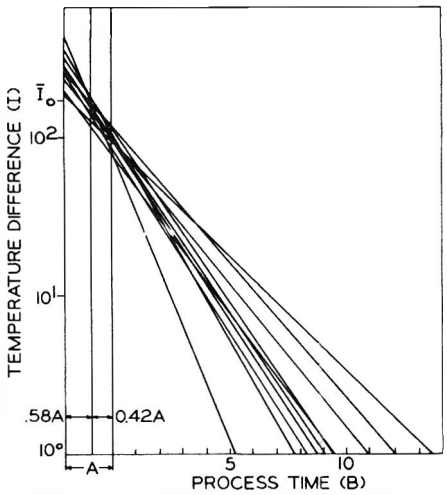

Fig. 1A—Heat penetration plots of whole kernel corn. $\bar{I}_o = \Sigma I_o/n = 170°F$; $\bar{J} = \log^{-1}$ (Σ log J/n) = 0.85.

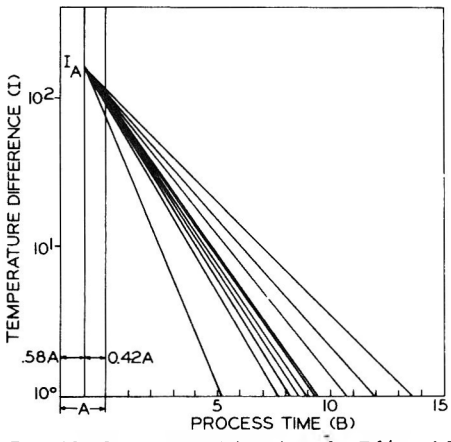

Fig. 1B—Common origin plot. $\bar{f} = \Sigma f/n = 4.5$ min; $\sigma_f = \sqrt{\Sigma (f - \bar{f})^2/n\text{-}1} = 1.2$ min.

$$t' = \frac{B - f_s (\log \bar{j} + \log \bar{I}_o - 2) + 0.42A}{f_s} \qquad [6]$$

For each hypothetical process time (t′) a corresponding hypothetical lethal process value (F′) is found using the Herndon-Griffin-Ball tables (Herndon et al., 1968).

It is also known that for each determined hypothetical process value (F′), there exists, for a given processing condition, a corresponding true process lethality ($F^z_{T_b}$). Using equation (7), the $F^z_{T_b}$ for each population segment is found.

$$F^z_{T_b} = (f_s)\,(F')\,(L) \qquad [7]$$

The $F^z_{T_b}$ value by itself will not show the level of sterilization achieved on a known number of specified bacterial spores. Equation (8) is used to determine this. The D value (decimal reduction time) is the number of minutes at T_b necessary to reduce the spore population by 90% and N is the number of decimal reductions achieved by a process lethal value of $F^z_{T_b}$.

$$N = \frac{F^z_{T_b}}{D_{T_b}} \qquad [8]$$

In determining the estimated number of surviving spores (S_g) remaining in each population segment after a lethal process equivalent to N decimal reductions, equation (9) is used. S_s is the effective initial spore population in a given segment before process.

$$S_g = \log^{-1} (\log S_s - N) \qquad [9]$$

Using equation (10), the number of packages (P_g) containing viable spores is now determined from the number of packages in each population segment (P_s) and the surviving spores (S_g) in each population segment.

$P_g = S_g$ With limits: (1) $P_g \leqslant P_s$; and (2) P_g be rounded off to closest whole number or zero if $S_g < 0.5$. [10]

Example: In a population segment of 100 packages an expected value of 0.4 remaining spores would make zero contaminated packages; an expected value of 53.6 remaining spores would make 54 contaminated packages; and an expected value of 100 or more would make 100 contaminated packages.

An alternate equation to equation (10)

Table 1—Spoilage for single time (B) print-out for whole kernel corn.

PA3679	Test spores identification	255.00	Retort temperature, °F
1000	Number of packages	0.00	Retort come up time (min)
10000.	Spores per package	150.00	Initial temperature, °F
18.00	Z value of spores, °F	4.50	Mean slope index, min
1.03	D_{250} value of spores, min	1.20	Sigma slope index
250.00	Base temperature, °F	0.85	Lag factor

% of Population	Slope index (f_s)	F Value	N Value	No. of packages (P_s)	Initial no. of spores (S_s)	No. of spores remaining (S_g)	Spoiled packages (P_g)
0.1	1.0	12.26	11.90	1	0.10000E 05[a]	**********[b]	0
0.2	1.1	12.12	11.76	2	0.20000E 05	**********	0
0.4	1.4	11.08	10.75	4	0.40000E 05	**********	0
0.8	1.8	10.04	9.74	8	0.80000E 05	**********	0
1.6	2.1	9.00	8.74	16	0.16000E 06	0.29343E- 03[a]	0
6.9	2.8	7.22	7.01	69	0.69000E 06	0.67265E- 01	0
10.0	3.3	5.90	5.73	100	0.10000E 07	0.18583E 01	2
10.0	3.7	4.85	4.71	100	0.10000E 07	0.19580E 02	20
10.0	4.0	4.09	3.97	100	0.10000E 07	0.10652E 03	100
10.0	4.3	3.48	3.38	100	0.10000E 07	0.41532E 03	100
10.0	4.7	2.96	2.87	100	0.10000E 07	0.13481E 04	100
10.0	5.0	2.48	2.41	100	0.10000E 07	0.38982E 04	100
10.0	5.3	2.03	1.97	100	0.10000E 07	0.10763E 05	100
10.0	5.7	1.56	1.52	100	0.10000E 07	0.30542E 05	100
6.9	6.2	1.15	1.11	69	0.69000E 06	0.53068E 05	69
1.6	6.9	0.77	0.75	16	0.16000E 06	0.28566E 05	16
0.8	7.2	0.61	0.59	8	0.80000E 05	0.20426E 05	8
0.4	7.6	0.49	0.47	4	0.40000E 05	0.13508E 05	4
0.2	7.9	0.38	0.37	2	0.20000E 05	0.84654E 04	2
0.1	8.4	0.28	0.27	1	0.10000E 05	0.53112E 04	1
		Mean 3.21	Mean 3.12			TOTAL	722

[a]Location of decimal point, i.e., 0.29343E-03 = 0.00029343, 0.19580E 02 = 1.9580.
[b]Less than 0.0001 spores.

would be equation (10a). The possible use of equation (10a) in this application is covered in the discussion section of this paper.

$$P_g = P_s - \frac{P_s}{\log^{-1}\left(\frac{S_g}{2.303\ P_s}\right)} \qquad [10a]$$

The total number of spoiled packages (P_c) in the group for a given process time (B) is the sum of the spoiled packages (P_g) in all the population segments:

$$P_c = \Sigma P_g \qquad [11]$$

The percentage of spoilage for a given process time (B) is thus the ratio of total spoiled packages (P_c) to total packages (P_t); i.e., % spoilage = P_c/P_t assuming P_t = 1000 as shown later, in the computer program development, % spoilage = $P_c/10$.

By repeating this procedure over a range of process times (B), a prediction of sterility over different process times can be made. By plotting on regular coordinate paper, a curve can be drawn showing the complete relationship between process time and percent sterility for the specified process conditions. The minimum process time (B_0) is the point at which the curve intersects the 0% spoilage line. This curve will intersect the 0% spoilage line only when a finite number of packages is considered. When an infinite number of packages is considered, the curve will not intersect the 0% spoilage line but will become asymptotic to it.

Computer program

To manually calculate a process using the above procedures would involve many time-consuming manipulations and be subject to human error. It was decided to let a computer do most of the work, and then develop a simplified manual procedure after examination of a series of computer curves.

The computer program developed for this purpose consisted of the program of relationship of hypothetical time and process values (Herndon et al., 1968) including the correction equations (6) and (7). Equations (8), (9), (10) and (11) were also incorporated into the program. Computer input values were:

P_t–Total number of packages. (To obtain spoilage values to 0.1%, P_t is usually given the value of 1,000.)

S_p–Effective number of spores per package.

z–Slope index of thermal death time curve.

D_{250}–Decimal reduction time at 250°F.

T_I–Retort temperature.

A–Retort come-up times.

T_o–Average initial temperature.

$\bar{f}$–Mean heat rise slope index.

σ_f–Standard deviation of slope index.

$\bar{j}$–Mean lag factor.

B values–A range of process times.

The base temperature (T_b) was made a constant in this program and given the value 250°F. The segment percentage population-number of standard deviations (K) from the mean relationships were also made constants in the program (Fig. 2).

The computer was programmed to print a percent sterilization for each designated process time (B) (See Table 1). From a series of such calculations, the computer also printed a second table (Table 2) which contains the process time (B) values and the corresponding total spoiled package values (P_c) for each of the working tables (equivalent to that shown in Table 1). From this table a graph (Fig. 3) was made and the points represented by (B) and (percent spoilage) were plotted and connected with a line. The resulting reverse S-shaped curve represents the complete relationship between process time and level of sterility of a package population under given processing conditions.

Process evaluation

To check the accuracy of this method, a process calculation was made using actual experimental data from a heat penetration test and an inoculated test pack. The product/package used was 180g of whole kernel corn with 60g of brine packed into a 5-½ in. × 7 in. flexible pouch. The pouch was fabricated from a laminate comprising 0.0005 in. polyester film/0.00035 in. aluminum foil/0.001 in. polyamid film.

A series of heat penetration studies were run using a laboratory Berlin Chapman test retort modified to give a high flow steam/air process on a rotating package load. Copper-constantan thermocouple readings from the center of kernels of corn at the thermal center of the food mass were recorded on a special Leeds and Northrup Multipoint recording potentiometer which had been designed especially for food heat penetration work. The resulting heat penetration plots are shown in Figures 1a and 1b.

The data obtained from the common origin heat penetration plots (Fig. 1b and Discussion) on the corn, along with proposed process conditions for a test pack series to be inoculated with 10,000 PA3679 spores per package, were fed into the computer and process lethalities for a series of process times were calculated. Table 1 shows one of the time print-outs and Table 2 summarizes results of all the time print-outs. Line #1 of Figure 3 is a plot of these values.

For comparison purposes, conventional process predicted spoilage levels are also illustrated for PA3679 where slope indices are assumed to have no variation. Line 2 is based on the mean slope index and Line 3 upon the slope index of the slowest heating package in the heat penetration test sample. In both cases, all pouches were treated as one segment.

A series of packages (100 per process time interval) were inoculated with 10,000 spores of PA3679 supplied by the Washington Laboratories of the National Canners Association. NCA

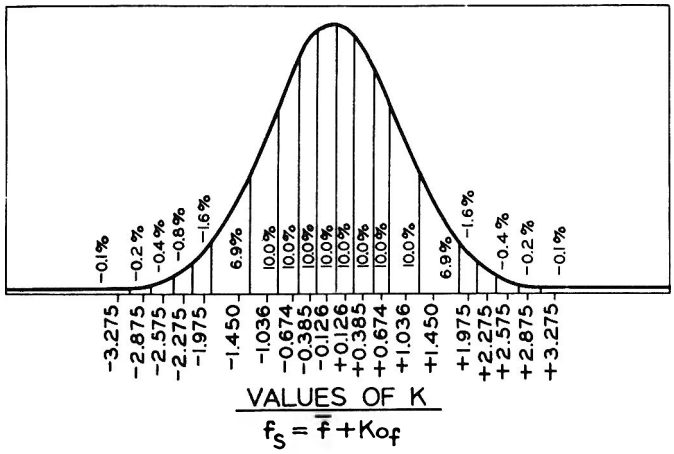

Fig. 2–Segmented normal distribution curve.

Table 2–Summary print-out of process time/sterility values. (Corn product)

PA3679	Test spore identification
1000	Number of packages
10000.	Spores per package
18.00	z Value of spores, deg
1.03	D_{250} Value of spores, min
250.00	Base temperature, deg
255.00	Retort temperature, deg
0.00	Retort come up, min
150.00	Initial temperature, deg
4.50	Mean slope index, deg
1.20	Sigma slope index
0.85	Lag factor

B Value (min)	No. of spoiled packages
3.00	1000
3.50	1000
4.00	997
4.50	993
5.00	981
5.50	970
6.00	919
6.50	842
7.00	722[a]
7.50	625
8.00	472
8.50	343
9.00	215
9.50	118
10.00	49
10.50	24
11.00	9
11.50	3
12.00[b]	0
12.50	0
13.00	0
13.50	0
14.00	0

[a]See Table No. 1.
[b]Minimum process for PA3679.

also determined the TDT values in corn and brine of the PA3679 spores which were used in the computer predictions. These packages were then processed over a range of process times at 255°F in a production prototype Robins Hydrolock continuous retort (Lawler, 1967) modified to process flexible packages in a high flow steam/air atmosphere. The processed pouches were then put into incubation at 85°F and checked for spoilage over a 3-mo period. The percent spoilage for each test process time is shown by the dots in Figure 3.

To illustrate the magnitude of the effect of an increase in variance of heat penetration slope indices, Figure 4 was prepared. This is a series of sterilization curves prepared by the computer where all variables remained constant except the value of the standard deviation of slope index (σ_f).

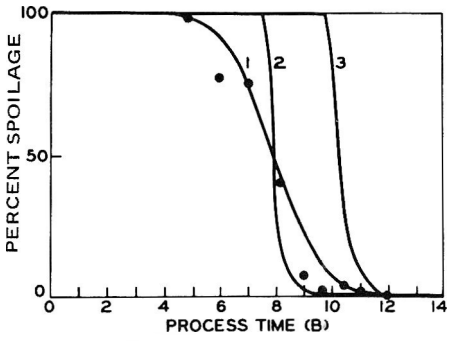

Fig. 3–Predicted and actual spoilage for whole kernel corn. Line #1–Computer prediction for spoilage based on 10,000 PA3679 spores per package (D_{250} = 1.03; z = 18°F). Line #2–Conventional prediction using mean slope index based on 10,000 PA3679 spores per package. Line #3–Conventional prediction using slowest test slope index (6.6) based on 10,000 PA3679 spores per package. Dots • = Actual spoilage from packages inoculated with 10,000 PA3679 spores per package. Relative to Line #1, the average of the squares of the residuals = 72. Relative to Line #3, the average of the squares of the residuals = 2,800.

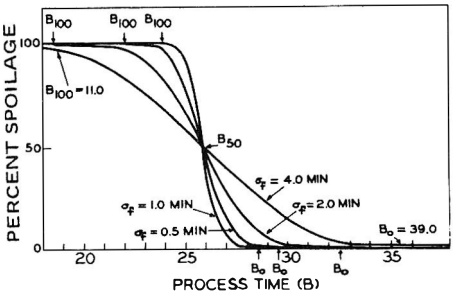

Fig. 4–Effect of slope index variance. Spore data: S_p = 10,000; D_{250} = 1.0 min; z = 18°F. Heat penetration data: $\bar{f}$ = 20.0 min; σ_f = as shown on lines; $\bar{j}$ = 1.41. Retort process data: T_o = 160°F; A = 0.0 min; T_1 = 250°F.

RESULTS & CONCLUSIONS

Data correlation

The actual spoilage results (in % of packages spoiled) from the inoculated test pack series on whole kernel corn are shown in Figure 3 (dots) to have close correlation (average of the squares of the residuals = 72) to the computer calculated predicted values based upon population distribution of heat rise curves (Line #1). Very little correlation (average of the squares of the residuals = 2,800 for Line #3) is shown between actual spoilage and the conventionally predicted values (Lines #2 and #3). The point 12 min (based upon destruction of PA3679 spores Line #1) is shown to be a minimum process (B_o) for PA3679.

The predicted spoilage results from postulated input data illustrated in Figure 4 show that as the value of standard deviation (σ_f) increases, there is a significant increase in the process time (B_o) necessary to achieve a minimim process for PA3679. At the median package lethality (50% package sterilization level) the process time (B_{50}) is not affected by changes in the value of σ_f.

Computer method

For those who have access to a digital computer determination of minimum process time (B_o) and process time-sterilization level relationships can be made by setting up a computer with a Fortran program and feeding in the input data consisting of retort conditions (T_1, A, T_o, and B's) bacteria spore values (S_p, z, and D_{T_b}), number of packages (P_t) and heat penetration data ($\bar{f}$, σ_f, and $\bar{j}$).

Manual method

For those who do not have access to a computer, determination of a minimum process time (B_o) for specified process conditions can be determined by calculating a process for the slowest heating package of a population of 1,000 as illustrated in the following steps:

1a–Using equation (12), determine a heat rise slope index (f_Ω) that is representative of the slowest heating package of a population of 1,000. (See development of equation 12 and definition of f_Ω in the Glossary.

$$f_\Omega = \bar{f} + 3.275\,(\sigma_f) \qquad [12]$$

1b–Using equation (13), determine the minimum number of decimal reductions (N_1) that will result in 0.5 spores in a package. Where S_p is the effective number of spores inoculated or postulated in a package,

$$N_1 = \log S_p + 0.301 \qquad [13]$$

[see Equations (9–9c) for derivation of equation (13).]

1c–Using equation (14), determine the lethal value (F_1) of the process at temperature T_b necessary to achieve the effect of N_1 decimal reductions.

$$(F_1) = (N_1)\,(D_{T_b}) \qquad [14]$$

1d–Using equation (15), determine the hypothetical lethal process value (F′) equivalent to F_1 for a specified process.

$$F' = (F_1)/[(f_\Omega)(L)] \qquad [15]$$

Note that f_Ω and F_1 were determined in Steps 1a and 1c. L is found in Table 2 of Herndon et al. (1968) from the value of I_L. If $T_b = T_1$ then $I_L = 0$ and L = 1.

1e–Using Table 1 of Herndon et al. (1968), find the hypothetical process time (t′) corresponding to F′ determined in 1d.

1f–Using equation (16), determine the minimum safe process time (B_o).

$$B_o = f_\Omega\,(t' + \log \bar{j} + \log I_o - 2) - 0.42A \qquad [16]$$

Note: f_Ω and t′ were determined in Steps 1a and 1e and $\bar{j}$ is determined from the heat penetration studies. I_o and A are conditions for the proposed process.

The B_o value determined in Step 1f is the minimum process time for a specified set of process conditions. For those who desire to know a more complete relationship between process time and spoilage level (for inoculated pack studies etc.) two additional points can easily be found: the process time (B_{50}) where one-half the packages are still contaminated; and process time (B_{100}), the maximum process time which will still leave 100% of the packages contaminated with spores. These points can be found using the following steps:

2a–Using equation (17), solve for the hypothetical process value (F′). F_1 was found in Step 1c.

$$F' = F_1/\bar{f}L \qquad [17]$$

2b–Using Table 1 of Herndon et al. (1968), find the t′ which corresponds to the determined F′.

2c–Using equation (18), determine the minimum process time (B_{50}) necessary to sterilize the median package.

$$B_{50} = \bar{f}\,(t' + \log \bar{j} + \log I_o - 2) - 0.42A \qquad [18]$$

3a–Using equation (19), determine a heat rise slope index (f_α) that is representative of the fastest heating package of a population of 1,000.

$$f_\alpha = \bar{f} - 3.275\,(\sigma_f) \qquad [19]$$

3b–Using equation (20), determine F′ equivalent to the F_1 determined in Step 1c.

$$F' = F_1/f_\alpha L \qquad [20]$$

3c–Using Table 1 of Herndon et al. (1968), find t′ corresponding to the F′ determined in Step 3b.

3d–Using equation (21), determine B_{100}.

$$B_{100} = f_\alpha(t' + \log \bar{j} + \log I_0 - 2) - 0.42A \qquad [21]$$

The points B_0, B_{50}, and B_{100}, determined in Steps 1f, 2c, and 3c, can be plotted on a graph similar to Figure 3 (Line #1) and Figure 4. The expected spoilage curve should follow a reverse S-shaped pattern intersecting the three points.

DISCUSSION

Slope index values

Semilogarithmic plots of heat rise data of package thermal centers vary considerably in both their slope index (f) and lag factor (j) values (Fig. 1a). Problems exist in comparing two or more slope index values as each line will have its own origin (I_A). Determination of a mean I_A makes it possible to relate individual lines, but this method (using original slope index values) would show distorted time values when compared to specified temperature differentials (I values). To correct for this time distortion, it was decided to determine from the original plots, the time values ($t_{I=1}$) at the point the curves intersected the I = 1 ordinate. Each $t_{I=1}$ point was then marked on a new plot and lines drawn from them to the common origin I_A (Fig. 1b). The mean slope index ($\bar{f}$) and standard deviation of slope index (σ_f) was determined from the slope index readings on the common origin plot. In other words; the possible process time error introduced by elimination of variance of the lag factors (j values) by the determination of a common origin ($\bar{I}_A$) was presumed to be corrected for by the increase in variance in the adjusted slope index values over the variance of the original slope index values.

Slope index values were found to have a fairly normal distribution in all cases examined, except where the range approached zero. To make accurate computations in this range, the computer was programmed to use a minimum slope index value of 1 min, where in normal distribution it would have been less. This method skewed the expected distribution in a manner similar to that of the actual distribution.

Source and deviation of equations

Equations (6), (7), (15), (16), (17), (18), (20) and 21 are simple transpositions and applications of the basic correction equation of a standard hypothetical process (Herndon et al., 1968) which in turn was derived from the accepted thermal processing relationships developed by earlier workers (Ball, 1923; 1928; Schultz and Olson, 1940; and Ball and Olson, 1957).

Equations (3), (8) and (14) are simple transpositions of accepted basic thermal processing relationships from Ball (1923).

Equation (9) is a simple illustration of decimal reduction using logarithms and thus the derivation of equation (13) as follows:

$$S_g = \log^{-1}(\log S_s - N) \qquad [9]$$

When a segment is made up of only one package, the final spore population (S_g) becomes:

$$S_g = \log^{-1}(\log S_p - N) \qquad [9a]$$

Taking the log of both sides and transposing

$$N = \log S_p - \log S_g \qquad [9b]$$

Assuming that a package is sterile when its spore population is 0.5 or less, then the minimum decimal reduction needed to sterilize a single package would be N_1:

$$N_1 = \log S_p - \log 0.5 \qquad [9c]$$

and by solving for log 0.5, equation (13) is obtained.

$$N_1 = \log S_p + 0.301 \qquad [13]$$

Equation (1), determination of a mean lag factor ($\bar{j}$), was made using the values of the logs of j values instead of the actual j values. It was originally conceived that $\bar{j} = \frac{\Sigma j}{n}$ but since the plots are made on a semi log graph, $\bar{j}$ solved using this equation would show a slight distortion. To be mathematically correct, the average lag factor ($\bar{j}$) was determined as the antilog of the sum of the logs of j values divided by the number of samples).

Equations (2) and (4) are simple applications of the standard equation for determining means from sample observations.

Equation (5) is a simple application of the accepted relationship used to calculate a standard deviation from a sample population (Gauss, 1880).

Equations (12) and (19) are interpolated from a table of standard distribution (Gauss, 1880). The slope indices f_Ω and f_α are predicted at K = ± 3.275 standard deviation from the mean for the probable extremes of a population of 1,000 in a normal distribution. These limits should also work well with a minimum of error for most population sizes that might be considered in process determinations.

To calculate value of K in equations (12) and (19) to be used for the estimated value of the mid area point of an end population segment (slowest or fastest package) of a population of any size, interpolation as described above is usually sufficient. However, in the consideration of K for end segments of extremely large package populations, K should be calculated directly from the equation of the area/K relationship in the normal curve (Gauss, 1880).

$$\int_K^\infty \frac{1}{\sqrt{2\pi}} e^{-x/2} dx = \alpha \qquad [22]$$

To solve for the expected value of K in large package populations, let the area (α) under the curve for various populations (P_t) be as follows:

P_t	α	P_t	α
10^0	5×10^{-1}	10^6	5×10^{-7}
10^1	5×10^{-2}	10^7	5×10^{-8}
10^2	5×10^{-3}	10^8	5×10^{-9}
10^3	5×10^{-4}	10^9	5×10^{-10}
10^4	5×10^{-5}	10^{10}	5×10^{-11}
10^5	5×10^{-6}		

Equation (10) was derived by assuming that all packages in a package population segment had identical slope indices and identical initial spore populations. Therefore, after a given process of equal decimal reductions, the estimated value of a spore being in a package would be the same for each package in the segment. Since a spore or package cannot be a fraction, it was decided that the best method to convert surviving spores (S_g) in a segment into contaminated packages in a segment (P_g) would be equation (10) which assumes maximum distribution of surviving spores among the packages in a segment.

$P_g = S_s$ With limits:

1. $P_g \leqslant P_s$
2. P_g be rounded off to closest whole number (or zero if expected value of $S_g \leqslant 0.5$) [10]

During a later review of this paper by C. R. Stumbo, it was suggested that the conversion of surviving spores in a package population segment into contaminated packages in that segment could better be represented by an adaption of

the Halvorson and Ziegler equation (1932):

$$P_g = P_s - \frac{P_s}{\log^{-1}\left(\frac{S_g}{2.303\,P_s}\right)} \quad [10a]$$

The author rechecked the calculation of B_0, B_{50} and B_{100}, using the manual method described herein substituting equation (10a) for equation (10). Using the calculated slowest, median, and fastest package, the answers were exactly the same as those using equation (10). To determine if there would be any differences for these times using population segments containing more than one package, the calculations were repeated for a package population segment containing the 10 slowest heating packages, a package population segment containing the center 100 packages, and a package population segment containing the 10 fastest heating packages. The B_0 and B_{100} times were exactly the same and the B_{50} time was approximately 2.5% shorter.

The data obtained using equation (10) were slightly closer to actual data taken from the spoilage curve of an inoculated test pack than were data obtained using equation (10a). However, since the differences were so small no conclusion could be made as to which, if either, was the better method.

Method discussion

In practice this author has found it simpler in calculating process times (B_0) or sterilization curves (construction of a reverse S-shaped curve through B_0, B_{50}, and B_{100}) to use the manual method described in this paper instead of the computer program. By use of a complete table of F′, t′ relationships (calculated from the Fortran Program of Herndon et al. (1968), processes or curves can be calculated quickly and easily without having to employ a computer for every test

GLOSSARY OF SYMBOLS

A = Retort come-up time. The elapsed time in minutes from steam-on until retort reaches its set processing temperature (T_1). In some continuous processing retorts, A = 0.

B = Processing time. The elapsed time in minutes at the set processing temperature (T_1). In general practice, processing timing begins when retort temperature reaches T_1.

B_0 = Minimum process time under a specified set of processing conditions which will assure complete sterilization of a food package population. (0.5 spores remaining from an initial total postulated or inoculation spore population).

B_{50} = Process time under a specified set of processing conditions that will sterilize 50% of a specified package population.

B_{100} = Maximum process time under a specified set of processing conditions which will leave 0.5 spores or more (from an initial postulated or inoculated spore population) in every package of a specified package population.

D = Decimal reduction time. The time in minutes at a temperature necessary to destroy 90% of the postulated or inoculated spoilage organisms.

D_{T_b} = Decimal reduction time at base temperature (T_b).

f = Slope index of the straight-line portion of the semilogarithmic plot of a heating curve expressed as the number of minutes required to traverse one logarithmic cycle, or for 1 to decrease by a factor of 10.

$\bar{f}$ = Mean slope index from a sample of slope indices of heat rise curves.

f_α = Slope index postulated for the fastest heating package in a package population of 1,000. $f_\alpha = \bar{f} - 3.275\,(\sigma_f)$

f_Ω = Slope index postulated for the slowest heating package in a package population of 1,000. $f_\Omega = \bar{f} + 3.275\,(\sigma_f)$

f_s = Slope index postulated for the mid-area point of a population segment. (One half the segment area on either side of the value of f_s) $f_s = \bar{f} + K\sigma_f$

$F^z_{T_b}$ = Lethal process. The number of equivalent minutes of sterilization at T_b applied to organisms or enzymes having a slope index of thermal death time equal to z°F.

F_1 = The $F^z_{T_b}$ necessary to achieve N_1 decimal reductions.

F′ = Hypothetical lethal process. The number of equivalent minutes of lethal process accumulated to a specified process time (t′) in a postulated hypothetical process (see definition of hypothetical process and t′).

Hypothetical process = Sterilization-by-heat process postulated by Herndon et al. (1968) to prepare computer tables to show the relationship of temperature difference (I), lethal value (F′) and process time (t′). In this hypothetical process, f = 1 min, j = 1, I_o = 100°F, A = 0 min, and I_L = 0. Tables of hypothetical processes were prepared for a range of z values from 2°F to 100°F. Use of these tables along with two corrective equations allows accurate and speedy calculations of real processes.

I = Temperature difference between the retort holding temperature (T_1) and the thermal center temperature of the package (T). $I = T_1 - T$

I_A = Theoretical origin of a heat rise curve. The temperature difference between the retort holding temperature (T_1) and the hypothetical temperature T_A located by extending the straight line portion of the heating curve to the time ordinate at 0.58A.

$$I_A = \log^{-1}(\log I_y - 0.58A/f)$$

$\bar{I}_A$ = Common origin point at time 0.58A for a sample population of heat rise curves. $\bar{I}_A = (\bar{j})(\bar{I}_o)$

I_L = Temperature difference between base temperature (T_b) and retort holding temperature (T_1). $I_L = T_b - T_1$

I_o = Initial temperature differential between the retort holding temperature (T_1) and the average initial temperature of the food mass in a package (T_o).

$\bar{I}_o$ = Mean I_o for a sample package population.

$$I_o = \Sigma I_o/n$$

I_y = Difference between the retort holding temperature (T_1) and the hypothetical temperature (T_y) located by extending the straight line portion of the heating curve to the initial time ordinate representing "steam-on."

j = Lag factor for a heating curve, used to establish the position of I_A.

$$j = \frac{\log^{-1}(\log I_y - 0.58A/f)}{I_o}$$

$$j = \frac{T_1 - T_A}{T_1 - T_o} = \frac{I_A}{I_o}$$

$\bar{j}$ = Mean lag factor of a sample population of heat rise curves.

$$\bar{j} = \log^{-1}(\Sigma \log j/n)$$

K = Number of standard deviations (σ) from the slope index population mean to the mid-area point of a package population segment.

L = Lethal rate used in correction equations (7), (15), (17) and (20) to relate a hypothetical process when $T_b \neq T_1$ and $I_L = 0$. The equation for L is programmed into the computer method and will be automatically calculated. To determine L for manual calculations use Table 2 of Herndon et al. (1968) or solve by using following equation: $L = \log^{-1}(\bar{I}_L/z)$

N = Number of decimal reductions (D_{T_b}) applied on a spore population in a specified process having $F^z_{T_b}$ lethal value.

$$N = F^z_{T_b}/D_{T_b}$$

N_1 = Number of decimal reductions necessary to reduce a postulated or inoculated spore population in a single package to 0.5 spores.

$N_1 = \log S_p - \log 0.5$ which reduces to $N_1 = \log S_p + 0.301$

n = Number of individuals in a sample population of packages.

P_c = Total number of packages containing surviving spores after a specified lethal process in a package population. $P_c = \Sigma P_g$

P_g = Number of packages containing surviving spores after a specified lethal process in a population segment of packages.

P_s = Number of packages in a package population segment.

P_t = Total number of packages of a package population.

Population Segment = A part or segment of a specified package population (P_t).

S_p = Effective number of spores postulated or inoculated in the thermal center area of a single package (see definition of thermal center). Lethal process studies on spore populations distributed throughout the package were made by Stumbo (1948, 1949, and 1953).

S_g = Number of surviving spores in a package population segment after a specified lethal process.

S_s = Effective number of spores postulated or inoculated into the packages in a population segment containing one or more packages.– $S_s = (P_s)\ (S_p)$. The effective number of spores is assumed to be the population at the thermal center.

T = Temperature of the thermal center of the food mass of a package during a sterilization-by-heat process.

T_o = Average initial temperature of the food mass of a package at the beginning of a sterilization-by-heat process.

T_1 = Retort holding temperature.

T_A = Hypothetical temperature located by extending the straight line portion of the heating curve to the time ordinate at 0.58A.

T_b = Base temperature or reference processing temperature. Unless otherwise specified, $T_b = 250°F$.

T_y = Hypothetical temperature located by extending the straight line portion of the heating curve to the time ordinate at the beginning of process (steam-on).

Thermal Center = That geometric point or region in the food mass of a package which is at any time during the heating part of a process lower in temperature than any other part. The particle(s) of food receiving the least amount of heat. In packages containing conduction foods, the thermal center is a certain volume of food near the geometric center. In packages containing convection foods, the actual thermal center is usually below the geometric center. However, since graduations in temperature in convection foods are relatively small, the lethal process at the thermal center is probably very close to the lethal process achieved throughout the entire package. Therefore, for calculation purposes the thermal center of a convection food package is 100% of the volume of the package.

t' = Hypothetical process time. The number of minutes required to reach a desired hypothetical lethal value F' in the postulated hypothetical process. (See definition of F' and hypothetical process.)

z = Slope index of the thermal death time curve of an organism. The z value is a measure of the effect of a change in temperature on the resistance of an organism or enzyme to the destructive effect of heat and is defined as the number of degrees F required for the thermal death time curve to traverse one log cycle.

σ_f = Standard deviation of slope index. A statistical value which, along with the mean slope index ($\bar{f}$), will define the parameters of a normal distribution of a slope index population.

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THEORETICAL FORMULAS FOR TEMPERATURES IN CANS OF SOLID FOOD AND FOR EVALUATING VARIOUS HEAT PROCESSES

SUMMARY—A general solution is obtained for transient temperature distributions in a finite cylinder by applying several integral transformations to heat conduction equation when it is subjected to time variable surface temperatures. From this general solution, various formulas for temperature distributions are derived for five different surface temperature-processing time relationships. By using these derived formulas, we obtain formulas for these two parameters: slope indices and intercept coefficients of heating or cooling curves of cylindrical cans of conductive food. Expressions are also derived for estimating sterilizing values during a come-up period of the heat process and also during the sinusoidal fluctuation of retort temperature.

INTRODUCTION

THEORETICAL formulas for transient temperature distributions in finite cylinders have been successfully utilized in predicting proper heat processes for canned food (Ball, 1923; Ball and Olson, 1957; Gillespy, 1951; 1953; Hicks, 1951; Stumbo, 1965). These formulas were obtained by solving a heat conduction equation with assumed surface temperatures. The surface temperatures can be approximated with retort temperatures because the coefficient of surface heat transfer is very large during the heat processing (Ball and Olson, 1957). There are many formulas for the heat conduction in finite cylinders in published articles. However, there are only a few available formulas applicable to the heat process estimation (Ball and Olson, 1957; Carslaw and Jaeger, 1959; Gillespy, 1953; Hayakawa and Ball, 1968; 1969; Hicks, 1951). These formulas were obtained by assuming simplified relationships between the surface temperature and processing time. Therefore, they cannot be utilized when there are complex relationships between these two variables. In the present paper, formulas for the transient temperature distributions are derived for various time variable surface temperatures frequently observed in the commercial heat processes.

When the surface temperature of a sample body changes with time, a formula for the temperature distribution can be derived by using Duhamel's theorem (Carslaw and Jaeger, 1959). In the present paper a general solution is derived by applying the finite Hankel, finite Fourier and Laplace transformations. From this general solution, formulas are derived for various time variable surface temperatures.

General solution and formulas for various surface temperatures

To derive the general solution, it was assumed that the initial temperature of the cylinder was uniformly equal to zero and that the cylinder was subjected to time variable surface temperatures.

By applying the finite Hankel, Laplace and Fourier transformations (Kondo, 1959, Mackie, 1965; Scott, 1955; Sneddon, 1951; Tranter, 1956) to the heat conduction equation, we obtained the following solution for the transient temperature distribution in the cylinder. (The derivation of Eq. [1] is given in Appendix B; all symbols used are defined in Appendix A.)

$$\theta = \frac{8}{\pi} \sum_{n=1}^{\infty} \sum_{k=1}^{\infty} \frac{J_0(p_k\rho)}{J_1(p_k)p_k} \cdot \frac{1}{2n-1} \cdot F(B) \cdot \sin(2n-1)\pi\zeta \quad [1]$$

where

$$F(B) = A \cdot L^{-1}\left\{\bar{\theta}_S(S) \cdot \frac{1}{A+S}\right\} \quad [1a]$$

in Eq. [1a], the function $\bar{\theta}_S(S)$ denotes the Laplace transform of $\theta_S(B)$, which represents any time variable surface temperatures. The symbol $L^{-1}\{\ \}$ in this equation represents the inverse Laplace transform of a function placed in $\{\ \}$. By using Eq. [1] and [1a], we can derive equations for the transient temperature, when the formula for the variable surface

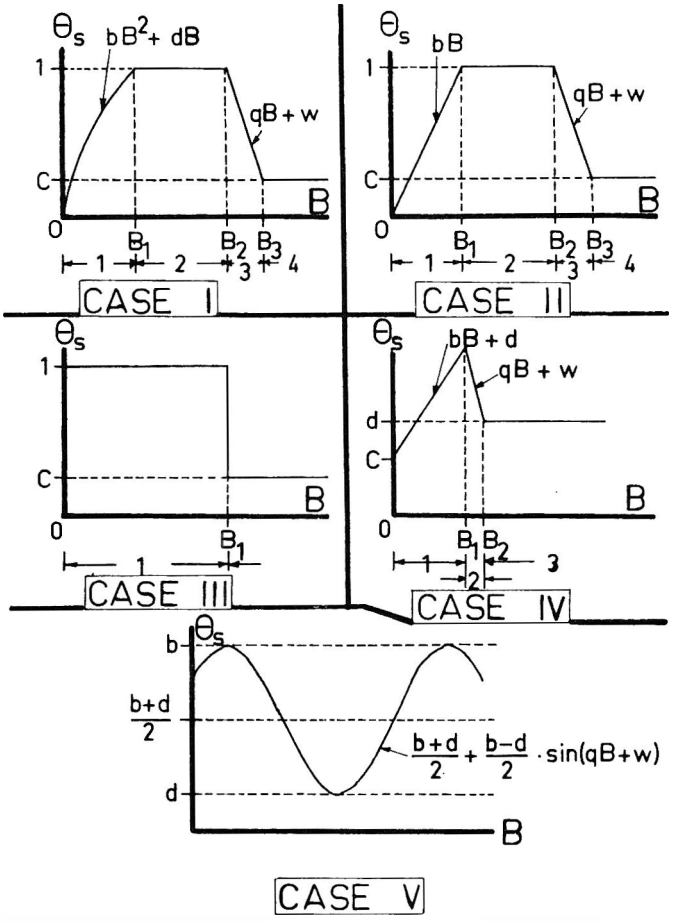

Fig. 1—Surface temperature history curves used to derive formulas for transient temperature distribution in a finite cylinder.

temperature, θ_S (B), has its Laplace transform, $\bar{\theta}_S$ (S), and when this expression, $\bar{\theta}_S$ (S)/(A+S), has its inverse Laplace transform.

Various formulas for the temperature distributions were obtained by assuming five different curves (Fig. 1) which show relationships between the surface temperature and processing time. These relationships were selected because in many commercial heat processings changes in heating or cooling medium temperature are approximated with either one of these curves, according to the present authors' experience in heat processing.

In cases I through III, $\theta_S = 1$ and $\theta_S = C$, respectively, represent the holding temperatures of the heating and cooling mediums. There are the come-up and go-down periods in cases I and II. In case III, it is assumed that there is neither a come-up nor a go-down period. The curve for case IV is considered because a poor manual operation of the retort might produce this curve. When a temperature regulator for the heating medium is poorly adjusted, it is frequently observed that the temperature of heating medium fluctuates sinusoidally. Therefore, case V is considered.

For each case except the last one, a formula is derived for the transient temperature distribution during the come-up, heating, go-down or cooling period whenever it is applicable. Subcases for each case except case V are also shown in Figure 1, with numerals placed under each abscissa. All resultant formulas are given in Table 1.

Application of derived formulas

The derived formulas may be utilized for analyzing the heat transfer characteristics of any cylindrical can of thermally conductive food during heat processes. These characteristics of the food are usually represented with a heating or cooling curve (Ball and Olson, 1957; Stumbo, 1965). A linear portion of this curve is estimated by using these two experimental parameters: a slope index and an intercept coefficient. In the present investigation these two parameters are computed from the derived formulas. In the following discussion, the slope indices of the heating and cooling curves are denoted by f and f_c, respectively, and the intercept coefficients by j and j_c.

The linear portion of each curve is estimated by using a formula for the asymptote, obtained by taking the first term in each summation series. The slope indices for the asymptotes are calculated by the following Equations [2a] and [2b], obtained by utilizing their definitions.

$$f = 1 \Big/ \left\{ -\frac{d}{dt} \log_{10} (T_1 - T) \right\}_{t \to \infty} \quad [2a]$$

$$f_c = 1 \Big/ \left\{ -\frac{d}{dt} \log_{10} (T - T_c) \right\}_{t_c \to \infty} \quad [2b]$$

The intercept coefficients are estimated by the following Equations [3a] and [3b]:

$$j = (T_1 - T_{OA}) / (T_1 - T_0) \quad [3a]$$

$$j_c = (T_{gA} - T_w) / (T_g - T_w) \quad [3b]$$

The formulas for these parameters are obtained for all cases except case V (Table 2). In all the cases examined, the f and f_c values are represented by the following common expression:

$$f = f_c = \frac{2.303}{\{(p_1/a)^2 + (\pi/l)^2\}\, \alpha} \quad [4]$$

Published literature (Ball and Olson, 1957; Stumbo, 1965) shows a formula for the f value exactly the same as Eq. [4]. Eq. [4] clearly indicates the following facts: The slope indices of the asymptote for the heating curve are equal to those for the cooling curve; also, these indices are not affected by the presence of a come-up or go-down period, because there are no constants or parameters for describing these periods in this equation. However, it should be mentioned that this result is applicable only when the coefficient of surface heat transfer is infinite during the heating and cooling phases. In most commercial heat processes, the coefficient for the cooling phase is smaller than that for the heating phase. Therefore, the relationship of Eq. [4] is not applicable to these cases.

Results for the intercept coefficients (j and j_c) are summarized in Table 2. The j value for case III is exactly identical to that in the published literature (Ball and Olson, 1957; Stumbo, 1965). We observe the following facts from this table: The j value is affected by the surface temperatures during the come-up period. The j_c

Table 1–Formulas of transient temperature distributions for various surface temperature history curves.

Case No.	Subcase No.	F1[a]	F2[a]
I	1	$bB^2 + d$	$2b/A^2 - (2bB+d)/A - (2b-d)\exp(AB)/A^2$
	2	1	$[2b\{\exp(B_1A)-1\}/A^2 + \{d-\exp(B_1A)\cdot(2B_1b+d)\}/A] \cdot \exp(-AB)$
	3	$qB+w$	$[2b\{\exp(B_1A)-1\} - A\{\exp(B_1A)\cdot(2B_1b+d)-d\}] \cdot \exp(-AB)/A^2 - q/A + q \cdot \exp\{-A\cdot(B-B_2)\}/A$
	4	C	$[2b\{\exp(B_1A)-1\} - A\{\exp(B_1A) \cdot (2B_1b+d) - d\}] \cdot \exp(-AB)/A^2 + q \cdot \{\exp(B_2A) - \exp(B_3A)\} \cdot \exp(-AB)/A$
II	1	bB	$b\{\exp(-AB)-1\}/A$
	2	1	$b[\exp(-AB) - \exp\{(B-B_1)A\}]/A$
	3	$qB+w$	$[b\cdot\exp(-AB) - B\exp\{-(B-B_1)A\} - q + q \cdot \exp\{-(B-B_2)\}]/A$
	4	C	$[b\cdot\exp(AB) - b\cdot\exp\{-(B-B_1)A\} + q \cdot \exp\{-(B-B_2)A\} - q\cdot\exp\{-(B-B_3)A\}]/A$
III	1	1	$-\exp(-AB)$
	2	C	$-\exp(-AB) + \exp\{-A(B-B_1)\} - C\exp\{-A(B-B_1)\}$
IV	1	$bB+d$	$-d\cdot\exp(-AB) + b\{\exp(-AB)-1\}/A$
	2	$qB+w$	$-d\cdot\exp(-AB) + q[\exp\{-A(B-B_1)\} - 1]/A + b\cdot\exp(-AB)\{1-\exp(AB_1)\}/A$
	3	C	$-d\cdot\exp(-AB) + q\{\exp(AB_1) - \exp(AB_2)\} \cdot \exp(-AB)/A + b\{1-\exp(AB_1)\}\cdot\exp(-AB)/A$
V		$(b+d)/2$	$-(b+d)\cdot\exp(-AB)/2 + \{(b-d)/2\}\cdot[\{A^2 \cdot \sin(qB-w) - Aq\cos(qB+w)\}/(A^2+q^2) + \{Aq \cdot \cos w - A^2\sin w\}]\cdot\exp(-AB)/(A^2+q^2)$

[a]These expressions, F1 and F2, must be placed in the following Eq. [A] to obtain formulas for the temperature distributions, θ, in a finite cylinder.

$$\theta = \boxed{F1} + \frac{8}{\pi} \sum_{n=1}^{\infty} \sum_{k=1}^{\infty} \frac{J_0(p_k\rho)}{J_1(p_k)\, p_k} \cdot \frac{1}{2n-1} \cdot \boxed{F2} \cdot \sin(2n-1)\pi\zeta \quad [A]$$

Table 2—Intercept coefficients of asymptotes for the heating or cooling curve.

Case No.	J1[a]	J2[a]	J3[a]
I	j	1	$2b\{1-\exp(B_1A_{11})\}/A_{11}^2 + [\exp(B_1A_{11})(2B_1b+d)-d]/A_{11}$
	j_c	$(T_1-T_0)/(T_g-T_c)$	$[2b\{\exp(B_1A_{11})-1\}/A_{11}^2-\{\exp(B_1A_{11})\cdot(2B_1b+d)-d\}/A_{11}+q\{\exp(B_2A_{11})-\exp(B_3A_{11})\}/A_{11}\cdot\exp(-A_{11}B_2)$
	j_c	$(T_1-T_0)/(T_g-T_c)$	$q[1-\exp\{A_{11}(B_3-B_2)\}]/A_{11}$, when $\exp(B_1A_{11}) \ll \exp(B_2A_{11})$
II	j	1	$\{\exp(B_1A_{11})-1\}\cdot b/A_{11}$
	j_c	$(T_1-T_0)/(T_g-T_c)$	$[b\{1-\exp(B_1A_{11})\}+q\{\exp(B_2A_{11})-\exp(B_3A_{11})\}]\cdot\exp(-A_{11}B_2)/A_{11}$
	j_c	$(T_1-T_0)/(T_g-T_c)$	$q[1-\exp\{A_{11}(B_3-B_2)\}]/A_{11}$, when $\exp(B_1A_{11}) \ll \exp(B_2A_{11})$
III	j	1	1
	j_c	$(T_1-T_0)/(T_g-T_c)$	$(T_1-T_c)/(T_1-T_0)-\exp(-A_{11}B_1)$
	j_c	$(T_1-T_c)/(T_g-T_c)$	1, when $\exp(-A_{11}B_1) \ll (T_1-T_c)/(T_1-T_0)$

[a]These expressions, J1, J2 and J3, must be placed in the following Eq. [B] to obtain formulas for the intercept coefficients.

$$\boxed{J1} = \boxed{J2}\cdot\frac{8}{\pi}\cdot\frac{J_0(p_1\rho)}{J_1(p_1)p_1}\cdot\boxed{J3}\cdot\sin\pi\zeta \qquad [B]$$

value is affected by the temperatures during the go-down period and also by the come-up period to a lesser degree than the influence of the go-down period. Because the length of the heating phase (B_2) is considerably longer than that of the come-up period (B_1) in most cases, the influence of the come-up period on the j_c value is negligibly small. In case III, the j_c value can be approximated by the j value when $\exp(-A_{11}B_1)$ is negligibly smaller than $(T_1-T_c)/(T_1-T_0)$ and when $(T_1-T_c)/(T_g-T_c)$ is approximately equal to unity.

A sterilizing value of heat treatment during the come-up period is another important parameter to be considered when this period becomes fairly long. Gillespy (1953) presented an analysis for this sterilizing value by using formulas for the heat conduction. Ball (1923) obtained a formula for the sterilizing value by analyzing experimental data. This value can be theoretically estimated from the formulas for case IV – subcase 1 and for case III – subcase 1. A sterilizing value at the center of the food is estimated from the former equation during the come-up period and another sterilizing value at the center is from the latter equation during the heating phase with no come-up period. Then, these two sterilizing values are equated to each other to find the following heating time, B_X, at the holding temperature, T_1. Heating the food for B_X at T_1 without any come-up period results in the sterilizing value identical to the one obtained during the come-up period. The resultant equation is given below.

$$10^{(T_0-T_1)/z}\int_0^{B_1}\phi_1\,dB = \int_0^{B_x}\phi_2\,dB \qquad [5]$$

where ϕ_1 and ϕ_2 are defined as follows:

$$\phi_1 = \exp\left[(\ln 10)\cdot\left\{\frac{T_1-T_0}{z}\right\}\right.$$
$$\cdot\left\langle bB + d + \frac{8}{\pi}\sum_{n=1}^{\infty}\sum_{k=1}^{\infty}\frac{1}{J_1(p_k)p_k}\right.$$
$$\cdot\frac{1}{2n-1}\cdot(-1)^{n+1}\cdot[-d\exp(-AB)$$
$$\left.\left.+\,b\{\exp(-AB)-1\}/A\right]\right\rangle\right] \qquad [5a]$$

$$\phi_2 = \exp\left[(\ln 10)\cdot\left\{-\frac{T_1-T_0}{z}\right.\right.$$
$$\cdot\frac{8}{\pi}\sum_{n=1}^{\infty}\sum_{k=1}^{\infty}\frac{1}{J_1(p_k)p_k}$$
$$\left.\left.\cdot\frac{1}{2n-1}\cdot(-1)^{n+1}\cdot e^{-AB}\right\}\right] \qquad [5b]$$

These formulas suggest that the heating time B_X is a very complicated function of B_1, T_0, T_1 and z, although Ball found the following simple relationship through his experimentation: $0.42\,B_1 = B_X$.

As previously mentioned, Eq. [5] was derived by considering the sterilizing value at the center of the food. Another formula can be derived for estimating the mass average sterilizing value during the come-up period through similar calculations. The resultant formulas are given below.

$$\int_0^1\rho d\rho\int_0^1 d\zeta\cdot\exp\left\{-(\ln 10)G_1/D_{250}\right\} = \int_0^1\rho d\rho\int_0^1 d\zeta\cdot\exp\left\{-(\ln 10)\cdot G_2/D_{250}\right\} \qquad [6]$$

where G_1 and G_2 are defined as follows:

$$G_1 = (l^2/a)\exp\{(T_0-250)/z\}\cdot\int_0^{B_1}\exp\{(\ln 10)(T_1-T_0)\psi_1/z\}\,dB \qquad [6a]$$

$$G_2 = (l^2/a)\exp\left\{(T_1-250)/z\right\}\cdot\int_0^{B_x}\exp\left\{(\ln 10)(T_0-T_1)\psi_2/z\right\}dB \qquad [6b]$$

and where ψ_1 and ψ_2 represent the following expressions.

$$\psi_1 = bB + d + \frac{8}{\pi}\sum_{n=1}^{\infty}\sum_{k=1}^{\infty}\frac{J_0(p_k\rho)}{J_1(p_k)p_k}\cdot\frac{1}{2n-1}\cdot\left\{\frac{b\{\exp(-AB)-1\}}{A} - d\cdot\exp(-AB)\right\}\cdot\sin(2n-1)\pi\zeta \qquad [6c]$$

$$\psi_2 = \frac{8}{\pi}\sum_{n=1}^{\infty}\sum_{k=1}^{\infty}\frac{J_0(p_k\rho)}{J_1(p_k)p_k}\cdot\frac{1}{2n-1}\cdot\exp(-AB)\cdot\sin(2n-1)\pi\zeta \qquad [6d]$$

In this case, the estimation of the heating time, B_X, requires extremely complicated calculations.

When the retort temperature fluctuates sinusoidally during the heating phase, it would be useful to find the following heating treatment at a constant holding temperature, T_X, of the retort. This treatment results in the central sterilizing value equivalent to the value obtained during the sinusoidal temperature treatment. A formula for estimating the temperature, T_X, can be derived from the formulas for III-1 and for V. The result is given below.

$$\exp\{(\ln 10)\cdot T_x/z\}\cdot\int_0^{B_b}\phi_3\,dB = \exp\{(\ln 10)(T_1-T_0)/z\}\cdot\int_0^{B_b}\exp[(\ln 10)\{(T_1-T_0)/z\}\cdot\Gamma]\,dB \qquad [7]$$

where Γ is defined as follows:

$$\Gamma = \frac{8}{\pi} \sum_{n=1}^{\infty} \sum_{k=1}^{\infty} \frac{(-1)^{n+1}}{J_1(p_k)p_k} \cdot \frac{1}{2n-1} \cdot \langle \{(b-d)/2\} \cdot \Big[\{ A^2 \sin(qB-w) - Aq\cos(qB+w) \} / (A^2+q^2) + \{ Aq\cos w - A^2 \sin w \} \cdot \exp(-AB)/(A^2+q^2) \Big] - (b-d) \cdot \exp(-AB)/2 \rangle \quad [7a]$$

An expression for ϕ_3 in Eq. [7] is obtained from Eq. [5b] through this substitution $T_1 = T_X$.

The formulas listed in Table 1 can be utilized in deriving many other equations useful in evaluating the heat process. However, discussion on these derivations is not presented in the present paper because of space limitations.

CONCLUSION

FORMULAS for the transient temperature distribution were derived for various time variable surface temperatures by using the integral transformations. The derived formulas were utilized to obtain expressions for the slope index and intercept coefficient of the linear portion in the heating or cooling curve. The expressions were also derived for estimating the sterilizing value for the come-up period and for estimating the sterilizing value when the retort temperature fluctuated sinusoidally.

Appendix A. Nomenclature

A	$= (p_k/m)^2 + \pi^2 (2n-1)^2$
A_{11}	$= (p_1/m)^2 + \pi^2$
a	Radius of finite cylinder (in.).
B	Fourier number, which is defined as $B = \alpha t/l^2$
B_1, B_2, B_3	Constant B values. They represent B values at which one phase or period terminates in heat processing. For their more detailed definition, see Figure 1.
B_b	$= \alpha t_b/l^2$
B_x	$= \alpha t_x/l^2$
b	Constant. This is used to represent a surface temperature history curve. See Figure 1.
C	Constant θ_s value during cooling phase. When a value for the surface coefficient of heat transfer is very large, C is approximately equal to $(T_c - T_0)/(T_r - T_0)$.
D_{250}	Decimal reduction time of microorganisms or nutrient at 250°F (min).
d	Constant. This is used to represent a surface temperature history curve. See Figure 1.
$F(B)$	Function defined as Eq. [1a].
$F1, F2$	Expressions used in Eq. [A]. See Table 1.
f, f_c	Slope indices for heating and cooling curves, respectively.
G_1, G_2	Defined as Eq. [6a] and [6b], respectively.
$J1, J2, J3$	Expressions used in Eq. [B]. See Table 2.
$J_0(x)$	Value for zeroth order Bessel function of first kind, evaluated at x.
$J_1(p_k)$	Value for first-order Bessel function of first kind, evaluated at p_k.
$J_1(p_1)$	Value for first-order Bessel function of first kind, evaluated at p_1.
j, j_c	Intercept coefficients of heating and cooling curves, respectively.
k	Integer subscript.
l	Height of finite cylinder (in.)
$L^{-1}[\ x\ ]$	Inverse Laplace transfer of x.
m	Shape factor of finite cylinder $m = a/l$.
n	Integer
p	Finite-Hankel-transformed variable of ρ.
p_1	First positive root of $J_0(x) = 0$.
p_k	k^{th} positive root of $J_0(x) = 0$.
q	Constant. This is used to represent a surface temperature history curve. See Figure 1.
r	Radial distance, measured from the central axis of finite cylinder (in.).
S	Laplace–transformed variable of B.
T	Temperature in finite cylinder (°F).
T_0	Initial temperature of finite cylinder (°F).
T_1	Holding temperature of retort during heating phase (°F).
T_{0A}	Phantom initial temperature of finite cylinder, obtained by extrapolating an asymptote for the heating curve (°F).
T_g	Temperature at any location in finite cylinder at end of heating phase (°F).
T_{gA}	Phantom temperature at any location in finite cylinder at end of heating phase, obtained by extrapolating an asymptote for the cooling phase (°F).
T_r	Reference temperature. It is convenient to use the highest temperature of heating medium (°F).
T_s	Surface temperature of finite cylinder (°F).
T_x	Imaginary holding temperature of retort. Heating a finite cylinder for t_b minutes at this temperature results in a sterilizing value at the center of the finite cylinder equal to a sterilizing value obtained when the cylinder is heated with sinusoidally fluctuating temperature for t_b minutes (°F).
t_b	Length of heating phase (min).
t_c	Cooling time variable, measured from the end of heating phase (min).
t_x	Imaginary heating time at holding temperature of retort T_1. Heating a finite cylinder for t_x minutes at T_1 °F results in a sterilizing value equal to a sterilizing value obtained during the come-up period of heating phase (min).
y	Axial distance measured from an end of finite cylinder (in.).
z	Slope index of thermal death time curve of microorganisms or nutrients (°F).
α	Thermal diffusivity of finite cylinder (in.^{2}/min).
Γ	Expression defined by Eq. [7a].
ζ	$= y/l$.
θ	$= (T - T_0)/(T_r - T_0)$, dimensionless temperature of finite cylinder.
θ_s	$= (T_s - T_0)/(T_r - T_0)$, dimensionless surface temperature of finite cylinder.
$\bar{\theta}_s$	Laplace transform of θ_s.
$\bar{\theta}$	Finite Hankel transform of θ.
$\bar{\bar{\theta}}$	Laplace transform of $\bar{\theta}$.
$\bar{\bar{\bar{\theta}}}$	Finite Fourier sine transform of $\bar{\bar{\theta}}$
ρ	$= r/a$
ϕ_1, ϕ_2	Expressions respectively defined as Eq. [5a] and [5b].
ϕ_3	Expression derived from Eq. [5b] through this substitution: $T_1 = T_X$.
ψ_1, ψ_2	Expressions respectively defined as Eq. [6c] and [6d].

Appendix B. Derivation of generalized solution

In the present paper, the following assumptions are imposed: i. The thermal diffusivity of sample is constant; ii. there is no heat-generation in the sample; iii. the temperature distribution in the sample is axially symmetrical. Under these assumptions, a partial differential equation for transient thermal conduction is expressed by Eq. [a].

$$m^2 \cdot \frac{\partial\theta}{\partial B} = \frac{\partial^2\theta}{\partial\rho^2} + \frac{1}{\rho} \cdot \frac{\partial\theta}{\partial\rho} + m^2 \cdot \frac{\partial^2\theta}{\partial\zeta^2} \quad [a]$$

Eq. [a] will be solved with the following initial and boundary conditions.

$$\theta(o, \zeta, \rho) = 0 \quad [a\text{-}1]$$

$$\theta_S(B) = \theta(B, \zeta, 1) = \theta(B, o, \rho) = \theta(B, 1, \rho) \quad [a\text{-}2]$$

$$o \leqslant \theta\,(B, \zeta, \rho) < \infty \quad [a\text{-}3]$$

Eq. [a] becomes the following Eq. [b] when the finite Hankel transform is applied to the variable ρ.

$$m^2 \cdot \frac{\partial \bar{\theta}}{\partial B} = p \cdot \theta_S\,(B) \cdot J_1\,(p) - p^2\,\bar{\theta} + m^2 \cdot \frac{\partial^2 \bar{\theta}}{\partial \zeta^2} \quad [b]$$

In the derivation of Eq. [b], the boundary condition $\theta\,(B, \zeta, 1) = \theta_S\,(B)$ was used. Thus, the other conditions which will be imposed on Eq. [b] are:

$$\bar{\theta}\,(o, \zeta, p) = 0 \quad [b\text{-}1]$$

$$\bar{\theta}\,(B, o, p) = \bar{\theta}\,(B, 1, p) = \frac{\theta_S\,(B)}{p} \cdot J_1\,(p) \quad [b\text{-}2]$$

$$\bar{\theta}\,(B, \zeta, p) < \infty \quad [b\text{-}3]$$

By applying the Laplace transform to Eq. [b], with respect to the variable B, we obtain Eq. [c].

$$m^2 S\,\bar{\bar{\theta}} = p\,J_1\,(p)\,\bar{\theta}_S\,(S) - p^2\,\bar{\bar{\theta}} + m^2 \frac{d^2\,\bar{\bar{\theta}}}{d\,\zeta^2} \quad [c]$$

The boundary conditions, which should be applied to Eq. [c], are:

$$\bar{\bar{\theta}}\,(S, o, p) = \bar{\bar{\theta}}\,(S, 1, p) = \frac{\bar{\theta}_S\,(S)}{p} \cdot J_1\,(p) \quad [d]$$

Eq. [d] becomes Eq. [e] by applying the finite Fourier sine transform (7) with respect to the variable ζ.

$$n \cdot \pi \cdot m^2 \cdot \bar{\theta}_S\,(S) \cdot \frac{J_1\,(p)}{p}\left\{(-1)^{n+1} + 1\right\} - n^2 \cdot m^2 \cdot \pi^2 \cdot \bar{\bar{\bar{\theta}}} - (p^2 + m^2\,S)\,\bar{\bar{\bar{\theta}}} + p \cdot J_1\,(p) \cdot \bar{\theta}_S\,(S) \cdot \frac{1}{\pi n} \cdot \left\{1 + (-1)^{n+1}\right\} = 0 \quad [e]$$

Therefore, we have Eq. [f].

$$\bar{\bar{\bar{\theta}}} = n \cdot \pi \cdot \frac{J_1\,(p)}{p} \cdot \left\{(-1)^{n+1} + 1\right\} \cdot \left\{m^2 + \frac{p^2}{\pi^2 n^2}\right\} \cdot \bar{\theta}_S\,(S) \cdot \left\{1/\{m^2(n^2 + S) + S^2\}\right\}$$

The solution for Eq. [a] is obtained by applying the inverse finite Fourier sine, the inverse Laplace and the inverse finite Hankel transforms one after another in the order named. The resultant solution is given in Eq. [g].

$$\theta = \frac{8}{\pi} \sum_{n=1}^{\infty} \sum_{k=1}^{\infty} \frac{J_o\,(p_k \rho)}{J_1\,(p_k) p_k} \cdot \frac{A}{2n-1} \cdot L^{-1}\left\{\bar{\theta}_S\,(S) \cdot \frac{1}{A + S}\right\} \cdot \sin\,(2n-1)\,\pi\zeta = \frac{8}{\pi} \sum_{n=1}^{\infty} \sum_{k=1}^{\infty} \frac{J_o(p_k \rho)}{J_1\,(p_k)\,p_k} \cdot \frac{1}{2n-1} \cdot F\,(B) \cdot \sin\,(2n-1)\,\pi\zeta \quad [g]$$

where

$$F(B) = A \cdot L^{-1}\left\{\bar{\theta}_S(S) \cdot \frac{1}{A + S}\right\} \quad [g\text{-}1]$$

The function F (B) will be called the surface temperature function because it represents the time variable surface temperature. The temperature distribution in a finite cylinder can be obtained for any θ_S (B) by using Eq. [g], provided that θ_S (B) has the Laplace transform, $\bar{\theta}_S$ (S), and that $\bar{\theta}_S$ (S)/(A + S) has the inverse Laplace transform.

In using Eq. [g], the following Eq. [h] is useful to simplify the derived formulas.

$$1 = \frac{8}{\pi} \sum_{n=1}^{\infty} \sum_{k=1}^{\infty} \frac{J_o(p_k \rho)}{p_k J_1\,(p_k)} \cdot \frac{A}{2n-1} \cdot \sin\,(2n-1)\,\pi\zeta \quad [h]$$

This formula is obtained by applying the finite Fourier sine transform and the finite Hankel transform to numeral 1.

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EFFECTIVENESS OF PNEUMATIC CONVEYING SYSTEMS FOR COOLING SPRAY-DRIED FOOD PRODUCTS

SUMMARY—The cooling effectiveness during pneumatic transport of a spray-dried food product has been investigated. Differential equations which describe the product and air temperatures as a function of distance from the initial mixing point have been derived and solved. The predicted results have been compared to experimental data obtained in a conveying tube equipped with thermocouples. The results indicated the equilibrium temperature of the air-product mixture could be predicted and was a function of loading ratio. Increased loading ratio decreased the distance required to reach the equilibrium temperature. The effectiveness of product cooling after reaching the equilibrium temperature was a function of conditions at the conveying tube wall. Results indicated that a water spray over the exterior surface was more effective than forced-air circulation which, in turn, was more effective than natural air circulation.

INTRODUCTION

THE TYPE of cooling system used for cooling of spray-dried food products will vary considerably depending on facilities available. In most cases, the cooling is accomplished by mixing the dry product with room temperature or cool air followed by conveying through a tube to the separators. Use of room-temperature air and long conveying tubes is normally adequate to reduce the product temperature to acceptable levels unless the ambient temperatures are too high. This situation does occur during the summer season in many parts of the United States and very frequently in countries with warm climates.

The dry product cooling problem is further complicated by the concern for providing purified air as a conveying medium. Recent encounters with *Salmonellae* contamination have emphasized the need for more effective control over product-air contact. Purified air can be provided by high-efficiency filters. However, large volumes are required as a conveying medium and power requirements become rather large. Through supplemental cooling methods, the power requirements for conveying and cooling of the dry food products may be kept to a minimum.

Objectives of this investigation were to a) investigate the mechanisms of product cooling during initial mixing as product and air temperature come to equilibrium and b) determine the effectiveness of supplemental cooling of the conveying tube wall on product cooling. The first objective was accomplished by performing an enthalpy balance on a section of conveying tube and solving the equations for air and product temperature as a function of distance. Preliminary verification was accomplished by conducting experiments in an insulated conveying tube. At locations beyond the equilibration region, the influence of forced-air movement and water spray over exterior portions of the conveying tube has been investigated.

There is an obvious lack of information in the literature on the effectiveness of cooling methods for powdered materials, although fluidized systems have been investigated to a considerable extent (Depew and Farbar, 1963; Soo, 1967; Soo et al. 1960; Tien, 1961; Wilkinson and Norman, 1967). In addition, there seems to be no published data which indicate the optimum storage temperatures for dry food products. Hanrahan et al. (1967) have made reference to slow cooling resulting in a cooked or scorched flavor in spray-dried milk, and indicated that sinkability of dry milk decreased significantly when the product was stored at 20°C rather than cooled to 5°C. The only logical conclusion is that maximum quality is attained only by effective cooling before the product reaches the storage container. Results by Farrall et al. (1968) and Chen (1969) indicate that thermal conductivities of dry food powders are sufficiently low to prevent rapid cooling of the product in the storage container.

Theoretical considerations

Cooling of dry food particles by cool air during pneumatic flow occurs in 2 steps a) a region immediately after the product is released into the air stream during which the product and air come to some equilibrium temperature and b) the region after the equilibration, during which the product and air cool by heat transfer to the walls of the conveying tube.

The following analysis will deal with the equilibration region, assuming adiabatic conditions exist at the conveying tube walls. By derivation of equations which describe both the enthalpy content of the air and the product as it moves through the conveying tube (Fig. 1), the change in temperature of product and air can be predicted, based on properties of product and air.

Symbols

A	=	particle surface area per unit volume of product-air mixture
C	=	specific heat
D	=	diameter
h	=	convective heat transfer coefficient
k	=	thermal conductivity
L.R.	=	loading ratio, lb product per lb air
q	=	heat flux
S	=	cross-sectional area of tube
T	=	cooling medium temperature
V	=	velocity
W	=	weigh density of powder per unit volume; Equation [19].
x	=	axial distance in tube
Pr	=	Prandtl number = $\frac{C_a \mu_a}{k_a}$
Nu	=	Nusselt number = $\frac{h_p D_p}{k_a}$

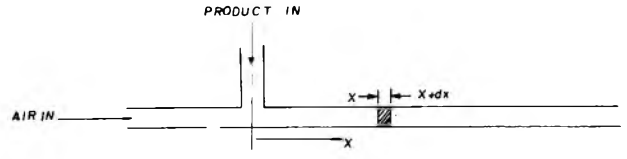

Fig. 1—Schematic diagram.

Re = Reynolds number for tube = $\frac{\rho_a D V_a}{\mu_a}$

$(Re)_d$ = Modified particle Reynolds number = $\frac{V_a D_p}{\nu}$

Re_p = particle Reynolds number = $\frac{\rho_a D_p (V_a - V_p)}{\mu_a}$

St = Stanton number defined in Equation [6]

B_1 = dimensionless number defined in Equation [6]

B_2 = dimensionless number defined in Equation [7].

θ = product temperature

ρ = density

μ = viscosity

Subscripts

a = air

p = product

o = initial conditions

w = wall conditions

Enthalpy balance on air

Using a control volume within the conveying tube as a reference, the enthalpy difference between x and x + dx would be:

$$V_a S \rho_a C_a (T + \frac{dT}{dx}) dx - V_a S \rho_a C_a T = V_a S \rho_a C_a \frac{dT}{dx} dx \quad [1]$$

Since the process being considered is adiabatic, heat transfer will occur only between the particles and air as described by the following equation:

$$q = - h_p A S (T - \theta) dx \quad [2]$$

The right-hand sides of Equations [1] and [2] can be equated to obtain the following differential equation:

$$\frac{dT}{dx} + \frac{h_p A}{V_a \rho_a C_a} (T - \theta) = 0 \quad [3]$$

which describes the temperature of the air during the equilibration region.

Enthalpy balance on products

Using an approach similar to the enthalpy balance on air, the enthalpy of the product will change some amount as it passes through the control volume as described by the following expression:

$$V_p \rho_p C_p S (\theta + \frac{d\theta}{dx}) dx - V_p \rho_p C_p S \theta = V_p \rho_p C_p S \frac{d\theta}{dx} dx \quad [4]$$

By equating the right-hand sides of Equations [2] and [4], the differential equation describing product particle temperature is obtained:

$$\frac{d\theta}{dx} = \frac{h_p A}{V_p \rho_p C_p} (T - \theta) \quad [5]$$

Solutions for differential equations

Equations [3] and [5] can be solved by methods normally used for solving ordinary differential equations.

By letting:

$$B_1 = \frac{h_p A}{V_a \rho_a C_a} = \frac{\frac{h_p D_p}{k_a} A}{\frac{V_a D_p}{\nu} \cdot \frac{C_a \mu}{k_a}} = \frac{(Nu)_d A}{(Re)_d Pr} = (St)_d A \quad [6]$$

$$B_2 = \frac{h_p A}{V_p \rho_p C_p} = \frac{\frac{h_p D_p}{k_a} A}{\frac{V_a D_p}{\nu} \cdot \frac{C_a \mu}{k_a} \cdot \frac{V_p \rho_p C_p}{V_a \rho_a C_p}} = \frac{(Nu)_d A}{(Re)_d Pr W} = \frac{(St)_d A}{W} \quad [7]$$

then: $$\frac{dT}{dx} + B_1 (T - \theta) = 0 \quad [8]$$

$$\frac{d\theta}{dx} = B_2 (T - \theta) \quad [9]$$

or: $$T = \frac{1}{B_2} \frac{d\theta}{dx} + \theta \quad [10]$$

By substitution of Equation [10] into [8], a new differential equation is obtained:

$$\frac{1}{B_2} \frac{d^2\theta}{dx^2} + (1 + \frac{B_1}{B_2}) \frac{d\theta}{dx} = 0 \quad [11]$$

Equation [11] can be solved to obtain:

$$\theta = \frac{1}{1 + w} \left\{ (w \theta_o + T_o) + (T_o - \theta_o) \exp \left[- B_1 (1 + \frac{1}{w}) x \right] \right\} \quad [12]$$

where:

$$w = \frac{B_1}{B_2} \quad [13]$$

while:

$$T = \frac{1}{1 + w} \left\{ (w \theta_o + T_o) + w (T_o - \theta_o) \exp \left[- B_1 (1 + \frac{1}{w}) x \right] \right\} \quad [14]$$

Equations [12] and [14] can be written in dimensionless form as follows:

$$\frac{\theta - \theta_o}{T_o - \theta_o} = \frac{1}{1 + w} \left\{ 1 - \exp \left[-(St)_d (1 + \frac{1}{w}) Ax \right] \right\} \quad [15]$$

and:

$$\frac{T - T_o}{\theta_o - T_o} = \frac{w}{1 + w} \left\{ 1 - \exp \left[- (St)_d (1 + \frac{1}{w}) Ax \right] \right\} \quad [16]$$

Application of derived equations

Equations [15] and [16] allow prediction of the air and product temperatures as a function of distance during pneumatic flow from the heat transfer coefficient between particle and air (h_p), particle surface area per unit volume (A), velocities of air and particles (V_a, V_p), density of air and particles (ρ_a, p_p) and specific heats of air and particles (C_a, C_p). Probably the most difficult of these parameters to measure is the heat transfer coefficients between particle and air. An empirical expression has been reported by Rowe et al. (1965) as follows:

$$Nu = 2 + b (Re_p)^a (Pr)^{0.33} \quad [17]$$

where:

b = 0.459 for air

a = 0.4 at Re_p = 1 and 0.6 at $Re_p = 10^4$

To use Equation [17] the particle Reynolds number (Re_p) must be computed which, in turn, is dependent on relative velocity between particle and air. The Hinkel equation for computing this relative velocity was reported by Zenz (1960):

$$\frac{V_p}{V_a} = 1 - 0.179 D_p^{0.3} \rho_p^{0.5} \quad [18]$$

Assumptions and limitations

The assumptions in the above derivation are as follows: The temperature range is sufficiently narrow that air and product properties do not vary significantly. The temperature differences are small enough to make radiation heat transfer insignificant. Temperature gradients do not exist within particles due to small size, and mass transfer does not occur due to low-moisture contents.

One potential limitation would be failure to consider heat transfer between particles and conveying tube walls. However, Farbar and Morley (1957) indicated this contribution should be very small due to small contact area between particle and wall, and short contact time. Probably the more significant limitations are related to predicting the heat transfer coefficient between particle and air. Use of Equation [18] for predicting relative velocity between particle and air assumes that the value is constant throughout the equilibration region. Actually, the particle may be accelerating in velocity during a considerable portion of the equilibration region. In addition, Equation [17] assumes the particles are well dispersed, which is not the situation during the early portions of the temperature equilibration between product and air.

EXPERIMENTAL

TWO TYPES of experiments were conducted. The region of flow which includes the equilibration of product and air temperature has been investigated under adiabatic conditions. The region of cooling after product and air have equilibrated has been investigated under three different surface-cooling conditions.

Experimental apparatus

The equipment used is shown schematically in Figure 2. It consisted of a section of 2-in.-

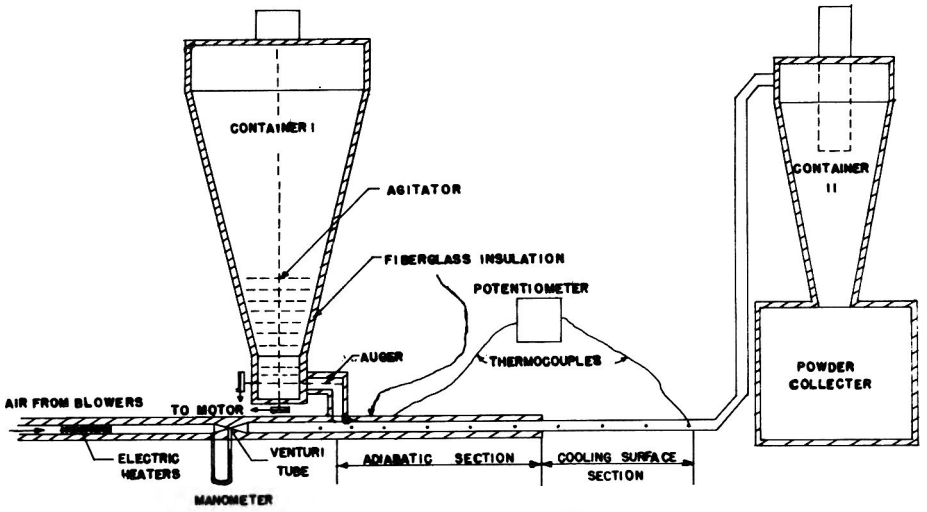

Fig. 2–Diagram of experimental apparatus.

diameter tubing into which a dry food powder could be metered and conveyed pneumatically to a powder collector.

A stainless steel powder collector (I) was used as a source of product. An agitator which turned around a vertical axis and a 2-in.-diameter auger were added to the container. Air was drawn from the room at flow rates measured with a venturitube and U-tube manometer.

Immediately downstream from the location of product injection, an 8-ft section of 2-in. (id) plexiglass tubing was insulated to provide the adiabatic conditions for product-air equilibration. The section of tubing used for testing the cooling effectiveness was connected to the adiabatic section and consisted of a 5-ft section of 2-in. (id) aluminum tubing. Temperature measurements in the tube were made at either 6-in. or 1-ft intervals, depending on location, using copper constantan thermocouples and 12-point recording potentiometer. The temperature sensors, located 0.75-in. from the bottom surface of the tube, indicated the mean temperature of the 2-phase flow. In addition, thermocouples were located in the product container and upstream from the site of product injection to provide initial product and air temperatures, respectively.

Product characteristics

Nonfat dry milk was used in all experiments. The mean particle diameter was assumed to be 60 μ, based on results reported by Hayashi et al. (1968) for products manufactured by the same procedures as in this investigation. A particle density of 1.46 was used, based on data by Hall and Hedrick (1966). A specific heat of 0.36 Btu/lb-°F was predicted, based on product composition and verified by experimental determination in a Differential Thermal Analyzer. This heat transfer surface area was computed by a method proposed by Zenz (1960) as:

$$A = \frac{6W}{\rho_p \, D_p} \qquad [19]$$

where W is the weight density per unit volume of fluidized power in air.

Test procedures

A typical experiment involved an initial heating of all powder to between 115 and 140°F. During experiments dealing with mixing of product and air resulting in equilibration of temperature in an adiabatic section of tubing, loading ratios (L.R.) of 0.54, 0.78 and 1.17 were utilized.

The effectiveness of various surface cooling methods was investigated in the tubing immediately downstream from the equilibration section. These tests were conducted using a loading ratio of 0.73 and three different surface-cooling methods: a) natural air circulation, b) forced-air circulation and c) water spray. The procedure involved the measurement of the air-product mixture temperatures at 0.5-ft intervals throughout the test section.

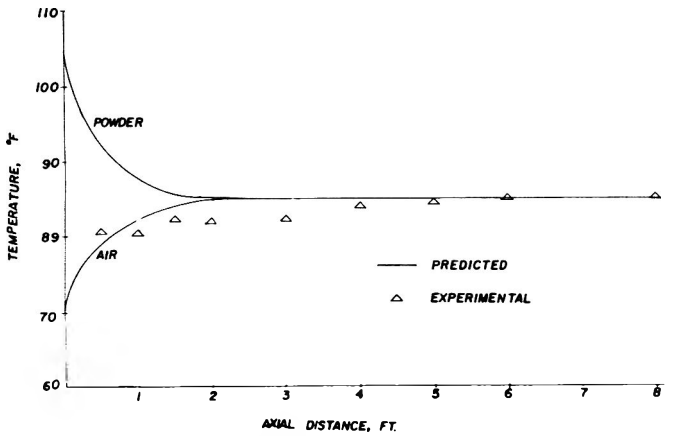

Fig. 3–Comparison of predicted and experimental temperatures during equilibrium with adiabatic conditions at wall.

RESULTS & DISCUSSION

TO ensure that temperature values ob-

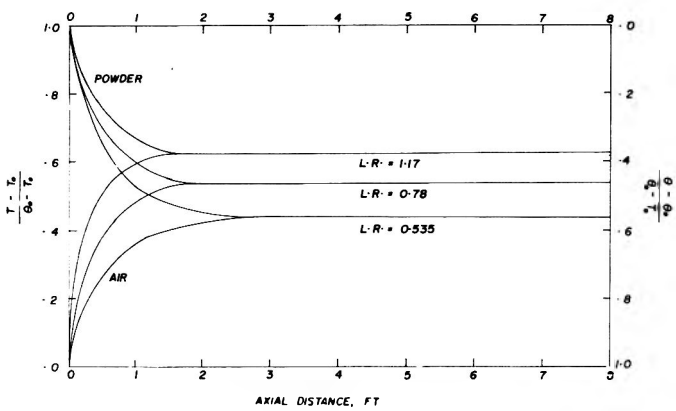

Fig. 4–Influence of product loading ratio on equilibration temperature and distance required to reach equilibration.

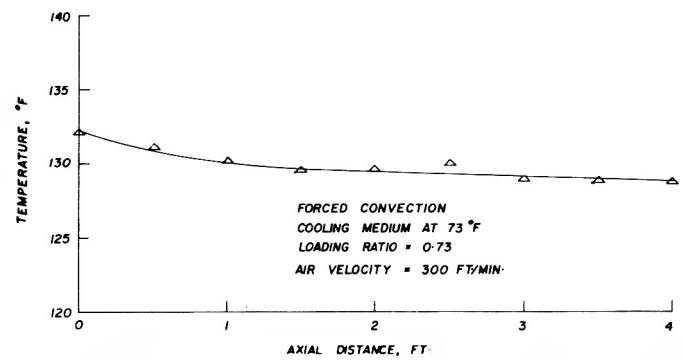

Fig. 5–Effectiveness of forced-air circulation on cooling product-air mixture after equilibration.

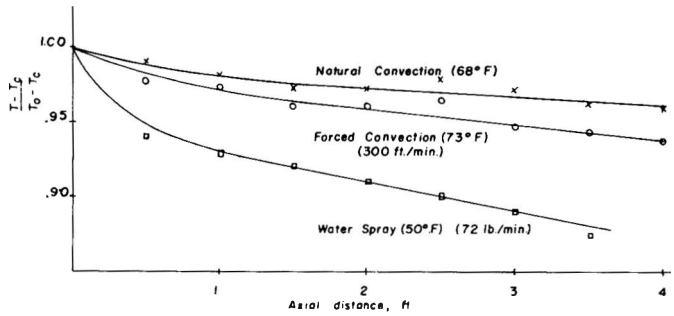

Fig. 6—Comparison of various conditions at the cooling surface on cooling effectiveness.

tained from measurements in the air-product mixture represented a mean value for conditions in the tube the temperature profile was measured. These measurements were accomplished by using 4 thermocouples, equally spaced in the 2-in.-diameter tube. Since the product particles were concentrated in the lower portion of the tube, it was reasonable to expect (Wen and Simons, 1959) the mean of the temperature distribution to be below the tube center. Measurements of temperature distribution indicated that a thermocouple located 0.75 in. from the bottom of tube would provide an acceptable prediction of the mean temperature.

Temperature equilibration region

The solid curves in Figure 3 indicate agreement between predictions from Equations [12] and [14] and experimental data obtained by the described methods. These results indicate the agreement for data collected at a loading ratio of 0.535 with each experimental point representing a mean of at least 10 temperature values. The initial product temperature and initial air temperature were 105 and 70°F, respectively, and came to equilibrium at approximately 85°F.

The experimental data tend to be lower than the predicted curves and longer distance is required to reach the predicted equilibration temperature. This is probably related to limitations of the prediction equations. The possibilities that relative velocity between particle and air is not constant and that the product powder is not well dispersed during early portions of the equilibration region would contribute to the type of experimental results obtained.

Figure 4 illustrates the influence of different loading ratios. Equations [15] and [16] were used as the prediction equations in generating the curves. As the loading ratio increased, the equilibrium temperature increased. This is as expected, since the larger amount of warm product in the tube will increase the product-air mixture temperature.

Interestingly, equilibration distance decreases with an increase in loading ratio (Fig. 4). Physically, this means that as larger amounts of product are released into the air flowing in the tube, shorter distance is required for the product and air to reach some equilibration temperature. The equilibration occurs at temperatures closer to the product temperature and, in the limit, equilibration would occur instantaneously at the initial product temperature for very high loading ratios. At very low loading ratios, equilibration would require infinitely long distances at temperatures near the initial air temperature. The significance of equilibration temperature is that the extent of cooling during this portion of conveying is known and can be utilized in the cooling system design.

Effectiveness of various cooling surfaces

By exposing the surface of the tube to various conditions, different rates of product cooling below the equilibration temperature can be achieved. Experiments were conducted to compare natural circulation of air, forced circulation of air and spraying of cool water over the exterior surface of the conveying tube with a loading ratio of 0.73 lb product per lb air.

The results (Fig. 5) illustrate the extent of product-air mixture cooling which occurs in a 4-ft distance. The cooling medium (air) was 73°F and was flowing at a free stream velocity at 300 ft/min. Each experimental point represents the mean of 10 temperatures recorded during the experiment.

To compare the results of the three cooling methods, a dimensionless temperature ratio $\frac{T - T_c}{\bar{T}_o - T_c}$ was used. Results in Figure 6 illustrate the effectiveness of the three methods. The water spray provides the most effective cooling of the product-air mixture, while forced-circulation air flow over the conveying tube is more effective than natural circulation. In the 4-ft distance investigated, water spray reduced the product-air mixture temperature by 12.5% of the initial temperature difference between the mixture and cooling medium. Force circulation and natural circulation reduced temperature by about 6 and 4%, respectively.

CONCLUSIONS

THE PRODUCT and air temperatures which exist during the equilibration region of pneumatic flow can be predicted with reasonable accuracy using equations derived from enthalpy balance.

An increase in loading ratio increases the equilibration temperature of the product-air mixture and reduces the distance required to reach equilibration.

Conditions at the conveying tube exterior surface influence cooling effectiveness significantly. A water spray over the exterior surface is the most effective of methods investigated, while forced air circulation is more effective than natural circulation of air.

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THE CENTRIFUGAL FLUIDIZED BED. 2. Drying Studies on Piece-form Foods

SUMMARY—Drying food pieces in a centrifugal fluidized bed with relatively high air flows may be self-limiting, even when only partial drying is desired. Rate increases obtained in early stages of drying may be more than offset through rate-retarding effects of a skin-like layer of collapsed surface tissue which forms on the pieces. This skin layer becomes increasingly resistant to heat and moisture transfer. Rate data and piece temperature patterns for potato, apple and carrot confirm visual observations that skin barriers 0.2–1.0 mm thick form in the first 8–10 min of drying with high air velocities and moderate temperatures. When air temperatures were 250°F with velocities of 15 fps, 1.2-cm cubes of potato could be blanched in only 6 min, suggesting a procedure for blanching some types of food products without leaching.

INTRODUCTION

A RATIONAL drying theory has not yet been developed for piece-form foods because the properties which govern their drying rates have not been identified or have resisted quantitative characterization. Empirical correlations (Van Arsdel and Copley, 1963) have served in the design of drying equipment and in the control of drying operations for some commodities. Several investigators (Coates and Pressburg, 1961; Saravacos and Charm, 1962; Dickerson and Read, 1968; and Harmathy, 1969) have introduced concepts to describe some of the observed drying phenomena, but a satisfactory model system has yet to be devised.

Fruit or vegetable tissues consist of cellular microsystems existing side by side, usually anisotropically. Drying operations often accentuate heterogeneity in resistances to heat and mass transfer by denaturing proteins, gelling starches, inverting sugars, modifying hydrates and causing other less understood actions and interactions. It is indeed an imposing task to characterize objectively the progressive changes in tissue that occur during drying, and to relate them to the effects on drying rates. This is particularly true in studies of the high-speed drying action of the centrifugal fluidized bed dryer (CFB dryer) (Farkas et al., 1969). Air velocities can be used in this unit in a range higher than the transport velocities of piece-form foods under normal gravitational fields. High air velocity maximizes surface transfer of heat and mass, so that internal factors can be readily assessed. Boulos and Pei (1969) investigated heat and mass transfer of a single sphere in a turbulent air stream and concluded that the presence of mass transfer at a given Reynolds number enhances the effect on heat transfer. This is in agreement with Clamen and Gauvin (1968), who concluded that interfacial velocity as well as turbulence affects the over-all heat transfer phenomenon. However, under constant external conditions, factors other than interfacial coefficients must be responsible for major reductions in drying rates. In food drying, internal resistances are clearly suspect, particularly the changing surface layers of the tissue, which form skins in high-rate drying environments.

Sykes and Kelly (1969) studied the kinetics of drying 1 by 1 by 1/2-in. apple pieces and found no constant rate period except under very slow drying conditions. They concluded that there were two falling-rate periods during most of their tests and observed that the surfaces of the apple pieces were very dry after the initial falling-rate period. From these measurements and temperature-gradient measurements, they estimated that a "skin" about 0.5 mm thick had formed and presented a barrier to evaporation.

Normally, more heat can be transferred to a piece during impeded drying than can be utilized as latent heat. The surplus appears as sensible heat and the piece temperature rises. This causes a reduction in the temperature difference between the piece and the heating medium and, consequently, the rate of heat flow is reduced. Some compensation should be obtained by increased piece temperature, which causes higher partial pressure of moisture and higher thermal diffusivity (Wadsworth and Spadero, 1969). However, an increasing rate was not observed, indicating the domination of internal resistances. The type of internal mechanism controlling the drying rate can often be predicted from the appearance of the piece during the drying (Fig. 1).

Our interest in the potential of the centrifugal fluidized bed dryer for preparing intermediate moisture foods (2- or 3-fold strength) led us to study the behavior of food pieces (potato, apple and carrot) exposed to various high-rate drying environments. Our investigations were designed to show the drying characteristics of real systems under select conditions, to gain an insight into the mechanisms involved during the early phases of high-rate drying when food pieces shrink and skin layers form. These situations deviate from the classical constant-rate and falling-rate cases described by Saravacos and Charm (1962) and others, for drying food pieces. Critical

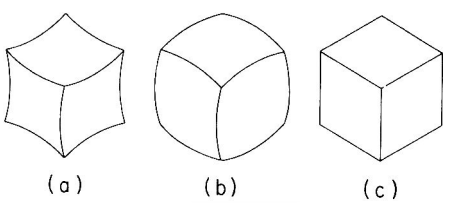

Fig. 1—Shape of a cube of food during drying. (a) Heat transfer controlled by skin layer. (b) Mass transfer controlled by skin layer. (c) Mass transfer and heat transfer balanced.

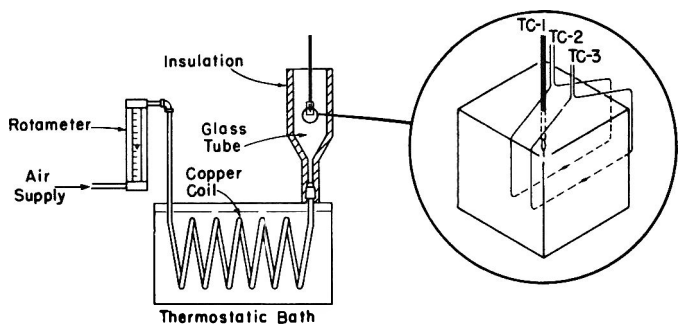

Fig. 2—Bench dryer for individual pieces (schematic). TC-1, center thermocouples; TC-2, midpoint thermocouple; TC-3, outside thermocouple.

moisture content (moisture content dividing constant-rate and falling-rate periods) is indeterminate because typical constant-rate periods are essentially nonexistent for common sizes of piece-form foods dried under the conditions of the CFB dryer.

EXPERIMENTAL

2 EXPERIMENTAL test units were used. 1 was a bench unit (Fig. 2) for preliminary studies on single pieces, consisting of an insulated airflow chamber made from a vertical Pyrex tube, 1 3/8 in. id and 10 in. long. Ambient air was heated to 125–175°F as it passed through a copper coil immersed in a thermostatically controlled bath and was blown up through the Pyrex tube at velocities up to 12 ft per sec. Food pieces were impaled on a 24 Brown and Sharpe gauge copper-constantan (C.C.) thermocouple ground to a needlepoint, with the junction positioned at the geometric center of the piece (Fig. 2). A second piece was impaled on the same thermocouple above the test piece, to dry simultaneously and minimize conduction error. The test piece was carefully weighed and suspended on its thermocouple support in the tube on the tube axis and weighed again after drying. Weight loss by evaporation during weighing was insignificant.

For temperature-gradient measurement, 2 more thermocouples (butt-welded 30 B. & S. gauge C.C.) were placed as shown in Figure 2 by threading through the piece with a fine sewing needle.

The second test unit was a pilot plant centrifugal fluidized bed dryer (shown in Fig. 3

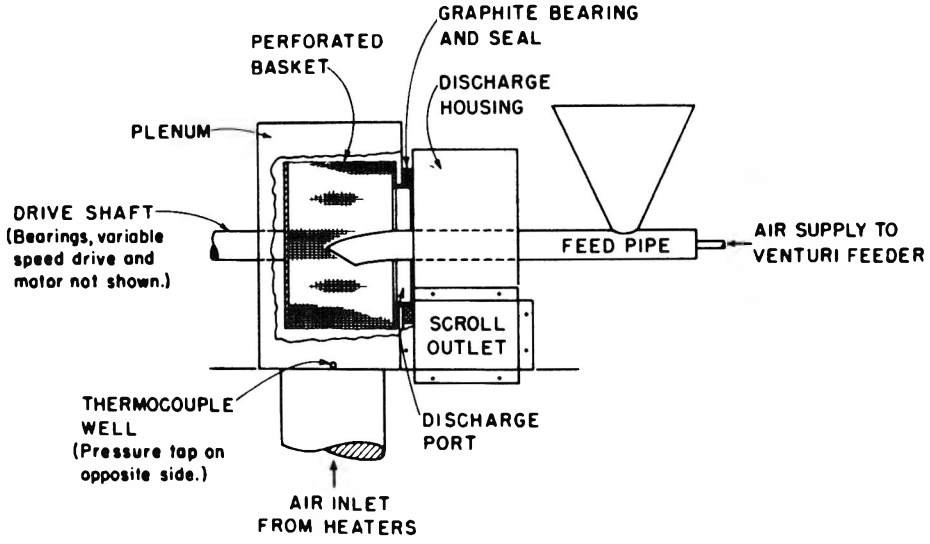

Fig. 3—Centrifugal fluidized bed dryer (Farkas et al., 1969).

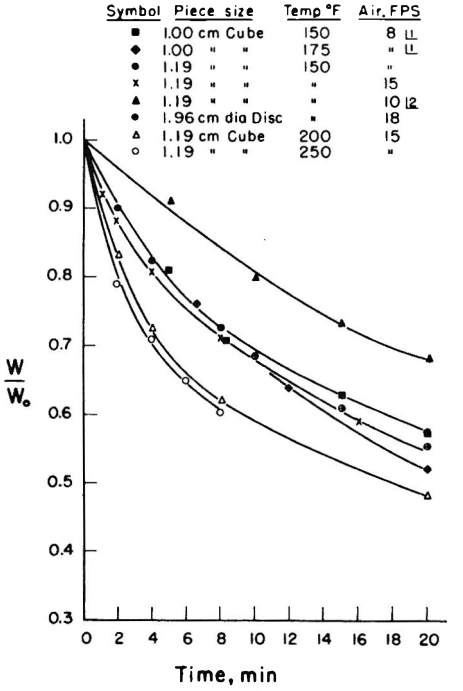

Fig. 5—Weight fraction (W/W_0) vs. drying time (θ) for several sizes of potato pieces with constant surface: volume ratio (5 cm^{-1}).

[1]Bench test (adjusted, 5 faces); [2]Cross-flow (conventional tray dried).

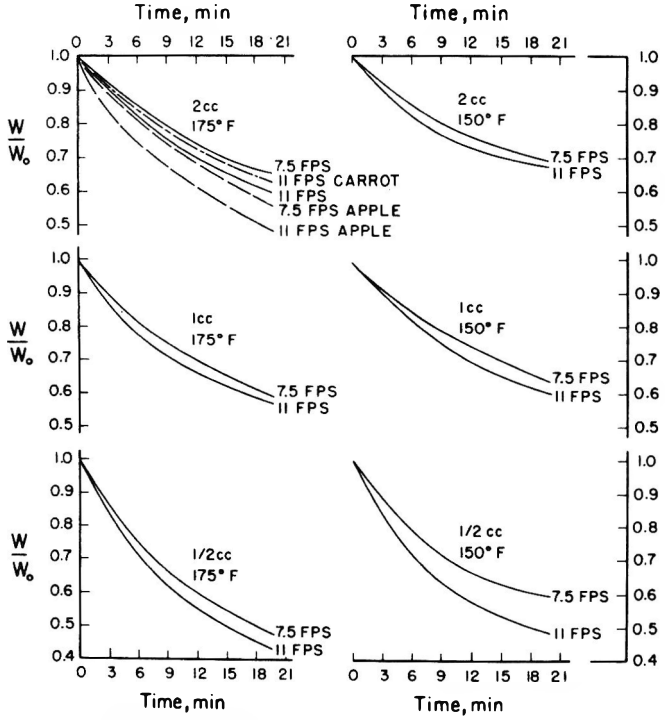

Fig. 4—Weight fraction (W/W_0) vs. drying time (θ) for 3 sizes of cubes of potato, apple or carrot. Curves are shown for 2 air temperatures and 2 air velocities. Data are from bench tests. All curves are for potato except where noted.

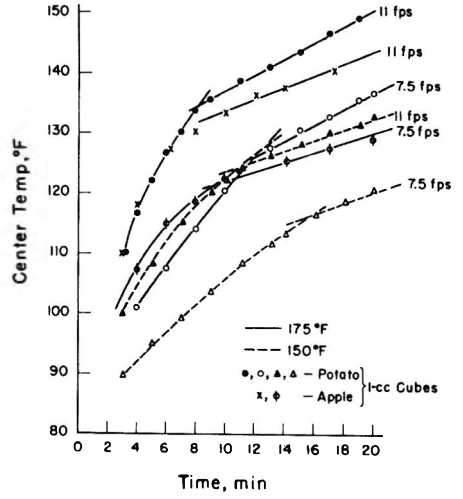

Fig. 6—Center temperatures vs. time during drying individual 1-cc pieces of potato and apple (5 faces exposed).

and described by Farkas et al., 1969). 400 g of cut food pieces were used during each batch run. About 65% of the support surface was covered. Drying was followed by periodically stopping the unit and quickly weighing the charge.

Potato (Russet Burbank), apple (Golden Delicious) and carrot (variety unknown) were dried. Only cubes were used in bench tests. Cubes were cut with a preset parallel razor blade jig into volumes of 2, 1 and 1/2 cc (see Table 1). The piece sizes were checked by weighing. To avoid the large variations in drying rates of single pieces that occurred from potato to potato, or even from piece to piece cut from the same tuber, uniformly sized potatoes were selected and "stock" sections cut from the same general portion, about midway between the skin and the heart. Midsection pieces were similarly obtained for apple and carrot. For the pilot plant tests these variations were averaged by using a large number of pieces (400-g charge). These were cut first into strips on a 3/8-in. (nominal) French-fry cutter, then by hand into cubes, half-cubes and double-cubes (see Table 1). Sizes were checked by weighing 100 or more pieces. Disc-shaped pieces were cut with a cork-borer and cross-cut with a razor blade to the exact disc thickness desired. Physical properties used in calculations are shown in Table 2.

Table 1—Dimensions of cut pieces.

Size (cc)	Dimensions (cm)	Area of surface (sq cm)	Ratio of surface to volume (cm^{-1})	Shape
2.00	1.26 by 1.26 by 1.26	9.53[a]	4.8[a]	Cube
1.00	1.00 by 1.00 by 1.00	6.00[a]	6.0[a]	Cube
0.50	0.79 by 0.79 by 0.79	3.74[a]	7.5[a]	Cube
1.70	1.19 by 1.19 by 1.19	8.50	5.0	Cube
3.40	1.19 by 1.19 by 2.39	14.20	4.2	Double-cube
0.85	1.19 by 1.19 by 0.60	5.69	6.7	Half-cube
2.08	1.96 dia. by 0.69 thick	10.30	5.0	Disc

[a]For bench tests where only 5 surfaces were freely evaporating, use 5/6ths of these values. This would make 1 cc test (bench) equivalent to S/V for 1.70 cc (pilot plant)

Table 2—Properties used in calculations.

	Undried			Dried skin[a]		
		Abs.		Abs. density at moisture contents:		
	Solids (%)	Density (g/cc)	Porosity (%)	50%	33%	25%
				(g/cc)		
Potato	20	1.09	–	1.23	1.33	1.40
Apple	15	1.06	24.5	1.23	1.33	1.40
Carrot	12	1.02	–	1.23	1.33	1.40

[a]Values estimated from sucrose tables.

RESULTS & DISCUSSION

THE DRYING curves obtained with the bench unit for 3 sizes of cubes are shown in Figure 4. Drying curves, "normalized" for constant surface-to-volume (S/V) ratio, are shown in Figure 5 for several sizes and shapes of potato in both test units. The data for the 2 bench tests shown were adjusted for only 5 faces of the cube exposed. Most of the points followed a common curve locus except those for cross flow and for 200°F or higher. The ratio of S/V appeared to be a reasonable basis for comparison of drying rates of different sizes and shapes of pieces.

The center-temperature patterns for 1-cc pieces are shown in Figure 6. Although not shown here, data for 1/2- and 2-cc pieces were quite similar to Figure 6 data. A "break" appeared in the curves at similar fractions of original weight $\frac{W}{W_0}$ for each size (Table 3). A plot of W/W_0 and S/V from Table 3 showed a simple linear relationship. The conclusion one might draw is that the thickness of the skin layer formed at the "break" point limits the drying. The moisture level in the skin also may be involved. Also shown in Table 3 are the skin thicknesses at the break points, calculated for 33% moisture in the skin. This will be discussed later.

Table 3—Calculated properties of cubes.

Piece size (cc)	S/V	W/W_0 at "break"	Cal'd skin thickness at "break" (mm)
2	4.8	0.74	0.24
1	6.0	0.70	0.22
1/2	7.5	0.65	0.21

To simplify analysis of results, the data obtained in the centrifugal bed dryer were divided into 2 short periods, 0–10 min and 10–20 min, and the rate of weight loss $\frac{\Delta(W/W_0)}{\Delta\theta}$ was treated as linear in each period as an approximation.

To compare the drying rates of different piece sizes and shapes, rates were based on unit surface areas $\frac{\Delta(W/W_0)}{S\Delta\theta}$, where S is the surface area at the start of each period. Since $S_0 = \frac{W_0}{d_0}\left(\frac{S}{V}\right)_0$ and $S_{10} = \frac{W_{10}}{d_{10}}\left(\frac{S}{V}\right)_{10}$, where $\frac{S}{V}$ is the surface: volume ratio and d is the apparent density, the rate equations are

$$R_{0\text{-}10} = \frac{d_0}{(S/V)_0}\,\frac{\Delta(W/W_0)_{0\text{-}10}}{\Delta\theta} \qquad [1]$$

and

$$R_{10\text{-}20} = \frac{d_{10}}{(S/V)_{10}}\,\frac{\Delta(W/W_0)_{10\text{-}20}}{\Delta\theta} \qquad [2]$$

It can be shown for rectilinear shapes that

$$\frac{S}{V}_{10} = \left(\frac{S}{V}\right)_0\left(\frac{W_0}{W_{10}}\right)^{1/3}\left(\frac{d_{10}}{d_0}\right)^{1/3} \qquad [3]$$

Equation [3] holds closely for discs also where the diameter is less than 3 times the thickness.

Substitute Equation [3] into Equation [2]

$$R_{10\text{-}20} = \frac{(d_{10})^{2/3}\,(d_0)^{1/3}}{\left(\frac{S}{V}\right)_0}\left(\frac{W_{10}}{W_0}\right)^{1/3}\frac{\Delta(W/W_0)_{10\text{-}20}}{\Delta\theta}$$

, and since

$(d_{10})^{2/3}\,(d_0)^{1/3}$ very nearly equals d_0 in the density ranges involved, then

$$R_{10\text{-}20} = \frac{d_0}{(S/V)_0}\left(\frac{W_{10}}{W_0}\right)^{1/3}\frac{\Delta(W/W_0)_{10\text{-}20}}{\Delta\theta} \qquad [4]$$

(Note that the shrinkage correction

$$(W_{10}/W_0)^{1/3}$$

was in the range 0.84–0.92 for all tests made.

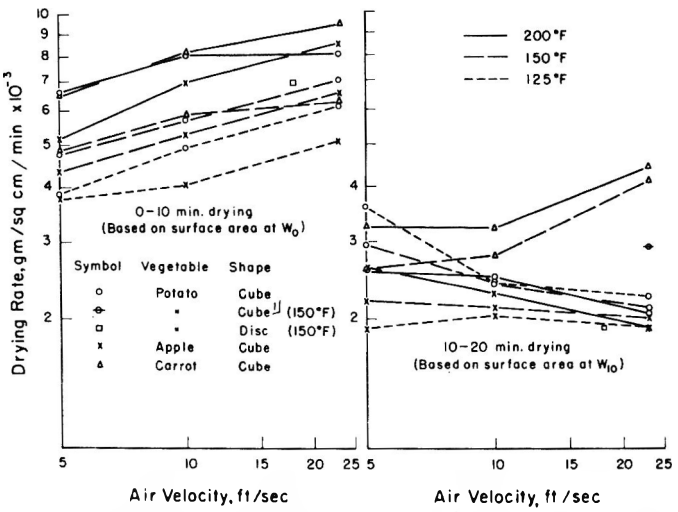

Fig. 7–Drying rates (R_{0-10} and R_{10-20}) vs. air velocity in centrifugal fluidized bed dryer. All pieces have surface:volume ratio = 5 cm^{-1}.

[1]Only one point for 6 hr hold after 0–10 min drying.

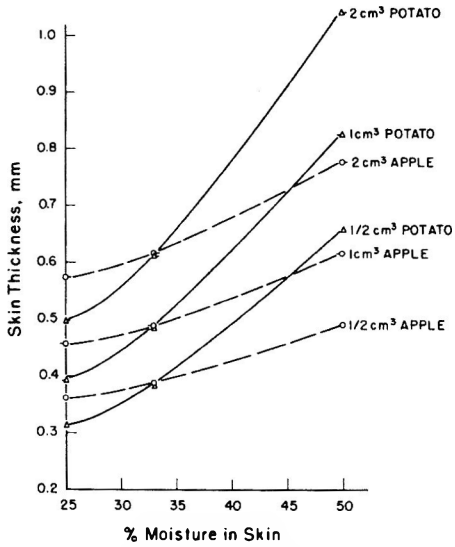

Fig. 8–Calculated skin thickness at 50% weight reduction, rapid drying of apple and potato cubes. Apple porosity 24.5%, potato porosity 0%.

The rates calculated from Equations [1] and [4] are shown in Figure 7 for potato, apple and carrot. Rate changes are "normal" for R_{0-10}, in that they increase both with temperature and air velocity. However, with potato, the effects of temperature and air velocity are inverted for R_{10-20}. For apple, R_{10-20} shows only slight increase with temperature (except at highest air velocities where temperature had no effect) and remains constant or slightly lower with increasing air velocity. Carrots appear to be "normal" for all conditions. Discs and cubes with equal S/V show nearly identical R_{0-10} values. R_{10-20} value for discs is 88% of value for cubes with equal S/V.

The above results for R_{10-20} reflect previous drying history more than any other factor. The pieces dried at very high rates developed a "skin" layer observed to vary from 0.3–1.0 mm in thickness. The "core" remained essentially the same as undried tissue (confirmed by Brix measurements). If it is assumed that a) all of the drying occurs in the outer layer and the inner tissue does not change significantly, b) the original porosity is unaffected, c) the change in absolute density is approximately the same as for sugar solutions and d) the cubic shape is retained, then the following equations may be derived for edge dimension of cubes and for skin thickness after drying:

$$E = (\text{vol.})^{1/3} = \left(E_0^3\ P + \frac{W_S}{\rho_S} + \frac{W\text{-}W_S}{\rho_0}\right)^{1/3} \quad [5]$$

$$L = \frac{\text{vol. of skin}}{\text{area of skin}} = \frac{1}{6E^2}\left(E_0^3\ P\ \frac{W}{W_0} + \frac{W_S}{\rho_S}\right) \quad [6]$$

where

W_0 = original wt of piece, g.
W = final wt of piece, g.
L = skin thickness at W, cm (6 faces exposed).
E = edge length at W, cm.
E_0 = edge length at W_0, cm.
P = porosity, as fraction of original tissue volume at W_0.
W_S = skin wt, g $= \frac{S_0}{S_S - S_0}\left(W_0 - W\right)$
S_0 = original solids content, g/g, at W_0.
S_S = skin solids content, g/g, at W.
ρ_0 = original absolute density, g/cc, at W_0.
ρ_S = skin absolute density, g/cc, at W.

Equations [5] and [6] were readily solved with a desk-model punched-card computer and the data obtained are shown in Figure 8. Skin thicknesses of 0.3–1.0 mm were calculated, confirming our observations and those of Sykes and Kelly (1969), with moisture contents varying from 25–50% for cubes of porous apple (P = 0.25) and nonporous potato (P = 0). It has been shown (Van Arsdel and Copley, 1963) that moisture diffusion resistance in potato tissue sharply increases manyfold for small decreases in moisture content (somewhere in the range 20–50% moisture). This effect and the increased skin thickness combine to create increasing barriers to transfer of moisture. Table 3 shows the thickness of skin that causes a break in the temperature pattern by affecting heat transfer. It is therefore reasonable to conjecture that extremely high-rate air-drying environments may be self-limiting in some cases, even when preparing intermediate-moisture piece-form foods.

Immediately after drying, the surfaces of the pieces appeared very dry. However, the surfaces soon remoistened by diffusion from within the pieces. The increased resistance caused by low moisture content in the skin layer was demonstrated by comparing the drying rates R_{10-20} (Equation 4) of 2 samples–1 dried without interruption and the other dried under the same conditions in 2 steps, with a 6-hr hold period between steps to allow the skin layer to remoisten by diffusion from within. R_{10-20} was 50% greater after the hold period (see Fig. 7).

It can be shown that 80–90% of the heat transferred to an evaporating moist body in a hot air stream goes into evaporation of water; the remainder goes into raising the piece temperature. From Figures 4 and 9 we may estimate the minimum value of h, the air film heat transfer coefficient using the equation $h = \frac{Q}{A\Delta t}$ where

Q is the heat (latent) transferred in unit time, or $\frac{\Delta W}{\Delta \theta}\ l_v$

A is the surface area exposed, and

Δt is the temperature difference between the drying air and the piece surface.

As an example, for a 2-cc cube of potato drying with 5 faces exposed (Fig. 9) and compensating for shrinkage of surface area during drying, we obtain $h = 34\ \frac{\text{Btu}}{\text{hr sq ft}\ ^\circ\text{F}}$ for most of the range of drying shown. This value is about 5 times as great as the result obtained using the equations of Charm (1963),

Table 4–Effect of blanching[a] 1.19-cm cubes of potato.

Drying temperature (°F)	Air velocity (fps)	R_{0-10}		R_{10-20}	
		Unblanched	Blanched	Unblanched	Blanched
150	10	5.10	5.70	2.46	2.42
150	23	5.43	7.00	2.08	2.10
200	23	7.15	8.00	2.33	2.07

[a]Blanched 3 min in steam at 212°F.

$h = 0.0128G^{0.8}$ for parallel flow and $h = 0.37G^{0.37}$ for perpendicular flow (where G is the mass air flow rate). If the quantity representing sensible heat increase in the piece were not neglected, or if there were significant temperature gradient between the outside thermocouple and the surface proper, even higher values for h would be obtained by our methods.

The effects of blanching on the rates of drying are shown in Table 4. Drying rates were 12–28% higher in the initial drying period for the blanched samples, but were not significantly affected after drying beyond 10 min. No effect of blanching on drying rates was observed by Saravacos and Charm (1962).

It is of interest to note that diced potatoes were air-blanched in 6 min when dried at 250°F (Fig. 5), which favored skin formation and rapid piece temperature rise. This suggests that a hot-air method of blanching might be developed to eliminate sources of water pollution which emanate from conventional water or steam blanchers.

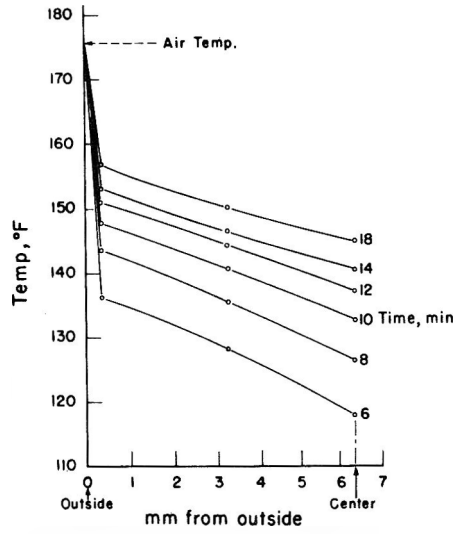

Fig. 9–Temperature distribution in a 2-cc piece of potato during drying at 175°F and 11.7 fps.

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FREEZE CONCENTRATION BY DIRECTIONAL COOLING

SUMMARY—It is generally assumed that as food materials are frozen, soluble solids move ahead of the "ice front." Under conditions tested in these studies the above was true only when the ice front moved in a descending direction. Thus, when foods were frozen in an ascending direction (as on a plate freezer) there was little, if any, movement of solids. When the freezing surface was placed above the material to be frozen, there was a rapid downward movement of solids. This "solids descent" was most apparent in true solutions such as drinks, and less apparent in structural cellular foods such as pieces of meat or potatoes. It is suggested that this phenomenon may be utilized for more efficient freeze drying or concentrating, or for the simultaneous production of low-solids and concentrated foods, particularly beverages.

INTRODUCTION

DURING freezing, water is removed from solution and transformed into ice crystals of variable but rather high degree of purity, Tressler and Joslyn (1961). The remaining material is left successively in a more concentrated state. The extent of concentration is influenced by the product characteristics and the rate of freezing, Fennema and Powrie, (1964). Fluid material such as fruit juice can be concentrated readily by freezing and in some cases this process is used commercially (Charm, 1963). The major limitation of this process is the substantial loss in yield caused by solids being occluded within or among the ice crystals. Less obvious is the fact that plant or animal tissues also undergo solute concentration during freezing, Van den Berg (1961). Slow rate of freezing was found to cause a greater degree of solute concentration than rapid freezing, Fennema and Powrie (1964).

No information was found in the literature that direction of freezing has any effect on solids movement. Consequently, in studies designed to determine rates of freezing on temperature of freezing, Wani (1967), the samples were all resting on the freezing medium and no significant concentration of solids ahead of the freezing front was noted. Additional experiments were, therefore, conducted to determine whether solids concentration is dependent upon direction of freezing. Generalized and empirical equations for unidirectional rates of freezing were developed by Wani (1967) and multidirectional by Sullivan (1969).

MATERIALS & METHODS

COMBINATIONS of different levels of water, sucrose and pectin were prepared to represent solutions or gels. Pasteurized whole milk was used to represent a fat-protein emulsion system. Apples, potatoes and turnips were used as plant cellular systems, and fresh beef as the animal cellular system. These products were purchased from local sources and trimmed to fit the sample cell used.

A square (tetragonal) sample cell was made from 5-mil cellophane film 1.5 by 1.5 and 2 in. high. The sides of the cell were insulated by multilayered aluminum. In addition, the entire cell was covered with a multilayered aluminum foil cap, except the bottom which was immersed into the cooling medium. The liquid samples were poured into this cell. Nonliquid samples such as the vegetables and meat were carefully trimmed to the cell dimension and inserted into the cell. For cooling the sample from the bottom up, the cell was placed over the cooling medium. For unidimensional descending cooling, the same cell was attached below the cooling medium.

The cooling media were mixtures of alcohol, acetone, water and dry ice which, upon continuous, gentle stirring provided a rather precise temperature ($\pm$ 0.5°C) at the interface of sample and cooling medium of 0, −50 or −100°F. Each value presented in Table 1 is an average of 6 determinations (duplicates frozen at each temperature of the cooling medium).

Round (tubular) cells were made by boring into a solid block of polystyrene insulation. Plastic (plexiglass) tubes, 1 in. inside diameter, 3 mm wall thickness and 8 in. long were inserted into these plugs, and the entire block of styrene placed in a freezer unit overnight at −20°C.

Following freezing, the frozen tetragonal samples were removed and cut into half-inch slices. Tubular samples were cut into 2-cm slices. Total solids were determined on each slice by drying overnight in a vacuum oven at 60°C.

RESULTS & DISCUSSION

Tetragonal cell

Some typical results on migration of solids as influenced by position of the

Table 1—Migration of solids in ascending and descending freezing.[a]

Material	Ascending or descending	Total solids—(%)[b] Distance from cooling surface				L.S.D.[c]
		< 0.5 in.	0.5–1 in.	1–1.5 in.	1.5–2 in.	(P = .05)
12% Sugar solution	Ascending	11.6	12.1	11.8	11.2	0.88
	Descending	12.6	8.0	9.6	15.6	1.06
40% Sugar + 3% pectin	Ascending	44.5	44.3	44.9	45.2	1.54
	Descending	45.4	45.1	45.1	45.2	1.24
Milk	Ascending	12.0	12.0	12.2	11.8	0.48
	Descending	12.1	11.3	11.7	11.5	0.70
Beef	Ascending	30.0	31.1	32.4	31.4	2.88
	Descending	29.8	29.2	29.0	30.7	2.60
Apple	Ascending	15.4	15.5	15.3	16.0	0.88
	Descending	15.6	15.2	15.3	16.7	0.95
Potato	Ascending	19.8	17.3	15.6	19.2	3.04
	Descending	23.6	18.4	17.0	21.5	3.68
Turnip	Ascending	10.9	11.1	11.0	11.0	0.28
	Descending	10.9	10.0	10.6	10.9	0.46

[a]Tetragonal cell.
[b]Means of 6 determinations—duplicates at each of 3 freezing temperatures.
[c]Least significant difference among distances from cooling surface.

Present addresses:
[1]Snow Brand Milk Products Company, Tokyo, Japan.
[2]McCormick and Company, Baltimore, Maryland.

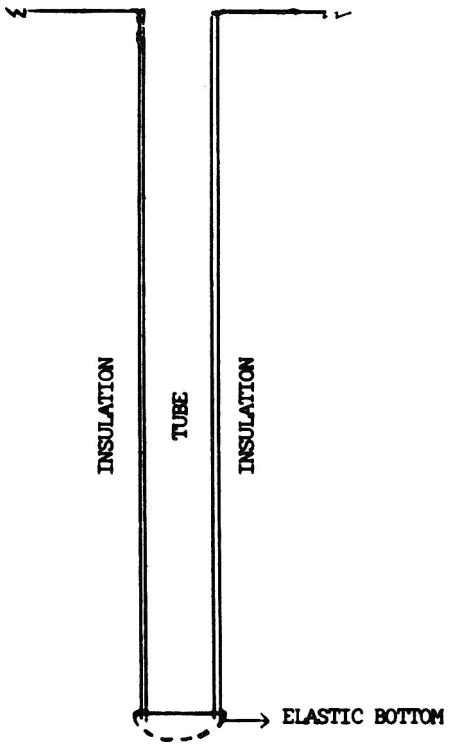

Fig. 1–Tubular cell with elastic bottom.

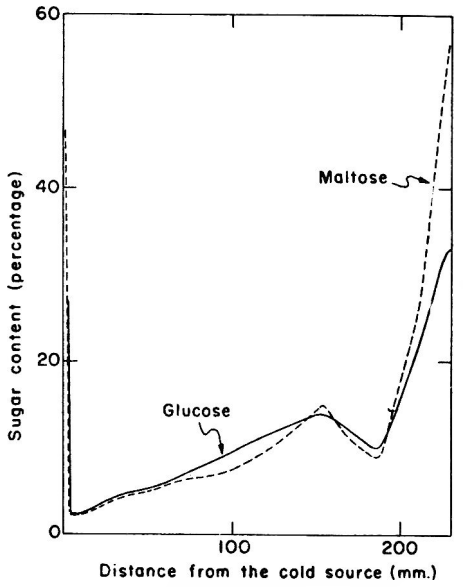

Fig. 2–Concentration of solids in relation to distance from cooling surface.

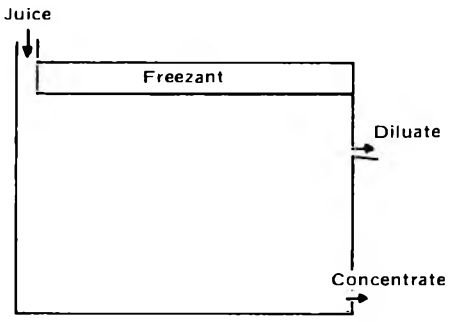

Fig. 3–Schematic diagram for eqipment to produce drinks and concentrates simultaneously.

sample cell in relation to the cooling medium are shown in Table 1. Whenever the specimen rested on the cooling medium and freezing proceeded from the bottom of the sample upwards (ascending), no statistically significant changes in solids content in relation to distance from the freezing medium were noted (except for potatoes whose centers were originally less dense). The reason for this inconsequential change in migration of solids during ascending freezing may be explained by the tendency of the ice crystals to form with the concentrated liquid phase occluded between the ice crystals without affecting the solids concentration in the next layer to be frozen.

When the cooling medium was placed above the sample, changes in freezing concentration in different positions of the samples were readily observed for the samples where the solids were largely in solution. Characteristically, the top of the sample which was in contact with the cooling medium, and the bottom which froze last. were higher in solids than the middle part. It was also noted that in those instances where the freezing medium was at very low temperatures (−100°F), and freezing occurred very rapidly, there was a greater tendency for a larger layer at the top of the sample to have essentially the same solids concentration as the original product. It was, therefore. concluded that where freezing occurs very rapidly, the solute might be occluded in a manner similar to ascending freezing in the upper layers. At lower layers, however, the soluble solids could migrate downwards, depending on the rates of ice formation and gravity. Another possible explanation for the relatively high concentration in the top layers may be the upward squeezing of solids as a result of expansion during freezing in a rather rigid container.

Samples that consisted of rigid gel (e.g., 40% sugar + 3% pectin) showed little solids movement in either ascending or descending cooling. Cellular materials showed a tendency towards solids migration during descending freezing, but not nearly as pronounced as the noncellular solution samples. This may be interpreted as indicating that the downward migration of solids ahead of the freezing front may not occur in gels at all, and may be attenuated but not prevented by cell wall membranes. The more pronounced migration of solids in potatoes and turnips as compared to milk and beef suggests that carbohydrates (and perhaps proteins) respond to the gravitational effect whereas the less dense lipids do not.

Tubular cell

The tubular cell (Fig. 1) was designed on the basis of the above hypotheses. With this equipment, it was possible to measure solids migration over a longer descent. With the elastic rubber bottom replacing a rigid bottom, it was thought that solids could accumulate in the distended lower portion of the tube rather than be forced up towards the top layers by pressure resulting from the ice expansion. The results shown in Figure 2 indicate that this, in fact, was the case. When 10% solutions of glucose and maltose were frozen in the tube, the soluble solids content of the sections showed a marked gradient, being very high–approximately 35% for glucose and over 60% for maltose in the distended bottom of the tube. Approximately 1 in. above this lowest layer the concentration decreased to approximately 10%, then increased again for the next inch to approximately 15%, then continued to decrease until it approached the very top layer consisting of 0.1 to 0.3% of the tube height, where the concentration again was very high. When the samples were thawed and refrozen several additional times, practically all of the solids were removed from this upper layer. The sample material was not stirred or agitated at any time during thawing or freezing.

CONCLUSIONS

THESE DATA indicate that solids, specifically sugars in solutions, do migrate ahead of the freezing front but mostly downward with the aid of gravity. The cooling chamber, however, must be open so as not to force some solids in an upward direction as a result of pressures devel-

oped during ice crystal formation. In such a system there should be little occlusion of solids among ice crystals so that such a method of freeze-concentration could be higher-yielding than other methods of freeze concentration.

Since actual removal of ice is not necessary, and also since it appears that solids begin to descend at the initiation of ice crystal formation, it may be possible to utilize this phenomenon not so much to concentrate by means of ice removal, but to separate a more dilute from a concentrated solution with only very little ice formation, thereby yielding simultaneously two products – a diluted drink-type product having 25–50% of the original solids, and a concentrate having 3–6 times the solids percent of the original solution. A schematic diagram for such a system is shown in Figure 3, where the solution enters the cooling tank and travels gradually under a cooling plate. Ice formation should be kept to a minimum. As the juice approaches freezing, solids should begin to descend towards the bottom of the cooling tank, where they should continue to move over the sloping bottom, to emerge at the lower orifice as concentrates. The diluted solutions would also move gradually across the top of the tank to emerge through an orifice located somewhere below the top of the freezing tank.

Such a process may be an efficient method of water reutilization or desalination, since it promises to require a minimum amount of washing and centrifuging to recover water crystals. However, its use for the separation of a concentrate from a dilute product appears more enticing, since there is a possibility that only a minimal amount of energy for actual change of state would be required.

Naturally, the products so treated would need to be fluid. The only conclusion drawn from these studies applicable to solid foods would be that to maintain uniform quality in a frozen product it is preferable to freeze from the bottom up rather than from the top down. These studies do explain the phenomenon frequently observed by users of syrup or fruit concentrates, that the refractive index towards the top of the drum may be much lower than that towards the bottom of the drum.

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HEAT AND CALCIUM TREATMENTS FOR FIRMING RED TART CHERRIES IN A HOT-FILL PROCESS

SUMMARY—Preheating of freshly pitted cherries at about 140°F for 5–20 min promoted firming sufficiently to permit pasteurizing the fruit in bulk without excessive tearing. Improved texture from this pretreatment was demonstrated in terms of increased extrusion force, drained weight and bulk volume. Addition of calcium up to .04% of final product led to 50% greater firmness. Hot-fill temperatures of 180°F or higher were required to protect color and flavor from enzymatic degradation, even though product character was adversely affected thereby. While firmness or chewiness of the fruit was notably greater in the pretreated, hot-filled product, the conventional cold-fill, exhaust and retort process provided superior drained weight and bulk, with about equal color.

INTRODUCTION

FIRMING red tart cherries for processing has been a focus of research effort and of industry concern for more than two decades. Though a soft fruit, it is handled in bulk and subjected to rather drastic pit removal by punching. The need for intrinsic or developed firmness of the fruit to protect its integrity through handling and processing is widely recognized. While cultural factors leading to intrinsic firmness are incompletely understood, the handling and processing procedures that promote a firm product have been carefully documented, if not always observed in practice.

At best, though, the cherry remains a delicate fruit with color and flavor characteristics so distinctive that any impairment is readily noticeable. Unfortunately, the most common practice for obtaining a firm product—a long soak during which damaged tissue toughens by an incompletely understood physiological process—leads at the same time to deterioration of color and flavor. In the preparation of pie mix, where firm fruit able to withstand mixing in the starch slurry is especially desirable, this deterioration can be corrected in part by formulation. In the canned product, or hot-pack, the red anthocyanin pigments are dispersed and diluted, and even slightly degraded, during the cook, so that previous losses of color quality are less noticeable than in the frozen pack. It has been common practice, therefore, to use a long soak for hot-pack and a shorter one, sacrificing some firmness to maintain fresh color, for frozen pack.

Prior to World War II the hot-pack predominated, but has since been gradually losing ground to frozen cherries, especially in short-pack years. More recently, the pack of canned cherry pie-mix has increased at the expense of the traditional pack of red tart cherries in water. Both of these developments can be traced in part to the less desirable quality of the hot-pack. The techniques outlined here for firming cherries after pitting by heat and calcium treatments are applicable either to the common water-pack, to a more desirable pack in sugar syrup or to the preparation of pie-mix.

The conventional process for hot-pack cherries seems at first glance something of an anachronism. Other high-acid products, such as applesauce and sauerkraut, are commonly pasteurized and filled into the can hot. After double-seaming and holding briefly to ensure pasteurization of the container, these products are cooled without further heat-processing. It is true, though, that the industry was slow to adopt the suggestion of Pederson and Beattie (1946) that effective pasteurization of sauerkraut could be obtained by hot-filling as low as 155–165°F. Applesauce is commonly filled at 190°F.

At present, canned cherries are filled into the can cold from the pitter and covered with hot water. The open cans are then conveyed through a hot water bath to be preheated slowly before closing. This operation exhausts air from the pack that might otherwise worsen can corrosion. Normally, a rather uneven center temperature of about 180°F is reached before closing the can. Then the pack is given an additional heat treatment, usually about 12 min at 212°F in a No. 303 can.

A filling temperature of 190°F for a high-acid product is ordinarily considered adequate to destroy nonspore-forming microorganisms, and the heat-resistant survivors, i.e., the bacterial spores, are unable to initiate growth at the lower pH values (3.1–3.5) encountered in the cherry medium. The apparent reason for not heating the cherries in bulk and filling at this temperature is that pitted cherries are too delicate to withstand the agitation necessary for rapid heating. The heat and calcium treatments to be discussed here provide means for circumventing this difficulty by a preliminary firming treatment that permits the pitted fruit to be heated in bulk to the required hot-fill temperature without excessive tearing. These two operations—the pretreatment and the heating in bulk to hot-fill temperature—would replace exhausting and retorting. Air is expelled during preheating, and sterilization, as opposed to pasteurization, is unnecessary.

The crux of this innovation lies in the pretreatment at moderate temperature in the presence of calcium ion. This activates the pectic demethylation process. Calcium-firming of the cherry tissue takes place before any thermal disruption or cooking of the tissues that would occur at pasteurization temperatures. The effect of calcium on plant tissues was pointed out by Kertesz as early as 1939, and he subsequently adapted it to the firming of canned tomatoes (Kertesz et al., 1940) and apple slices (Kertesz, 1947). Various commercial applications, especially in canned tomatoes, have been common for two decades. Recently the Standard of Identity for canned tomatoes has been amended to permit an increased level of added calcium salts to 0.1% (as Ca) to aid in the preparation of sound packs of cut tomato pieces (Food and Drug Administration, 1970).

The effects of calcium treatment on red tart cherries were investigated by Whittenberger, 1952; Whittenberger and Hills, 1953, and by Bedford and Robertson, 1957, in the one case showing an improvement of firmness and drained weight by canning cherries with added calcium by the conventional process and in the other demonstrating only an increase in firmness. The chemistry of the pectic system has been extensively covered by Kertesz (1951) and Deuel and Stutz (1958), and further elaborated by Collins and Wiley, 1963; Collins, 1965. Dastur et al. (1968) have recently studied the thermal process for canned cherries, including consideration of both thermal death time and peroxidase inactivation. LaBelle and Moyer (1960) showed that bruising and elapsed time after harvest, and not soaking in cold water, were the important factors affecting product firmness and drained weight.

The present method has been anticipated in part by the low-temperature blanching studies of Van Buren et al. (1960) on green beans and Hoogzand and Doesburg (1961) on cauliflower. The latter attributed the firming of cauliflower during a 15-min blanch at 158°F

Table 1—Effects of pretreatment temperature on texture of canned cherries.

	Pretreatment temperature (°F)				
	120	130	140	150	160
Extrusion force (kg)					
1969 (6P)[a]	52.6	54.3	53.8	48.2	44.3
1968 (18P)	(54.2)[b]	59.5	59.5	54.5	50.8
1968 (6L)	52.4	58.0	57.5	56.2	52.2
1967 (8L)	37.3	42.0	45.6	44.8	42.0
Avg	49.1	53.4	54.1	50.9	47.3
Drained weight (%)					
1969 (6P)	70.2	71.3	71.0	70.2	67.3
1968 (18P)	(77.2)[b]	75.7	76.0	76.3	74.5
1968 (6L)	81.9	81.9	80.0	81.3	80.8
Avg	76.4	76.3	75.7	75.9	74.2
Bulk volume (L/kg)					
1969 (6P)	.958	.971	.965	.944	.920

[a]Data are averages of the number of replicates noted in parentheses for pilot-plant (P) or laboratory (L) experiments.
[b]Three replicates only at 120°F, but data normalized.

Table 2—Effects of pretreatment time on texture of canned cherries.

	Pretreatment time (min)			
	0	5	10	20
Extrusion force (kg)				
1969 (6P)	31.0	43.4	48.7	53.0
1968 (8P)	37.3	47.0	58.6	59.0
1968 (8L)	35.7	51.7	53.0	58.4
1967 (9L)	–	34.3	38.8	43.6
Avg (1968–1969 only)	34.7	47.4	53.4	56.8
Avg	–	44.1	49.8	53.5
Drained weight (%)				
1969 (6P)	63.5	67.8	68.2	66.5
1968 (8P)	73.7	77.5	76.6	75.7
1968 (8L)	81.0	82.3	80.7	81.2
Avg	72.7	75.9	75.2	74.5
Bulk volume (L/kg)				
1969 (6P)	.880	.926	.932	.915

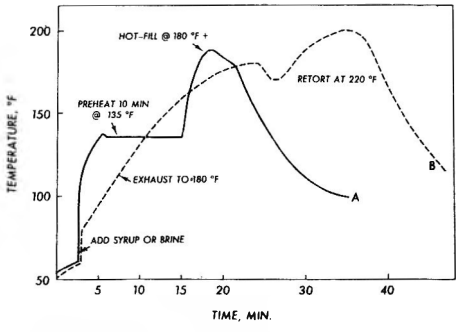

Fig. 1—Schematic representation of cherry processes for canned cherries: A) Pretreatment with calcium and hot-fill; B) conventional cold-fill, exhaust and retort.

Table 3—Effects of added calcium on texture of canned cherries.

	Level of $CaCl_2$ in added brine (%)[a]			
	0	0.1	0.2	0.5
Extrusion force (kg)				
1969 (12P)	33.0	35.2[b]	42.1	46.8[b]
1968 (8P)	38.6	48.0	55.8	59.6
1968 (8L)	40.0	46.2	53.3	59.3
1967 (3L)	30.5	37.5	40.2	46.7
Avg	35.5	41.7	47.8	53.1
Drained weight (%)				
1969 (12P)	65.8	67.1	67.7	67.7
1968 (8P)	76.3	76.5	75.8	75.0
1968 (8L)	82.0	81.1	81.2	80.7
Avg	74.7	74.9	74.9	74.5
Bulk volume (L/kg)				
1969	.900	.922	.932	.956

[a]To obtain level in final product (as % Ca), multiply by 40/111 × 1.14/5.14 = 0.80.
[b]Six replicates only at these two levels.

to partial de-esterification upon activation of pectinesterase.

EXPERIMENTAL

RED TART cherries (var. Montmorency) were harvested from a neighboring commercial orchard and soaked overnight for 16 hr at 34°F. All of the fruit was then drained and held in air at this temperature until needed. This handling treatment resulted in fruit of moderate firmness but excellent scald-free color. Sufficient fruit was sorted and pitted in a Dunkley junior-size pitter at one time to provide a 4-kg lot of pitted cherries for further processing. To this was added 1.14 kg of a hot aqueous solution of 0, 0.1, 0.2 or 0.5% calcium chloride, giving overall calcium content in the product of 0, 0.08, 0.16 and 0.40%. The slurry was then carefully brought to the desired pretreatment temperature (120–160°F) in a 20-gal jacketed kettle with just sufficient steam pressure and manual stirring to prevent local overheating at the wall. Product temperature during the typical 3-min come-up to the desired level was monitored with a thermocouple and recording potentiometer.

After the desired pretreatment time (0–20 min) had elapsed, the batch—now more fluid than before—was quickly brought to hot-fill temperature (150–200°F), again with just sufficient steam and stirring to maintain a fairly uniform temperature throughout. The whole batch was then quickly filled into No. 303 cans. All but the last can was immediately closed, then rolled for 3 min to pasteurize the can interior before cooling in a spin-cooler. Nine cans were stored at 65°F (75°F in 1968), while the tenth can was opened the following day for determination of color parameters with the Hunter Color Difference Meter and spectrophotometer (Beckman Model DK). After 6 to 8 months' storage, the remaining cans were opened for determination of drained weight, bulk volume, extrusion force, color and subjective evaluation of color, flavor and texture. Since the fill of container was not standardized as to net fill-in weight or proportion of fruit to liquid, it was necessary to obtain the summation of drained weight and bulk volume on all filled cans and also on the small leftover.

Drained weight was measured after a 2-min drain at 75–80°F on an 8-mesh screen sloping at 20°. The fruit was then carefully returned to its juice in a 500-ml graduated beaker (high form), and a nesting 250-ml beaker weighted with 200g of water was superimposed to immerse the fruit. After 60 sec the volume of fruit was estimated to 5 ml. Texture of the canned fruit was measured in the compression-extrusion cell of an Instron universal testing machine. A 225-g sample of freshly drained fruit at 75°F was compressed with a ram speed of 20 cm/min into a piston clearance of 6 mm, extruding more than half of the sample back through the 4-mm annulus.

In addition to the pilot plant tests carried out in 1968 and 1969, bench-scale tests employing 0.45-kg lots were carried out at the same pretreatment times, temperatures and calcium levels in 1967 and 1968. Only drained weight and texture differences were observed in these tests.

RESULTS & DISCUSSION

IN THE DEVELOPMENT of this mode of processing, the central theme is that of filling the acid product into the container hot, so as to obviate subsequent retort processing. However, hot-filling pitted cherries would be practical only if the fruit could be firmed sufficiently beforehand to withstand heating in bulk. The proposed hot-fill process, together with the conventional procedure, is shown schematically in Figure 1.

Pretreatment

Our earliest experiments were directed toward a firming pretreatment, though effective levels of temperature, time and added calcium were then unknown. But several guides were available, among them the observation by Matz (1962) that a semipermeability of cell membranes is lost at about 150°F, the need for keeping treatment times short enough to fit into the general scheme of a continuous process and the now superseded precedent (in canned tomatoes) for incorporating up to .026% of calcium in the product. Accordingly, the earliest tests bracketed what were considered promising levels of these variables, namely, 10 min at 140°F with .20% of $CaCl_2$ in sufficient brine to make a fluid slurry of pitted fruit.

In Table 1 the results of pretreating pitted cherries at temperatures from 120–160°F are presented in terms of texture and the related parameters–drained weight and bulk volume. In four separate experimental series on either pilot-plant or laboratory scale over three seasons, a consistent result was obtained: maximal firmness as measured by extrusion force occurred close to and usually slightly below 140°F. Drained weight, obtained only in the latter two seasons, and bulk volume, only in 1969, also peaked at this temperature level, showing a close association among these measurements.

A somewhat different relationship among them was demonstrated for the effect of elapsed time of pretreatment (Table 2). Although cherry firmness continued to increase as treatment was prolonged to 20 min, albeit with diminishing unit effect, the drained weight and bulk volume reached maxima between 5 and 10 min and, if anything, declined slightly thereafter. This unexpected departure from the close association of these texture parameters might be explained in terms of selective tissue maceration–disruption of the cell body but continued toughening of the cell wall.

The effects of the third variable–added calcium–are different again, as shown in Table 3. Continued response to calcium addition was experienced through 0.5% $CaCl_2$, or about 0.04% as Ca in the final product. Firmness was increased by 50% at this high level, and chewiness of the product developed to a very marked extent. Bulk volume, or plumpness of the fruit was likewise affected, but drained weight effects were inconsistent and on the average nil. Again, we can only suggest that the treatment is affecting cell walls rather than cell contents, and that to strengthen the framework is not necessarily to retain the fluids.

Table 4–Effects of hot-fill temperature on texture of canned cherries.

	Added Ca (%)	Hot-fill temperature (°F) 150	160	170	180	190	200	Avg
Extrusion force (kg)	0	54.0	45.0	43.8	38.6	34.4	35.2	41.8
	.02	70.9	67.4	59.4	58.0	51.6	52.8	60.0
Drained weight (%)	0	70.2	68.3	69.2	64.8	64.9	66.0	57.4
	.02	67.3	67.3	66.8	68.8	65.7	66.8	57.3
Bulk volume (L/kg)	0	.948	.920	.933	.878	.874	.882	.906
	.02	.950	.962	.934	.935	.910	.918	.934

Two replicates at each added calcium level.

Table 5–Effect of hot-fill temperature on color of canned cherries.

	Storage time	Hot-fill temperature (°F) 150	160	170	180	190	200
Hunter *a*:	1 day	17.2	19.0	20.4	21.2	20.8	20.9
	7 months	15.2	15.9	16.3	16.8	16.6	16.6
Hunter *L*:	1 day	24.8	25.8	26.0	27.1	26.0	26.6
	7 months	22.1	23.6	24.3	26.0	25.0	25.4
Optical density at 510 mμ:	1 day	3.72	3.81	4.09	3.77	3.93	3.92
	7 months	3.30	3.22	2.71	3.14	3.35	3.20

Table 6–Comparison of hot-fill and conventional processes for canned cherries.

	Hot-fill[b] only	Hot-fill + retort	Pretreatment[c] + hot-fill	Cold-fill, exhaust and retort
Extrusion force (kg)	31.0	26.1	48.7	31.8
Drained weight (%)	63.5	62.9	68.2	73.3
Bulk volume (liter/kg)	.880	.882	.932	1.008
Hunter color:				
a (1 day)	24.4	23.1	21.9	22.7
(7 months)	17.4	18.6	17.3	18.4
L (1 day)	27.6	28.2	27.0	26.8
(7 months)	26.9	26.8	25.3	24.7

[a] Average of six replicates at various levels of added calcium.
[b] All hot-filling at 180°F.
[c] Pretreatment–10 min at 140°F.

Hot-filling

Having demonstrated the practicality of firming the cherry tissue by carefully chosen conditions of pretreatment, we were then concerned with the requirements of the hot-fill itself. It is commonly understood that thermal processing is directed toward rendering harmless both microorganisms and potentially destructive enzymes. Of the two, the latter have been shown by Dastur et al. (1968) to be the more difficult to inactivate. While not dismissing the importance of avoiding microbial spoilage in even this very acid product, effective pasteurization seems assured if our hot-filling procedure maintains color and flavor integrity against the relatively heat-resistant peroxidase system. We experienced no apparent microbial spoilage at filling temperatures of 170°F and above.

In Table 4 the effects on cherry character of hot-filling temperatures in the range 150–200°F are presented. A notable softening was found, accompanied by diminishing drained weight and bulk volume, as the hot-fill temperature was increased up to 190°F, but the effect then leveled off at 200°F. As in the study of pretreatment temperatures in the immediately lower range, there was a close

association among the three texture parameters.

Included in these data is a differentiation between two levels of calcium that was incidental to the replication of this 1969-season experiment. Results are similar to those discussed from Table 3, showing a 50% increase in firmness and a corresponding improvement in bulk, but no effect on drained weight.

It was in this survey of hot-fill temperatures that the only pronounced differences in color were noted (Table 5). Both the L, or brightness, and a, or redness measurements obtained with the Hunter color meter improved as the hot-fill temperature was raised by 10-degree steps to 180°F. This variation was as apparent on the day following processing as 7 months later, though at different levels consistent with the expected deterioration of color during storage. These differences could readily be detected by eye, as well, with the products filled at the two lower temperatures of 150–160°F definitely oxidized and substandard. However, the optical density data contributed nothing to this picture.

The cherry pack hot-filled at 150°F smelled and tasted bad in three of the four replicates, though the cans were not swelled. The nature of this spoilage was not determined.

Process comparison

While we had earlier hoped that the carefully programmed pretreatment and hot-fill process would give a product better on all counts of character and color, the results were mixed. We compared 1) an abbreviated hot-fill with no pretreatment, 2) the same plus retorting and 3) the hot-fill with a normal pretreatment of 10 min at 140°F against 4) a conventional cold-fill (of an identical slurry) followed by exhausting and retorting. The data developed in Table 6 from this comparison replicated six times show that the programmed hot-fill did indeed improve, or at least toughen, texture. But it was characteristic of the product from the conventional process that the cherries were both plump and very tender, while the pretreated, hot-fill product was somewhat less plump but much chewier. There was little to choose between product colors in these two processes. As expected, a rapid hot-fill process gave a very poor texture, but also provided the best-protected color, even when followed by retorting. Apparently, further degradation of color did not occur to any great extent during the moderate cook.

The conventional process, unlike the accelerated hot-fill not preceded by a low-temperature pretreatment, should promote firming to some extent, particularly if heating during the exhaust operation is slow. The pitted fruit in the filled can warms up slowly and remains in the effective range of pretreatment temperature (120–150°F) for an appreciable time. That the cherries remained undisturbed once filled into the can was probably responsible for the retention of plumpness, even though firmness or tissue toughness was not most efficiently promoted. On the other hand, frequent stirring of the fruit in our hot-fill process may have damaged the fruit sufficiently to effect plumpness.

Brief trials conducted on hot-filling, using a pilot-plant model screw cooker, flooded with cherry juice and heated by direct steam injection, may have provided one answer to the problem of gentle bulk-heating. Pitted fruit conveyed in a semifloating state through the hot fluid could be heated rapidly or held at a uniform temperature without damage. However, this technique introduces other practical problems, such as the nature of the heating medium; cherry juice or sugar syrup has been suggested. It is also necessary to separate the solid product to obtain a uniform fill of container, though this may reintroduce air into the product.

In addition to these problems, there are microbiological and enzymatic ramifications that need further investigation. While adoption of a completely new process is premature in view of these unanswered questions, the information developed here may lead to useful modifications of existing hot-pack and pie-mix procedures.

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DIFFUSIONAL PROCESS IN THE DRYING OF TAPIOCA ROOT

SUMMARY—Experimental results obtained in the through-circulation drying of tapioca root slices were analyzed to obtain the effective diffusivity in the material. Results are in good agreement with the theoretical solution which assumes the diffusivity to be constant; however, for a better comparison, a model with variable diffusivity appears to be necessary. It can also be seen that as temperature increases, the constant diffusivity model represents closely the experimental results. The assumption that water migrates within tapioca root by a process of liquid diffusion is also confirmed by the Arrhenius type temperature dependence of calculated values of the effective diffusivity.

INTRODUCTION

ALTHOUGH MOST methods of tapioca drying are traditional and primitive, there is an impelling need to apply modern techniques, with the two-fold aim of increasing productivity and obtaining closer control of the process to achieve a uniform product. This requires that basic data on drying be available together with a knowledge of the fundamental principles involved. The present study was undertaken to obtain such data and to provide a basis for a detailed understanding of the process.

In previous research (Chirife et al., 1969) conducted to study the effect of external conditions on the through-circulation drying of tapioca root, it was noted there is no constant rate period. Therefore, the internal water movement is the controlling step from the beginning of the drying process. Two different mechanisms were also found to occur in the falling rate period; however, only one will be considered here because it takes place in the range of 66–14% water content–the usual range in commercial tapioca drying.

It will be assumed that Fick's Law is applicable to the movement of water in tapioca root; thus, for a rectangular parallelepipede,

$$\frac{\delta C}{\delta \theta} = D_x \frac{\delta^2 C}{\delta x^2} + D_y \frac{\delta^2 C}{\delta y^2} + D_z \frac{\delta^2 C}{\delta z^2} \quad [1]$$

where D_x, D_y, D_z represent the diffusion coefficients along rectangular axes x, y, z, respectively.

If the medium is isotropic,

$$D_x = D_y = D_z = D_e \quad [2]$$

D_e being the effective diffusion coefficient. For unidirectional diffusion (i.e., infinite slab) Eq. [1] becomes,

$$\frac{\delta C}{\delta \theta} = D_e \frac{\delta^2 C}{\delta x^2} \quad [3]$$

Sherwood (1929) solved Eq. [3] assuming: (1) the initial moisture distribution is uniform; (2) the diffusivity is constant; and (3) the resistance to moisture removal from the surface is negligible compared to the resistance to internal diffusion.

Solution is given by the well known expression that relates $\bar{E}$, the average fraction of evaporable water remaining in a slab of half-thickness, and the dimensionless term, $D_e\theta/a^2$:

$$\bar{E} = \frac{C - C_e}{C_o - C_e} = \frac{8}{\pi^2}\left[e^{-D_e\theta(\pi/2a)^2} + \frac{1}{9} e^{-9\, D_e\theta(\pi/2a)^2} + \frac{1}{25} e^{-25\, D_e\theta(\pi/2a)^2} + \ldots \right] \quad [4]$$

$\bar{E}$ can also be defined approximately in terms of moisture content, W. Thus, $\bar{E} = (\bar{W} - W_e)/(W_o - W_e)$, where W_o, W_e, and $\bar{W}$, are respectively the initial moisture content, the final equilibrium moisture content, and the average content at the time θ. Newman (1931) extended the mathematical treatment to different geometries other than infinite slab, and considered the most general situation where the surface resistance to mass transfer is not negligible compared to the resistance to internal diffusion. Figure 1 shows a graphical representation of Newman's solution for an infinite slab. $\bar{E}$ is shown as a function of $D_e\theta/a^2$ for different values of S, the mass transfer surface resistance ratio. The curve for S = 0 corresponds to the solution (Eq. [4]) given by Sherwood (1929). The use of Figure 1 to determine the coefficient D_e, requires the measurement of the time required for $\bar{E}$ to be reduced to some arbitrary value, say $\bar{E} = 0.4$. The value of $D_e\theta/a^2$ which is associated with $\bar{E} = 0.4$ is obtained from the particular curve of Figure 1 which applies. After $D_e\theta/a^2$ has been obtained, D_e can be calculated, since a, the half thickness of the drying specimen, and θ, the time required for $\bar{E}$ to decrease to 0.4, are known.

EXPERIMENTAL

Apparatus

Consists of a laboratory through-circulation drier previously described (Chirife et al., 1969).

Material samples

To facilitate subsequent calculations, pieces of tapioca root were cut in the form of rectangular parallelepipedes 0.3 cm thick, 3–4 cm wide, and 6–7 cm long. The thickness, relative to the other dimensions, is small enough to approach the condition of infinite slab.

RESULTS & DISCUSSION

IN THE DRYING of many common materials, a constant rate period is followed by a falling rate period in which internal liquid diffusion controls throughout. During the constant rate period there may set up an appreciable difference between the moisture contents at the surface and in the interior of the solid (Sherwood, 1932). Since Eq. [4] was derived on the assumption of uniform distribution of moisture in the solid at the start, it can be applied to the drying of tapioca, because, as previously demonstrated, the whole drying process takes place in the falling rate period.

The assumption of a constant effective diffusivity may or may not be a realistic approximation, depending upon the properties of the material to be dried. The differential equation of moisture diffusion which must be solved for the case where the effective diffusivity varies with the moisture concentration, is

$$\frac{\delta C}{\delta \theta} = \frac{\delta}{\delta x}\left[D_e \frac{\delta C}{\delta x} \right] \quad [5]$$

which reduces to Eq. [3] when D_e is constant. Unfortunately there is no general solution to Eq. [5] analogous to that for Eq. [3]. There are however a few numerical solutions for the case where

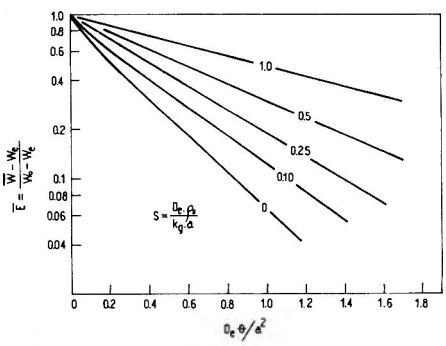

Fig. 1—Fraction of evaporable water remaining in a slab as a function of $D_e\theta/a^2$, for different values of the mass transfer surface resistance ratio.

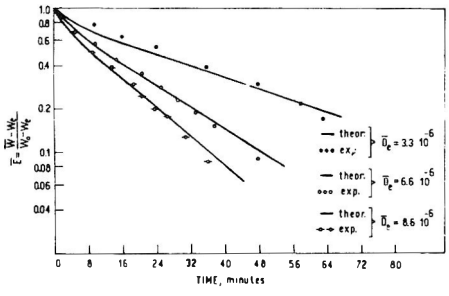

Fig. 2—Comparison of experimental data with theory.

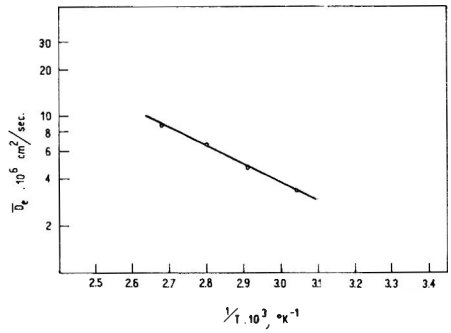

Fig. 3—Effect of temperature on effective diffusivity.

the surface resistance is neglected. Crank and Henry (1949) obtained numerical solutions for Eq. [5] for a number of cases where the diffusion coefficient varies in particular ways with the concentration. Crank and Henry (1950) also published a method for determining empirically the variation of D_e with concentration by observing the curve of $\bar{E}$ as a function of time. This curve is then compared with the theoretical curve, where D_e is presumed to be constant. By a series of approximations the behavior of D_e as a function of concentration is obtained.

The solution given by Eq. [4] assumes that external mass transfer resistance is negligible. This is easily obtained in practical air drying operations, because of the forced convection conditions at the surface, added to the low values of effective diffusivity. However, care must be taken before assuming that in through-circulation drying the external mass transfer resistance is always negligible. This is due to the low range of air velocity commonly employed in this kind of drying operation.

By analogy to unsteady heat transfer, the thermal diffusivity, $k/\rho_s \cdot C_p$ (cm²/sec) is analogous to the liquid diffusivity, D_e (cm²/sec). The surface resistance ratio of heat transfer associated with the well known Gurnie-Lurie charts, $k/h \cdot a$, is analogous to the mass transfer surface resistance ratio, $D_e\rho_s/kg \cdot a$. The mass transfer equivalence of the heat transfer is determined from:

$$\frac{h(t_a - t_s)}{L_v} = k_g(H_s - H_a) \qquad [6]$$

In order to evaluate the mass transfer surface resistance ratio, for our particular case, it is necessary to estimate the value of the mass transfer coefficient, as defined by Eq. [6]. This can be done applying the following relationship:

$$j_d = 2.0Re_o^{-0.50} \qquad [7]$$

where $Re_o = v_o \rho D_p/\mu$, and v_o is the air velocity based in the cross section of the bed. Eq. [7] represents the experimental results of solid-fluid mass transfer in the Reynolds range normally used in through-circulation drying (Chirife, 1968), and it has been theoretically justified according to a laminar boundary layer model.

Thus, for the air mass velocity employed in the drying runs, k_g becomes,

$$k_g = 0.028 \quad g/cm^2 \cdot sec$$

In order to evaluate D_e when a surface resistance is presumed to exist, it is necessary to proceed as follows:

(1) assume a value for D_e and evaluate the surface resistance ratio;

(2) Employing Figure 1 determine $D_e \cdot \theta/a^2$ for an experimental value of $\bar{E}$; and

(3) Evaluate D_e from this term and check with the assumed D_e.

Let us assume, $D_e = 10^{-6}$ cm²/sec; the surface resistance ratio becomes:

$$S = \frac{D_e \rho_s}{kg \cdot a} = \frac{10^{-6} \quad 0.84}{0.28 \cdot 0.15} = 0.0096 \cong 0 \qquad [8]$$

Application of the aforementioned trial and error method allows deducing that Eq. [8] is correct, and consequently the mass transfer surface resistance is negligible.

Application

At this point we have to assume that the slab thickness is constant, i.e., there is no shrinkage of the tapioca during drying. It is interesting, however, to mention that in the moisture content range considered, the observed shrinkage of tapioca is considerably smaller than that observed in other vegetable materials (i.e., apple, potato, etc.).

Effective diffusivities, D_e, can now be evaluated from the curve for S = 0 on Figure 1, since $\bar{E}$, geometric characteristics, and drying times are known. This was done repeatedly on the experimental curve. The values of D_e were averaged, giving an average effective diffusivity for each slab, $\bar{D}_e$, with about 6% relative deviation. This procedure was done for different air temperatures, ranging from 55–100°C. The experimental values of $\bar{D}_e$ were then used to test the assumption of constant effective diffusivity. Figure 2 compares the results based on theory and the corresponding experimental data. Log $\bar{D}_e$ is plotted against the reciprocal of absolute temperature, 1/T, in Figure 3. The results may be represented by an Arrhenius type relationship of the form:

$$\bar{D}_e = D_o \exp(-Q/RT) \qquad [9]$$

where D_o is a constant, Q is the energy of activation for diffusion, and R is the gas constant. The activation energy for diffusion was calculated to be, Q = 5.4 x 10³ cal/gram mole.

Figure 2 compares the theoretical and experimental results and shows a fairly good correlation of both curves. However, the curve of experimental data does not follow exactly the theoretical pattern, which indicates that the effective diffusivity cannot be considered constant. Also, it can be seen that as temperature increases, the constant diffusivity model approaches the experimental values.

Throughout this paper it has been assumed that liquid water migrates within tapioca root by a process of diffusion. The experimental evidence which supports this assumption consists in the exponential relationship between $\bar{E}$ and the drying time, and the Arrhenius type temperature dependence of calculated values of the effective diffusivity. It is interesting, however, to consider the experimental results in relation to other mechanisms of internal flow likely to operate: namely, capillary flow, viscous flow of water vapor, gaseous diffusion, Knudsen's flow, and thermal diffusion.

Krischer (1938, 1940, 1942, 1956) regards the early stages of drying as being controlled by capillary flow relationships. He used the following expression for correlating experimental data on transfer of moisture within the wet body, during the stage of pure capillary flow mechanism:

$$N_A = - k_w \rho_s \frac{dW}{dx} \qquad [10]$$

This Ohm's law type of expression is completely analogous to the simple diffusion law. The conductivity factor, k_w, lumps together the effect of surface tension and viscosity of the liquid, and the distribution of pore diameters within the material. Gorling (1958) found a good correlation between experimental

data during the first phase of drying of potato slices, and values of the quantity $\rho\,\sigma/\mu$, that is, the product of surface tension and density of water divided by the viscosity. This would be expected if the flow through the solid were determined by the capillary properties of the material. Experimentally, k_w is always found to be strongly dependent of the water content of the material. As might be expected, even slight changes in the internal structure of a material, such as those accompanying shrinkage, can change the moisture conductivity by several orders of magnitude; Gorling (1956, 1958) found the relationship between moisture content and conductivity in potato. This pattern is not quite in agreement with the approximate constant "conductivity" experimentally found during the drying of tapioca slices. On the other hand, the tendency of tapioca to exhibit a constant diffusivity with increasing temperature is similar to that observed by other authors during diffusional processes in vegetable solids; i.e., Krasuk et al. (1967) found that in oil extraction of tung seed by diffusion, the system tends to reach constant diffusivities as extraction temperature increases, independent of oil concentration in the solid.

The flow of gases in a capillary system is similar to that observed in diffusion through a solid medium and can be characterized by a permeability coefficient, P. According Poiseuille's law, in a capillary of diameter d and length l,

$$N_A = \frac{d^2\, g_c}{32\mu\, l RT}\, p_{av}(p_1 - p_2) \qquad [11]$$

Then, if the rate of flow is measured in terms of a gas volume V, at the average pressure flowing per unit time per unit cross section of the solid, Eq. [11] becomes:

$$p_{av}V = P\,\frac{p_{av}(p_1 - p_2)}{z} \qquad [12]$$

where z is the solid thickness, and P is the permeability, which dimensions are the same as those of a diffusion coefficient. Eq. [11] shows that P varies inversely as the gas viscosity, which in turn increases with temperature; P then varies with 1/T and not with exp(− 1/T). Therefore, the possibility of Poiseuille flow in the tapioca must be excluded.

Simple kinetic theory for gaseous diffusion gives the relationship between the diffusion coefficient, D, the mean thermal velocity $\bar{v}$, and the mean free path, λ, as:

$$D = 1/3\,\bar{v}\,\lambda \qquad [13]$$

But since $\bar{v}$ is proportional to $T^{1/2}$ the temperature dependence of D will be $T^{3/2}$, which is again different from the experimental value.

If the capillary diameters in tapioca are small in comparison with the mean free path of the gas molecules, flow will take place following Knudsen's law. For a single straight capillary of radius r, the Knudsen diffusivity is given by:

$$D_K = \frac{2}{3}\,r\,\bar{v} \qquad [14]$$

From Eq. [14] is seems that D_K varies with $T^{1/2}$, which is not the temperature dependence experimentally found.

The hypothesis of thermal diffusion is rejected because the effect is negligible for the small temperature gradients experimentally observed during the drying of tapioca root slices. On the basis of these comments we may conclude that the liquid water diffusion is the most appropriate mechanism to describe the drying behavior of tapioca root. However, it is interesting to compare the experimental results with other published data on the diffusion of water in food materials. The most detailed study of the behavior of water in a colloidal food material, especially from the standpoint of theoretical interpretation, is the investigation by Fish (1957) of the diffusion and equilibrium properties of water in a clear gel prepared from pure potato starch. Fish also obtained data (1958) on the diffusivity of water in the flesh of scalded potatoes; i.e., at 25°C the value for 0.2 moisture content is, $D_e = 2 \cdot 10^{-6}$ cm²/sec. The diffusivity is only slightly different from that of water in pure starch gel; this fact indicates that the cell walls and other non-starch constituents of potato have only a minor influence on the transport of water through the scalded material. Saravacos and Charm (1962) have reported experiments on the air drying of potato slices which also appear to be consistent with the molecular diffusion mechanism. The reported diffusivity of water in scalded potato was derived as a mean over a moisture range 1.0–0.10 for temperatures of 54.5°C ($\bar{D}_e = 2.5 \cdot 10^{-6}$ cm²/sec), 60°C, 65.5°C and 69°C ($\bar{D}_e = 6 \cdot 10^{-6}$ cm²/sec). The activation energy for this diffusion was estimated to be 12,500 cal/gram mole of water transferred in this moisture range.

The observed activation energy for the diffusion of water in tapioca root, Q = 5,400 cal/gram mole, is smaller than that reported by Saravacos and Charm (1962) (which could be partially attributed to the different moisture ranges considered), but is near to the value of Q = 6,300 cal/gram mole computed by Fish (1958) for the diffusion of water in starch gel at 14% moisture content. It is also comparable with the reported value of Jason (1958), Q = 7,190 cal/gram mole, for the first falling rate period of drying of fish muscle.

NOMENCLATURE

a	=	one half thickness of the slab, cm
C	=	water concentration, g/cm³
C_o, C_e, $\bar{C}$	=	initial water concentration, equilibrium water concentration, and average concentration at the time θ, respectively.
C_p	=	specific heat, cal/g°C
D_e	=	effective diffusion coefficient, cm²/sec
$\bar{D}_e$	=	average diffusion coefficient, cm²/sec
D	=	diffusion coefficient, cm²/sec
D_p	=	particle diameter, cm
d	=	capillary diameter, cm
$\bar{E}$	=	fraction of evaporable water remaining in the slab
h	=	heat transfer coefficient, cal/(sec) (cm²) (°C)
H_a	=	absolute humidity, g water/g dry air
H_s	=	saturated absolute humidity at wet bulb temperature, id.
j_d	=	Chilton & Colburn mass transfer factor, dimensionless
k_g	=	mass transfer coefficient, g/(sec) (cm²)
k_w	=	moisture conductivity, cm²/sec
K_D	=	Knudsen diffusivity, cm²/sec
k	=	thermal conductivity of the solid, cal/(sec) (cm) (°C)
L_v	=	latent heat of vaporization at t_s, cal/g
l	=	capillary length, cm
N_A	=	rate of flow g/(sec) (cm²)
P	=	permeability coefficient, cm²/sec
p_1, p_2	=	pressure, dynes/cm²
Q	=	energy of activation, cal/gram mole
R	=	universal gas constant, 1.98 cal/(gram mole) (°K)
Re_o	=	Reynolds number = $v_o \rho\, D_p/\mu$
r	=	capillary radius, cm
S	=	mass transfer surface resistance ratio = $D_e \rho_s / k_g \cdot a$
T	=	absolute temperature, °K
t_a	=	air temperature, °C
t_s	=	surface temperature, °C
v_o	=	air velocity based on the cross section of the bed, cm/sec
v	=	gas mean thermal velocity, cm/sec
W	=	moisture content, grams of water per gram of bone dry solid
W_o, W_e, $\bar{W}$	=	initial moisture content, equilibrium moisture content, and average moisture content at the time θ, respectively

Greek symbols

ρ	=	air density, g/cm³
ρ_s	=	dried solid density, g/cm³
θ	=	drying time, minutes
μ	=	viscosity, g/(cm) (sec)
λ	=	mean free path, cm
σ	=	surface tension, dynes/cm

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MONILINIA AND RHIZOPUS DECAY CONTROL DURING CONTROLLED RIPENING OF FREESTONE PEACHES FOR CANNING

SUMMARY—*Decay was controlled when freestone peach fruit, harvested at various stages of maturity ranging from green to straw-color, were dipped in 50% 2,6-dichloro-4-nitroaniline (DCNA) at 1-1/2 and 4 lb/100 gal water and ripened for 5–17 days at 20°C and 90% R.H. Treated fruit had less than 10% decay while untreated fruit developed as much as 61% decay during this period. Regardless of treatment, straw-blush and full-blush fruit held for only 3 days did not develop decay. The most commonly occurring fungus pathogens,* Monilinia fructicola *and* Rhizopus stolonifer, *were controlled with these treatments. Postharvest DCNA plus captan dip treatments gave more effective decay control than from preharvest field sprays. Concentrations of DCNA or DCNA plus captan required for* Monilinia *decay control, suggested by preliminary laboratory tests on fruit, were verified by these commercial-size experiments.*

INTRODUCTION

THE QUALITY OF freestone peaches (*Prunus persica* L.) for canning can be improved by picking fruit at a firm, mature stage and ripening in a controlled-environment (Leonard et al., 1957). However, conditions for optimum ripening (extended periods at 20°C and over 85% RH), promote development of postharvest decays. Therefore, some assurance of decay prevention is needed before controlled ripening of fruit for canning can become a standard practice.

Ogawa et al. (1964) found that decay of Fay Elberta peaches from the primary decay pathogens *Rhizopus stolonifer* and *Monilinia fructicola* was reduced by preharvest applications of 2,6-dichloro-4-nitroaniline (DCNA), or DCNA in combination with Difolatan. Also postharvest DCNA spray treatments reduced decay in mature but unripe fresh market stonefruit during storage and transit (Ogawa, 1965). DCNA was effective in control of *R. stolonifer* on fruit at deposits of 2 ppm, whereas higher deposits were required for control of *M. fructicola*. *Rhizopus arrhizus* was not controlled with DCNA treatments (Ogawa et al., 1963).

This paper reports tests for preventing decay of freestone peaches by treating the fruit before and after harvest with mixtures of DCNA and captan, DCNA and Difolatan, and DCNA alone.

MATERIALS & METHODS

THE FUNGUS PATHOGENS, *Monilinia fructicola* (Wint.) Honey, *Rhizopus stolonifer* (Fr.) Lind., and *R. arrhizus* Fischer isolated from decayed fruit in commercial orchards were used for laboratory studies. Chemicals used for laboratory tests, field sprays and postharvest dip treatments were 50% or 75%, 2,6-dichloro-4-nitroaniline (DCNA, "Botran," Upjohn Company, Kalamazoo, Michigan), 50% n-trichloromethylthiotetrahydrophthalimide (captan, "Orthocide;" Chevron Chemical Company, Richmond, California), and 80% N-(1,1,2,2-tetrachloro-ethyl-sulfenyl)-cis-Δ-4-cyclohexene-1,2,dicarboximide "Difolatan;" Chevron Chemical Company, Richmond California). Chemical treatments were made by hand atomization in the laboratory, by airblast sprayers in the field, and in specially designed dip-tanks holding 30 50-lb boxes of peaches.

Small scale laboratory tests with fruit

Tests to determine the concentrations of DCNA and captan necessary for decay control were made on eight mature but unripe Fay Elberta peaches for each chemical treatment (Table 1). 1 hr after surface-sterilization with sodium hypochlorite (400 ppm ^{C}l for 5 min), a shallow 1 mm dia puncture was made on the center of the right cheek (facing the suture) with a glass rod. The puncture was inoculated with 0.2 ml of *Monilinia* spores (20,000/ml), and various concentrations of the chemical suspensions were sprayed with a hand atomizer attached to an air-pressure line. Spraying was continued until the fruit was thoroughly covered but the liquid did not drip from the fruit. These fruit were placed in plastic containers with a false wire-mesh floor and incubated at 20°C. The lesion diameter was measured daily, and if chance contamination occurred, these fruit were removed from the experiment to avoid infection of adjacent fruit.

Small-scale commercial test in 1966

20 boxes (50 lb and about 100 fruit/box) of mature Kirkman Gem peaches were randomly selected from a commercially harvested lot. 5 boxes of fruit were immersed in chemical suspensions for 3 min for each of the three chemical treatments and 5 boxes to serve as control were not dipped. Treatments were DCNA at 2 lb, DCNA plus captan at 1 lb each, and DCNA plus Difolatan at 1 lb each/100 gal of water. One-half pint of liquid was reduced for each 50-lb box of fruit from the dip tank. The peaches were then trucked from the San Joaquin Valley to Salinas Valley and stored in an open shed with an average temperature of 21°C. Data on decay were taken 8 days after treatment.

Large-scale commercial tests

Shortly before harvest 1-1/3 lb of 75% DCNA plus 2 lb/100 gal of captan were applied at the rate of 400 gal/acre with a "Bean" airblast sprayer to 8 rows of Regular Elberta and 8

Table 1—Effect of DCNA, captan, and their combinations on lesion diameter in mm of Monilinia decay on Fay Elberta peaches.

DCNA concentration (10^{-4}M)	Captan concentration (10^{-4}M)					
	0	9	18	36	72	144
0	63.0	62.0	55.5	53.0[a]	32.0	44.3
	a[b]	a	ab	bc	f–h	c–f
9	55.5	36.6	40.1	12.4	35.5	15.7
	ab	e–h	e–g	m–r	g–i	l–p
18	20.1	11.7	6.8	6.2	9.4	5.6
	j–m	m–r	p–s	p–s	n–s	p–s
36	11.2	7.0	8.0	6.5	4.8	6.3
	m–r	q–s	p–s	p–s	q–s	p–s
72	8.7	6.9	6.5	8.1	5.8	4.2
	o–s	p–s	p–s	p–s	p–s	q–s
144	5.7	4.9	5.3	3.9	4.1	4.2
	p–s	q–s	q–s	q–s	q–s	q–s

[a] Areas within lines indicate similar range in lesion diameter.

[b] Statistical significance. Statistical groupings (P = .05) for chemical concentrations and combinations comparisons are shown by letters following the numbers. Values having a letter in common do not differ significantly.

[1] Dept. of Food Science & Technology; all others Dept. of Plant Pathology.

rows of Fay Elberta peach trees. The orchard was located in the Sacramento Valley. Fruit sprayed on July 12, 23, and August 4, were harvested on July 26, 29, and August 5, at which time the fruit was sorted according to color and the Magness test-pressure values to green, green-straw, and straw-colored fruit, respectively. The designation of maturity was as follows:

Maturity code	Pressure test (lb)*	Fruit color
M-1	15	green
M-2	13–14	green-straw
M-3	8–11	straw

*(Magness pressure tester using 5/16 in. tip. The range in maturity for Fay Elberta was uniform while that of Regular Elberta was not.)

Three replications of 30–40 lb boxes for a total of 90 boxes of fruit were harvested on each of three dates. In addition the same number of boxes of fruit were harvested from the adjacent unsprayed control plots for postharvest dip treatment which consisted of a 1-min dip in a suspension containing 2 lb each of 50% DCNA and 50% captan. The same number of boxes of control fruit were also harvested from the unsprayed plot.

The treated and untreated fruit were immediately placed in a ripening room at Libby, McNeil and Libby Cannery in Sacramento, California and held at 20°C and 55% RH. Disease data were obtained by examining 3 boxes of fruit from predetermined locations: top, middle, and bottom of each pallet of fruit. Fruit were recorded as diseased regardless of the size of the decayed area. (Table 2.)

Table 2–Amount of Monilinia and Rhizopus rots on Regular and Fay Elberta peaches treated before and after harvest with a 50% DCNA plus 50% captan mixture and ripened at 20°C and 55% RH.

Time of treatment	Harvest dates and amount of *Monilinia* rot			Harvest dates and amount of *Rhizopus* rot		
	7/26[a]	7/29[b]	8/5[c]	7/26	7/29	8/5
	%	%	%	%	%	%
Fay Elberta peaches						
Postharvest dip[d]	--	8.8a[f]	5.2a	--	0.8a	0.0a
Preharvest sprays[e]	--	51.2b	5.8a	--	0.7a	0.0a
Untreated control	--	87.2c	59.8b	--	0.6a	1.5b
Regular Elberta peaches						
Postharvest dip	1.2a	1.7a	2.2a	0.4a	3.4b	0.0a
Preharvest sprays	23.8b	54.2b	12.5b	3.5a	1.7a	0.2a
Untreated control	25.5b	44.0b	29.0c	10.2a	11.8b	2.0b

[a]7/26 harvested fruit (green-colored M-1) had field sprays 14 and 3 days before harvest and were ripened for 7 days.
[b]7/29 harvested fruit (green-straw colored M-2) had field sprays 17 and 6 days before harvest and were ripened for 6 days.
[c]8/5 harvested fruit (straw-colored M-3) had field sprays 24, 13, and 1 day before harvest and were ripened for 3 days.
[d]Postharvest dip, in 50% DCNA plus 50% captan at 2 lb each per 100 gal of water, made after transport from field to cannery.
[e]Preharvest spray applications using 1-1/3 lb of 75% DCNA and 2 lb of captan 50W.
[f]Statistical significance data were converted to arc sin $\sqrt{\text{percentage}}$ for statistical treatment. Statistical groupings (P = .05) for vertical comparisons are shown by letters following the numbers. Values having a letter in common do not differ significantly.

First large-scale tests in 1967

Tests were conducted with Fay Elberta peaches selectively hand picked to insure uniformity from unsprayed commercial orchards in the San Joaquin Valley near the city of Modesto.

Green-straw maturity fruit were harvested from the Olivera Orchard for studies on postharvest decay. The treatments were: (1) control; (2) 1-1/2 lb of 50% DCNA; and (3) 4 lb of 50% DCNA. Each treatment consisted of 10 40-lb boxes of fruit with three replications for a total of 30 boxes. The fruit were dipped for 3 min and placed in a ripening room at the University of California, Davis, and held at 20°C and 90% RH. (Table 3.)

Second large-scale test in 1967

Unsprayed fruit were harvested at different maturities (green, green-straw, straw, straw-blush, and full-blush) to study the effect of maturity on postharvest decay. These fruit were dipped for 3 min in 4 lb of 50% DCNA/100 gal of water and placed in a ripening room held at 20°C and 90% RH. Processing dates were determined by pressure tests. A University of California firmness pressure tester with a 5/16 tip was used to test for 6–7 lb for maturity code 3-1/2 (straw blush) and 3-1/2 lb for maturity code 4 (full blush). Prior to processing, the fruit were examined for decay; if any decay were present, the fruit was removed and weighed. For each processing date, 6 untreated and 6 treated boxes of fruit were examined. (Table 4.)

Table 3–Decay control of mature Fay Elberta peaches dip-treated with 50% DCNA and held in ripening room at 20°C and 90% RH for 10 days.[a]

Treatment, lb/100 gal water	Avg amounts of fruit weights			Avg amt of diseased fruit			Total disease	Amount cannable
	Field wt	Wt. lost in 10 days		*Monilinia*	*Rhizopus*	Other		
	lbs	lbs	%	%	%	%	%	%
Control	391.4	35.7	8.2NS[c]	22.0A[c]	1.6A	0.2NS	23.8	67.9A
DCNA[b] 1-1/2	387.6	29.4	7.6NS	0.9B	0.2B	0.0NS	1.1	91.1B
DCNA[b] 4	394.7	34.6	8.8NS	0.0C	0.5B	0.2NS	0.7	90.5B

[a]Fruits harvested at green straw (M-2) on August 13, 1967 at Olivers orchard in San Joaquin Valley.
[b]Active ingredients of DCNA is 50% 2,6-dichloro-4-nitroaniline. Residues immediately after dipping in concentrations of 1-1/2 and 4 lb in 100 gal of water were 16.07 and 33.65 ppm respectively and after 10 days of storage 16.12 and 51.98 ppm respectively.
[c]Data were converted to arc sin $\sqrt{\text{percentage}}$ for statistical treatment. Duncan's multiple-range test was used. Statistical grouping (P = 0.05) for vertical comparisons are shown by letters following the numbers. Values having a letter in common do not differ significantly. NS indicates no significant difference.

RESULTS & DISCUSSION

Laboratory in vivo tests

Table 1 compares the effectiveness of DCNA, captan, and their combinations on the suppression of lesion diameter of *Monilinia* on inoculated, treated fruit. DCNA concentrations of 18 x 10^{-4}M and captan at 72 x 10^{-4}M significantly reduced the size of lesion development, with DCNA significantly more effective. DCNA combined with captan was more effective than either chemical alone. Lesion diameters were comparable with 36 x 10^{-4}M DCNA, 18 x 10^{-4}M, DCNA plus 9 x 10^{-4}M captan and 9 x 10^{-4}M DCNA plus 36 x 10^{-4}M captan. Maximum effect of the chemical was shown with DCNA at 72 x 10^{-4}M, or combinations of DCNA at 36 x 10^{-4}M plus captan at 9 x 10^{-4}M or DCNA 18 x 10^{-4}M plus captan at 18 x 10^{-4}M. In terms of pounds of active chemical in 100 gal of water, maximum effectiveness was obtained with DCNA alone at 2.48 lb, or DCNA plus captan combinations of 1.24 plus 0.25 lb, respectively, or DCNA plus captan combination of 0.62 plus 0.51 lb, respectively. At concentrations tested, captan alone failed to control *Monilinia* as effectively as DCNA or DCNA-plus-captan combinations.

An identical test was made with DCNA and Difolatan. Synergistic effects

were also shown, though less striking than with captan.

Small-scale commercial test in 1966

Results from the initial small-scale dipping tests on Kirkman Gem peaches showed that out of 5 boxes of fruit for each treatment the nontreated fruit showed 3% *M. fructicola* and 16% *Rhizopus* rots. The chemical treatments of DCNA alone or in combination with captan or Difolatan resulted in no fruit decay.

Large-scale commercial test in 1966

Table 2 shows that, regardless of maturity, postharvest dip treatment effectively reduced *Monilinia* rot of Fay Elberta peaches which verifies the results of the small-scale tests. Two preharvest sprays gave significant reduction compared with the untreated control, but when fruit were harvested 6 days after the second spray, control was not as effective as a postharvest dip. Three field sprays, with the last spray applied 1 day before harvest, gave control equal to a single postharvest dip. Explanation for this result is found in previous published information that the half-life of DCNA residue in the field on fruit under an arid environment is 4–6 days and that about 10 ppm chemical residue is required for effective control (Ogawa et al., 1964). Figure 1 shows the development of both *Rhizopus* and *Monilinia* in the control box (A), *Monilinia* only in the two preharvest DCNA-captan spray treatments (B), and no decay in the DCNA-captan dip treatment (C).

Since the percent of *Rhizopus* rot was very low in the Fay Elberta experiment, the results were not significant; though control fruit harvested at straw color developed 1.5% decay, the postharvest dip and the preharvest spray-treated lots developed no decay.

On Regular Elberta, *Monilinia* rot was controlled more effectively with a postharvest dip treatment than preharvest spray treatments. As expected, *Rhizopus* rot developed, in an erratic fashion, with decay counts differing greatly between boxes because of the nesting effect. Postharvest dip or preharvest sprays were equally effective against *Rhizopus*. This result can be explained by the small amount of DCNA residue required for effective control of *Rhizopus* decay on peaches.

First large-scale test in 1967

Table 3 shows the percent decay of Fay Elberta fruit harvested at green-straw stage of maturity on August 13, 1967, trucked about 70 miles to Davis, dipped in DCNA suspension, excess liquid drained naturally, weighed, and placed immediately in the ripening room. No significant differences in weight loss were shown between the control and the DCNA field spray treatments although some shriveling was noticed on the upper layer of fruit treated at the 4-lb rate. No shriveling was noticed at the 1-1/2 lb rate. Significant reduction in decay from *Monilinia* and *Rhizopus* was shown with the DCNA treatments. In comparing the two concentrations of DCNA, 4 lb/100 gal afforded better control of *Monilinia* than the 1-1/2 lb, but no significant difference in decay from *Rhizopus* rot. Traces of decay from *Alternaria, Penicillium*, and *Aspergillus* were noted. The difference in amount of cannable fruit was very striking–only 67.9% for the control, compared with over 90% for the DCNA treatments. There was no significant difference in the amount of cannable fruit between the two concentrations of DCNA used.

Table 4–Percent loss in weight from fungus decay of Fay Elberta peaches harvested at various maturities, dipped, and stored in ripening rooms for various time limits.[a]

Fruit maturity and date of harvest	Decay pathogens	Number of days from harvest to canning on treated (T)[b] and nontreated (NT) fruits									
		T	NT	T	NT	T	NT	T	NT	T	NT
		%	%	%	%	%	%	%	%	%	%
		9		11		15		17			
	Monilinia	0.1	1.2	0.2	2.7	0.0	2.2	0.0	5.7		
Green (M-1)	*Rhizopus*	0.2	1.5	2.0	31.2	3.1	17.8	8.3	4.5		
Aug. 7	Others	0.0	0.5	0.2	0.8	0.3	0.9	0.7	10.8		
	Total	0.3	3.2	2.4	34.7	3.4	20.9	9.0	21.0		
		6		7		9		11		13	
Green-straw	*Monilinia*	0.0	1.8	0.1	0.7	0.0	7.4	0.0	5.6	0.0	30.4
(M-2)	*Rhizopus*	0.2	0.9	0.9	0.9	0.2	3.6	5.1	4.7	6.7	25.9
Aug. 11	Others	0.1	0.9	0.2	1.0	0.2	1.0	1.1	1.4	0.7	5.9
	Total	0.3	3.6	1.2	2.6	0.4	12.0	6.2	11.7	7.4	62.2
		1		3		5		7			
	Monilinia	0.0	0.0	0.0	0.2	0.2	2.7	0.0	7.1		
Straw-blush	*Rhizopus*	0.0	0.0	0.2	2.0	2.8	15.3	6.5	30.3		
(M-3 1/2)	Others	0.0	0.0	0.4	0.0	0.6	0.1	1.0	1.3		
	Total	0.0	0.0	0.6	2.2	3.6	18.1	7.5	38.7		
		1		3							
Straw-blush	*Monilinia*	0.0	0.0	0.0	0.0						
(M-3 1/2	*Rhizopus*	0.0	0.0	0.0	1.1						
Aug. 21	Others	0.0	0.0	0.0	0.0						
	Total	0.0	0.0	0.0	1.1						
		1		3							
Full blush	*Monilinia*	0.0	0.0	0.0	0.0						
(M-4)	*Rhizopus*	0.0	0.0	0.0	0.0						
Aug. 21	Others	0.0	0.0	0.0	0.0						
	Total	0.0	0.0	0.0	0.0						

[a]Fruits harvested at Yoshino orchard, San Joaquin Valley in 1967.
[b]Dip treated in 4 lb of 50% DCNA in 100 gal of water.

Second large-scale test in 1967

Table 4 shows the effects of 3-min dip treatments on various maturities of fruit with 4 lb of 50% DCNA in 100 gal of water. The fruit were in the ripening room until ready for processing, and examined for decay at that time. If any decay showed, the fruit were removed and weighed.

Decay control was effective in fruit harvested green (M-1), DCNA-dip-treated, and held for extended periods. After 11 days in storage, the percent total decay of untreated fruit rose rapidly from 3.2–34.7%, while decay of treated fruit increased from 0.4–2.4%. The only reversal in percent decay was shown on the 17th day when more *Rhizopus* developed in treated fruit than in untreated fruit. In this instance the organism involved was *Rhizopus arrhizus* (Ogawa et al., 1963), as was also the case in other instances where *Rhizopus* was observed.

Fruit harvested at the green-straw (M-2) stage of maturity showed a sudden increase in decay from the 9th to the 11th day. The treatment appeared effective at each inspection, and even on the 13th day the weight of decayed fruit was reduced from 62.3–7.4 lb.

No loss occurred in fruit harvested at straw color (M-3) and held in the ripening room for 1 day; losses were evident on the 3rd day and decay from *Rhizopus* was considerable on the 5th day as well as the 11th. The treatment gave effective control of *Monilinia* and *Rhizopus stolonifer* but not *Rhizopus arrhizus*.

The straw-blush colored fruit (M-3-1/2) showed only slight loss from *Rhizopus* on the 3rd day, and no loss of treated fruit. Fruit harvested at full-blush (M-4) showed no decay on the 1st or 3rd day of ripening.

The data clearly show the effectiveness of DCNA at 1-1/2 or 4 lb in the control of *Monilinia* rot. Of 12 lots of fruit, treated fruit showed no decay in 8 lots and the maximum decay in any lot was less than 1/4%. Control of *Rhizopus stolonifer* was perfect, but *Rhizopus arrhizus* accounted for losses as high as 8.3% on treated fruit. These tests indicate that green fruit (M-1) can be stored for 11–15 days, green-straw fruit (M-2) for 9 days, and straw-colored fruit (M-3) for 5 days. The straw-blush colored (M-3-1/2) and full-blush (M-4) fruit used in these tests did not require treatment to control decay during storage for 1 day before they were ready for canning.

Studies related to the quality of the final processed product from these chemical treatments on fruit of different maturities are forthcoming.

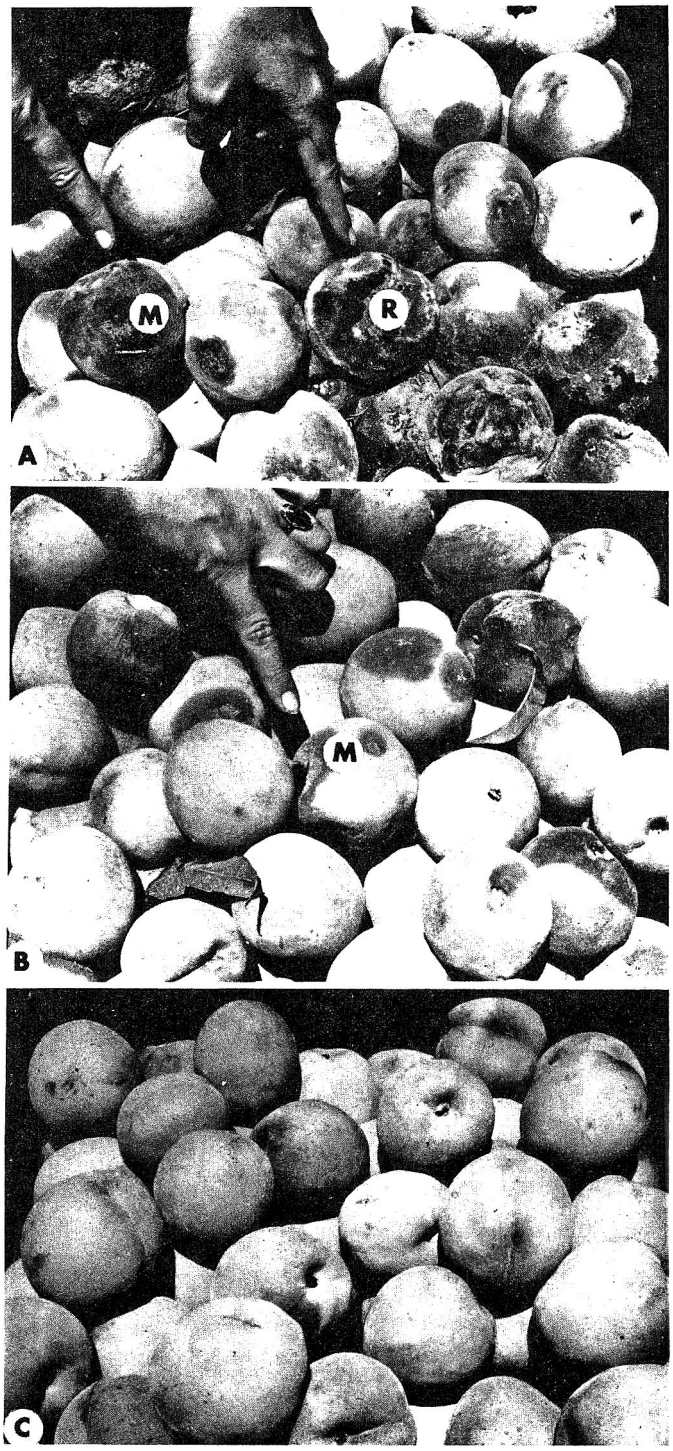

Fig. 1—Fay Elberta peaches harvested green-straw color and ripened for 6 days at 20°C and 55% RH. (A) No treatment lot showing Monilinia (M) and Rhizopus (R); (B) Two preharvest sprays 17 and 6 days before harvest showing only Monilinia (M); and (C) Postharvest dip-treated fruit showing no decay.

CONCLUSIONS

RESULTS from the laboratory *in vivo* tests clearly support the decay control results on peaches made on commercial-sized experiments which followed and served as a guide for the chemical concentrations to be tested. First, the chemical combinations of DCNA and captan at 0.62 lb and 0.51 lb, respectively, were altered to equal amounts of each chemical. Such tests, using 2 lb each of 50% proprietary DCNA and captan, gave excellent control of both *Monilinia* and *Rhizopus* decay. Both organisms were also controlled effectively in large scale tests using 1-1/2 or 4 lb of 50% proprietary DCNA in 100 gal of water. The lower dosage of DCNA gave significantly less *Monilinia* decay than the higher 4 lb dose. The dip treatments afforded better control of decay than did the preharvest field spray treatments except when fruit received three field treatments. Fruit harvested at maturities of green, green-straw, straw, and straw-blush ripened with little decay when given dip treatments of DCNA at 4 lb/100 gal. Fruit harvested at full-blush did not require decay control treatments. *Rhizopus arrhizus* continues to be important as a postharvest decay pathogen even though *Monilinia fructicola* and *Rhizopus stolonifer*, the primary rot pathogens, were effectively controlled.

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COMBINED EFFECT OF HEAT AND ALKALI IN STERILIZING SUGARCANE BAGASSE

SUMMARY—Bacterial spores contained in sugarcane bagasse were subjected to various combinations of heat exposure and alkali concentration and the rate of destruction determined for each set of conditions. A series of survival, thermal death time and alkaline destruction curves revealed a different mode of death by heat exposure than alkali treatment. Addition of alkali into the heating menstruum caused the death rates of bacterial spores to be much greater than with heat alone at a given temperature. Exposure of the spores to a temperature of 75°C for 130 min was required to reduce the spore population by 90% with heat treatment alone. Incorporation of a 1% NaOH solution into the heating menstruum effected the same degree of destruction of the spores within a 2 min period at the same temperature. From a series of thermal destruction and alkaline destruction curves, an empirical equation expressing the relationship between the death rate of bacterial spores, and the intensity of temperature and the concentration of alkali was established. The equation reveals that the death rate of bacterial spores is affected in an exponential manner by temperature and in a direct relationship by alkali concentration. Using the equation, sterilization time for various combinations of temperature and alkali concentration was determined and the overall correlation index between the experimental data and computed value was 0.877.

INTRODUCTION

A PROCESS for the conversion of sugarcane bagasse to microbial protein or single cell protein has been developed at Louisiana State University (Callihan et al., 1969). The process utilizes alkali treatment of bagasse, whereby the cellulose fibers are caused to swell and the bagasse is delignified, thus making the substrate more susceptible to enzymatic hydrolysis (Tarkow and Feist, 1969). After the alkali treatment, the bagasse is subjected to a series of heat treatments involving infrared radiation and high pressure steam before inoculating with the culture.

The alkali used for this process, sodium hydroxide, is a good dissolving and deflocculating agent and is considered to be the most germicidal of the commonly used alkalies. The germicidal effect of caustic soda has been recognized for some time (Dunn, 1950; Levine, 1952) and the alkali has been used for bottle washing and cleansing operations in various food processing plants (Greenfield, 1964). In spite of its common employment as a detergent, the scientific basis of its germicidal efficiency has not been well established.

In the present report an attempt has been made to establish the death rate of bacterial spores upon exposure to varying heat and alkali treatments in order to determine the time-temperature requirements of the steam utilized to sterilize the substrate.

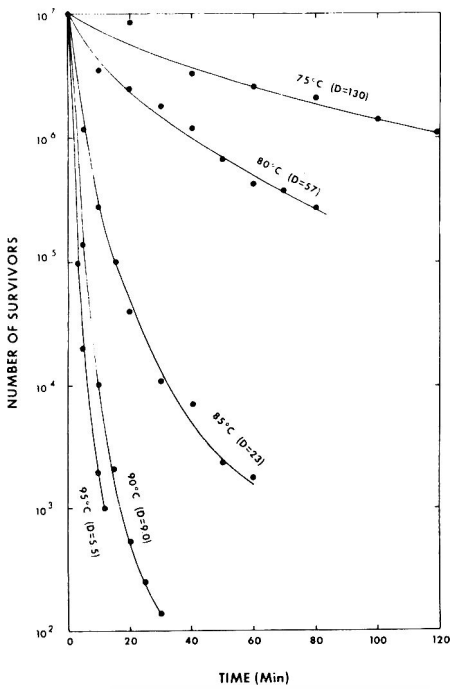

Fig. 1—Survival curves of bacterial spores subjected to 0% NaOH and various temperatures.

EXPERIMENTAL

Test organism

A spore forming bacterium was isolated from sugarcane bagasse by a series of enrichment and plating techniques and the organism was identified as a strain of *Bacillus cereus*. The organism was grown in nutrient broth and the culture was permitted to sporulate on nutrient agar. At the end of 7 days incubation at 30°C, spores were removed from the surface of the agar passed through sterile cheese cloth and collected in a Waring Blendor where they were agitated for 5 min. The spore homogenate was centrifuged for 20 min in an international B-20 centrifuge at 10,000 rpm and sediment containing the spores was resuspended in cold sterile distilled water and heated for 20 min at 75°C to kill the remaining vegetative cells. Alternate exposure of the material to blending and centrifuging was continued until a clean, uniform spore preparation was obtained. Microscopic examination of the preparation revealed a homogeneous population of spores with no detectable vegetative cells. This material was suspended in a small amount of cold distilled water with a layer of 4 mm glass beads and stored at 4°C until further use.

Determination of death rate

A 500 ml three-neck flask containing 100 ml of sterile caustic solution and 1.0g of washed sugarcane bagasse was heated and maintained at a predetermined temperature in a constant temperature oil bath as illustrated by Stumbo (1969). The caustic-bagasse mixture was agitated continuously throughout the experiment using a motor driven stirrer. A 1 ml volume of stock suspension was introduced through one neck of the flask at zero time. The volume of inoculum was so small that its introduction did not cause an appreciable reduction in the temperature or the concentration of the reaction mixture. In this manner, virtually instantaneous exposure of the inoculum to the selected temperature and alkali concentration could be attained. After inoculation, a 1 ml volume of the sample was withdrawn at predetermined time intervals and diluted immediately into a 9 ml volume of chilled water containing sufficient HCl to neutralize the effect of alkali on the test organism. After appropriate dilution in 0.1M phosphate buffer, pH 7.0, the sample was subcultured on Brain Heart Infusion Agar for 3 days and the number of survivors counted.

Analysis of data

Survivor curves for the spores of the test organisms were constructed following exposure of spores to various combinations of time, temperature and alkali concentration. Decimal reduction times (D value) were determined for each set of conditions. The D values were derived from the straight line portion of the survivor curve, since the survivor curves did not yield a straight line throughout the logarithmic cycles, especially in early phase of death when alkali was incorporated. The D values were then incorporated into thermal destruction curves and alkaline destruction curves in which log D values were plotted against temperature and log alkali concentrations, respectively. From a series of thermal destruction and alkaline destruction curves, an empirical equation expressing the relationship between death of organism and heat and alkali concentration applied was developed. To evaluate the fitness of experimentally obtained data to the equation, the correlation index, r^2, was determined using the following formula:

$$r^2 = 1 - \Sigma(D_o - D_c)^2 / \Sigma(D_o - \bar{D}_o)^2$$

where D_o = experimental D values, D_c = computed D values, $\bar{D}_o$ = average experimental D values.

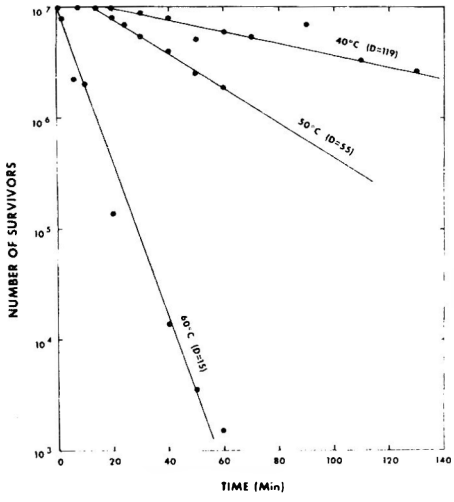

Fig. 2—Survival curves of bacterial spores subjected to 1% NaOH and various temperatures.

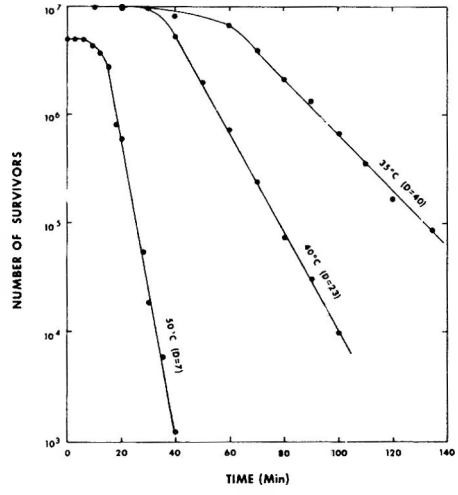

Fig. 3—Survival curves of bacterial spores subjected to 2% NaOH and various temperatures.

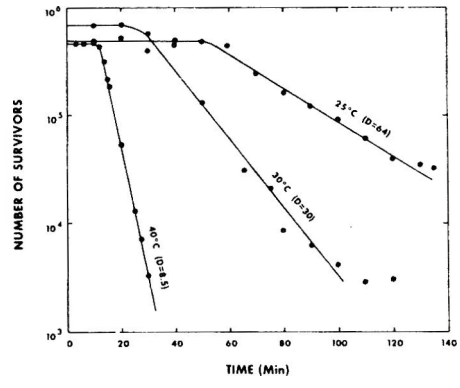

Fig. 4—Survival curves of bacterial spores subjected to 4% NaOH and various temperatures.

RESULTS & DISCUSSION

DATA IN Figure 1 show that the survival curves of bacterial spores subjected to heat were generally logarithmic in nature, even though the organisms showed a tendency to be more resistant as the time of exposure increased as reflected in the tailing of the curves. A mathematical expression describing the increased heat resistance of bacterial spores during the prolonged heating period was proposed by Han and Zhang, (1970). As shown in Figures 2, 3, 4 and 5, the survival curves of the spores exposed to the combination of heat and alkali at much lower temperature showed a plateau in early phases of the death period.

The information of Figure 6 shows that the slope of the thermal death time curve in the absence of NaOH differed from those obtained at the various alkali concentrations. In the absence of NaOH the z value, the degree of temperature required for the thermal death time curve to traverse one log cycle, was 13°C; the z values for the curves at each alkali concentration were 18°C. However, there was no significant difference in the slope of the curves among the alkali concentrations of 1–6%.

The data in Figure 6 also show that the sterilization time can be reduced greatly by incorporating alkali into the heating menstruum. With heat treatment alone a period of 130 min was required at 75°C to reduce the population by 90%, but the same degree of destruction could be achieved in 2 min at the same temperature by addition of 1% NaOH into the heating menstruum.

Alkaline destruction curves were constructed in a similar manner to the thermal destruction curves, plotting log D vs alkali concentration. The resulting curves showed a curvelinear relationship between the log D and alkali concentration, while the thermal death time curves showed a linear relationship between log D and temperature. The alkaline destruction curves, however, revealed a straight line when log D values were plotted against log alkali concentration (Fig. 7). The differences in the nature of the survival curves, the changes in the slope of the thermal destruction curves and different relationships between D values and temperature and D values and alkali concentrations indicate a different mode of death of bacterial spores by heat exposure than by alkali treatment.

From the thermal destruction curves (Fig. 6) and the alkali destruction curves (Fig. 7), an empirical equation expressing the relationship between bacterial death and heat and alkali concentration was

Table 1—Experimental and computed D values.

Temp. (°C)	Alkali Concentration (%) 0	1	2	4	6	8	10
Experimental D values							
25	–	–	–	64.00	–	–	–
30	–	–	–	30.00	14.00	–	–
35	–	-	40.00	–	7.50	–	–
40	–	119.00	23.00	8.50	3.80	–	–
50	–	55.00	7.00	–	–	–	–
60	–	15.00	–	–	–	–	–
70	–	–	–	–	–	–	–
75	129.00	–	–	–	–	–	–
80	56.80	–	–	–	–	–	–
85	22.80	–	–	–	–	–	–
90	9.00	–	–	–	–	–	–
95	5.50	–	–	–	–	–	–
100	–	–	–	–	–	–	–
Computed D values							
25	–	727.91	193.18	51.27	23.60	13.61	8.88
30	–	389.00	103.24	27.40	12.61	7.27	4.74
35	–	207.89	55.17	14.64	6.74	3.89	2.54
40	–	111.10	29.48	7.83	3.60	2.08	1.35
50	–	31.73	8.42	2.23	1.03	.59	.39
60	–	9.06	2.40	.64	.29	.17	.11
70	–	2.59	.69	.18	.08	.05	.03
75	–	1.38	.37	.10	.04	.03	.02
80	–	.74	.20	.05	.02	.01	.01
85	–	.39	.10	.03	.01	.01	–
90	–	.21	.06	.01	.01	–	–
95	–	.11	.03	.01	–	–	–
100	–	.06	.02	–	–	–	–

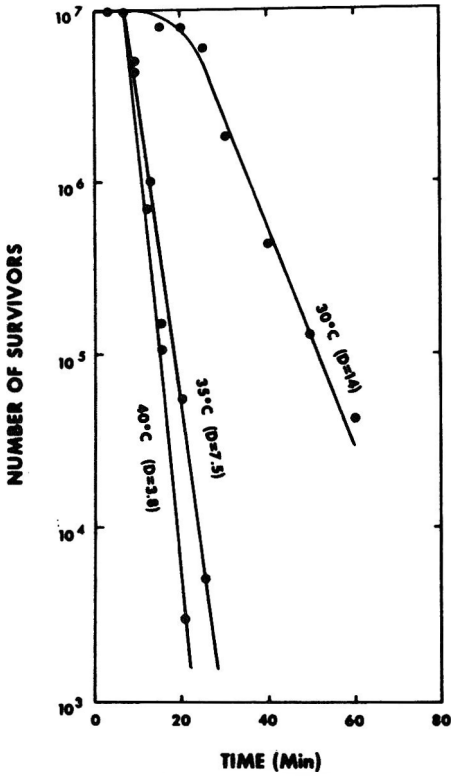

Fig. 5–Survival curves of bacterial spores subjected to 6% NaOH and various temperatures.

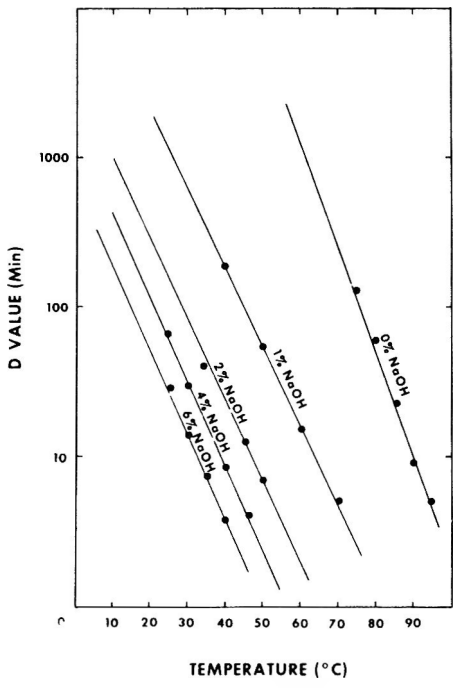

Fig. 6–Thermal destruction curves of bacterial spores at various alkali concentrations.

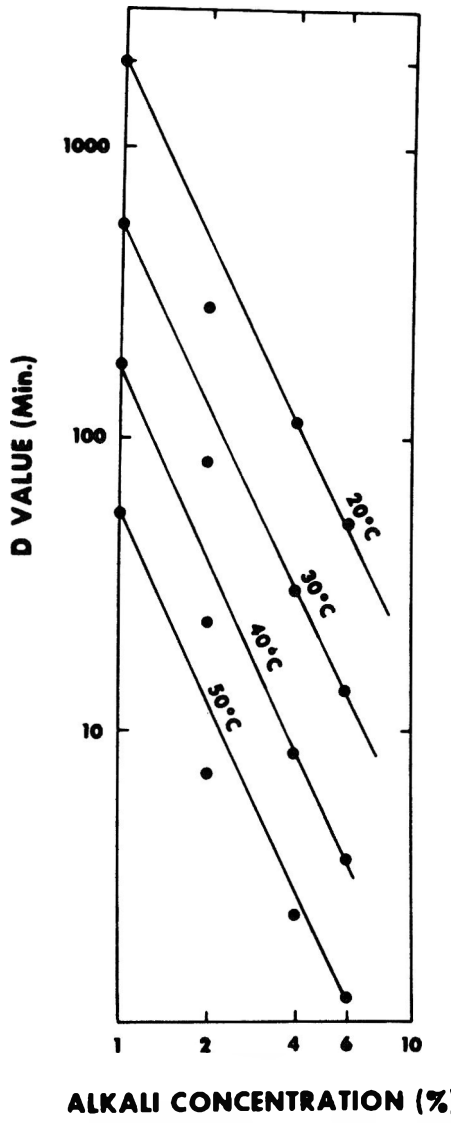

Fig. 7–Alkaline destruction curves of bacterial spores at various temperatures.

developed as follows:

From thermal destruction curve

$$\text{Log } D = m_1 T + \log b \quad [1]$$

Where:

- D = 90% destruction time (min) at any temperature and at any alkali concentration
- m_1 = average slope of thermal destruction curve
- T = temperature (°C)
- b = D value of any alkali concentration at 0°C

From alkaline destruction curve

$$\text{Log } D_o = m_2 (\log C) + \log a \quad [2]$$

Where D_o = D values at 0°C

- m_2 = average slope of alkali destruction curve
- C = alkali concentration (%)
- a = D_o at 1% alkali concentration

Since b = D_o, substitute (2) into (1):

$$\text{Log } D = m_1 T + m_2 (\log C) + \log a \quad [3]$$

$$\text{or } D = a \cdot C^{m_2} \cdot 10^{m_1 T} \quad [4]$$

m_1, m_2 and a are characteristic of each bacteria. These values for the test organism were as follows:

m_1 = −.054425
m_2 = −1.913775
a = 26,000

The equation, $D = a \cdot C^{m_2} \cdot 10^{m_1 T}$, expresses the relationship between the death rate of bacterial spores and the concentration of alkali and the intensity of the temperature applied to kill the organism. From the equation, it is apparent that the temperature plays a significant role since the temperature term in the equation affects the death of the organism exponentially. The concentration of alkali, however, directly influences the death of the organism; therefore, the effect of increasing alkali concentration is not as significant as increasing temperature for killing the bacterial spore.

Since the terms a, m_1 and m_2 in the equation are characteristics of individual organisms, they must be determined experimentally. Once these terms are obtained, utilizing the minimum amount of experimentation, sterilization time can be calculated for any combination of temperature and alkali concentration. Table 1 illustrates the D values obtained experimentally and by calculation using equation (4). As the table shows, these two values agree closely.

To evaluate the accuracy of the fit of the experimentally-obtained data to the equation, the correlation index, r^2, was determined. The correlation indices of 0.79, 0.49, 0.89 and 0.95 were obtained for 1%, 2%, 4% and 6% NaOH solutions, respectively. The r^2 values were high for all concentrations of alkaline solutions except for the 2% caustic solution. The overall correlation index was 0.877. Therefore, the empirical equation closely expresses the relationship between bacterial death, intensity of temperature and concentration of alkali employed to destroy the organism.

In the process of single cell protein production from cellulosic waste the alkali treatment is not only effective to facilitate the degradation of cellulose but also contributes greatly to the reduction of heat requirement to sterilize the substrate. The alkali effect in heat sterilization is more pronounced with lower concentrations of alkali.

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A Research Note
RADIATION DESTRUCTION OF *Vibrio parahaemolyticus*

DATA ARE available in the literature on the resistance of food pathogens to radiation in broth, in buffer and in complex media (Erdman et al., 1961; Dyer et al., 1966). These data were published prior to the first isolation of *Vibrio parahaemolyticus* in the United States by Liston et al. (1967) and Baross and Liston (1968) from Puget Sound and Washington coast sediments. This food pathogen has since been isolated from seafoods in the United States by Baross and Liston (1970). Radiation pasteurization has been proposed as a means of prolonging the shelf life of seafoods and has been found to be a useful and feasible process by Masurovsky et al. (1963) and Spinelli et al. (1965). To be effective as a means of prolonging the storage life of a food, radiation must be sufficient to free the food product from low numbers of pathogens which might be present and grow during possible mishandling of the product.

This is a report of the destruction of *Vibrio parahaemolyticus* by irradiation. Twenty-seven *Vibrio* strains obtained from Dr. Sakazaki, National Institute of Health, Japan; Dr. R. R. Colwell, Georgetown University, Washington, D. C., and strains isolated from sediment, seawater and shellfish in the Puget Sound area were tested. The organisms were suspended in 0.1% peptone made up with seawater, in fish homogenate (English Sole, *Parophrys vetulus*) made up with both seawater and fresh water and in fresh picked crab meat (*Cancer magister*). Fish homogenate was made up by blending 1 part fish and 4 parts seawater in a Waring Blendor.

Numbers of viable cells surviving irradiation were determined at 22°C by the drop plate method with trypticase soy agar medium made up with seawater or by the pour plate method with the same medium. A starch-containing medium (Baross and Liston, 1968) was used in the crab meat experiments for differentiation of the *Vibrio* inoculum from the other flora. Samples were irradiated in the Cobalt 60 Mark II food irradiator at a dose rate of 300 Krad/hr and a temperature of 24°C.

Twenty-seven *Vibrio* strains suspended in seawater peptone were irradiated with from 0–40 Krads. Fifteen strains suspended in seawater fish homogenate were irradiated with from 0–90 Krads. Six strains were suspended in fresh water fish homogenate and irradiated with from 0–30 Krads. Thirteen strains were inoculated onto fresh picked crab meat and irradiated with from 0–100 Krads. These dose ranges were chosen to give from very little to almost complete destruction of the test organisms in the four test media.

Table 1–Reduction of Vibrio parahaemolyticus after irradiation in peptone water, fish homogenate, and crab meat

	Seawater + 0.1% Peptone		Seawater Fish Homogenate		Freshwater Fish Homogenate		Crab Meat	
Strain	Dose, Krad	Log Reduction	Dose, Krad	Log Reduction	Dose, Krad	Log Reduction	Dose, Krad	Log Reduction
K-3	30	4.7	30	3.3	10	2.7		
K-4	30	4.3	30	2.7	10	2.3		
K-12	30	6.6	30	5.6	10	4.1	25	5.4
K-15	30	6.7	30	5.9	10	4.0	25	4.7
SAK-1	25	4.9	30	3.0	10	4.6	25	2.3
SAK-2	25	4.9						
SAK-3	25	4.7					50	4.6
SAK-4	25	4.7	30	3.0	10	3.6	25	2.9
SAK-6	30	5.9	30	3.1			75	4.9
SAK-7	30	5.3						
SAK-8	30	5.6						
SAK-9	30	5.7	30	2.8			100	2.8
SAK-10	30	4.7						
SAK-18	30	6.8						
SAK-19	30	6.5						
SED-2	30	6.5						
SED-4	20	6.5	30	5.8			25	4.5
SED-12	30	4.8	30	3.3			25	3.8
6604	30	6.8						
6267	30	4.7						
6651	30	6.1	30	4.0			50	5.0
SW16	30	5.9						
SW50	30	6.0						
OY310	30	6.2	20	5.2			75	3.8
OY14	20	4.5	30	6.1			50	3.3
OY32	30	6.2						
OY26	–	––	20	4.9			25	1.9

The results of up to five replications of duplicate samples for the four media are shown in Table 1. At 20–30 Krads in seawater peptone all strains tested were reduced 4–7 logs from 10^7 organisms/ml; and in fish homogenate made up with seawater they were more resistant and were reduced 2.7–6.1 logs. In fresh water fish homogenate at 10 Krads the six strains tested were reduced rapidly 2.3–4.6 logs from an inoculum level of 10^7 cells/ml. In most cases complete destruction was obtained with 30–40 Krads. It is not known whether the rapid destruction was due entirely to radiation damage or in part to the undesirable osmotic properties of the fresh water fish homogenate since *Vibrio parahaemolyticus* requires salt. Salt in the seawater fish homogenate would satisfy the osmotic requirements but it might also offer some protection to the irradiated organisms. The test organisms inoculated onto nonsterile crab meat were approximately twice as resistant as those in fish homogenate made up with seawater. Reduction varied from 2.8 logs at 100 Krads to 5.4 logs at 25 Krads, but sensitivity to gamma irradiation was still quite high.

Irradiation was especially lethal at very low doses for *Vibrio parahaemolyticus* in the media tested. Sensitivity varied between strains but appeared to depend

upon the suspending medium, as was reported also for *Staphylococcus aureus* by Slabyj et al. (1965). Thus, it appears that *Vibrio parahaemolyticus* can be controlled easily in seafoods by exposure to pasteurizing doses of irradiation.

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DETINNING IN CANNED TOMATOES CAUSED BY ACCUMULATIONS OF NITRATE IN THE FRUIT

SUMMARY—The 1965 experimental crop of Floradel variety tomatoes accumulated no nitrate and removed only about 15% of the tin from their containers in a 2-yr storage period. The 1966 Florida crop of Homestead 24 variety tomatoes accumulated from about 50–80 ppm in the fruit and removed about 70% of the tin from their cans. All fertilizer application variables except the lowest resulted in about the same level of nitrate accumulation. The nitrate in most of these variables was exhausted in about 6 mo and the rate of detinning leveled off soon after. The correlation between loss of nitrate and tin removal was significant at the 5% level. Detinning in these cans approached a "problem level" in that about 70% of the tin was removed in 18 mo. In the 1965 tomato crop at Ohio State University there was no apparent connection between the levels of applied nitrogen and the nitrate accumulated in the fruit. In the 1966 Ohio tomatoes, the treatments with no applied nitrogen accumulated less nitrate in the fruit than the other treatments with varying levels of nitrate fertilization. The differences among the detinning histories of the various treatments followed in a general way the differences in nitrate accumulation. Detinning in individual cans varied between extremely wide limits. There were no differences apparent among the treatment variables in the 1965 crop. In the 1966 tomatoes the zero applied nitrogen treatment consistently showed less detinning than the others. In both the 1965 and 1966 crops the nitrate in the canned samples was exhausted after about 2 mo of storage. Essentially all of the detinning in these tomatoes occurred during the first 6 mo. Regression equations from pooled results of field studies and work on nitrate fortification of non-aggressive tomatoes suggest that an initial nitrate concentration of the order of 100 ppm can constitute a rapid detinning problem in tomatoes in a 303 can having a tin coating weight of 1.00 lb/bb.

INTRODUCTION

RAPID DETINNING in canned food products caused by nitrate was studied in an extensive research program sponsored by the tin plate producers, the Can Manufacturers Institute, and the National Canners Association. The organization of the program, container and tin plate specifications for the cans used throughout the study, and the general plan of the research were previously described by Farrow (1970). A study of the action of nitrate in model systems simulating foods of various acidities (Farrow et al., 1970) was a part of this coordinated program.

Johnson (1966) reported a study of the effect of high nitrate content in canned tomatoes and green beans on the internal can corrosion of plain tin plate containers. Hoff (1970) investigated factors which influence nitrate accumulation in tomato fruit.

This paper reports studies on experimental tomato packs canned at Ohio State University and the University of Florida, and analyzed at the laboratories of the National Canners Association as part of the cooperative program mentioned.

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EXPERIMENTAL

Field procedures

Work at the University of Florida extended over two seasons. In 1965, spaced applications of nitrogen were used for the production of nine experimental packs differentiated on the basis of harvest time and fertilization rates. The total nitrogen in lb/acre was 92, 167, 232; 153, 288, 414; 214, 349, 596 for three harvests, representing early, midseason, and late picking, respectively. Floradel variety was used.

In 1966 there were two harvests, each using five fertilization levels. Total nitrogen applications were 348, 428, 508, 468 and 588 lb/acre for both the first and second harvests. Homestead 24 variety was used.

Field studies on tomatoes were carried out at Ohio State University in the 1965 and 1966 seasons. The experimental plan utilized in 1965 made provisions for two harvests each consisting of ten fertilization treatments. There were five levels of total applied nitrogen, a native soil level plus four applications ranging from 80–480 lb/acre. In five of the treatments all of the nitrogen was applied at planting. A second set of five variables provided for the same quantities of nitrogen but in split applications. The total pounds of nitrogen per acre therefore corresponded to 0, 80, 160, 240, 480, 40/40, 80/80, 120/120, 240/240 and 0/80 for each of two harvests.

In the 1966 season the plan was similar, but with fewer nitrogen treatment variables. There were three harvests each using seven fertilization treatments. The total pounds of nitrogen per acre were 0/0, 80/0, 160/0, 320/0, 0/80, 0/120, 0/240 for each of the three harvests. For both seasons Heinz 1370 variety was used.

Methods

The experimental tomato packs were grown and canned at the University of Florida and Ohio State University. "Zero time" controls were canned in enamel-lined containers and frozen immediately. They were shipped to the Washington, D.C. laboratory of the National Canners Association for storage and examination.

Analytical data collected included headspace, vacuum, net weight, pH and mineral composition. Nitrate content in the canned products was followed until the nitrate was exhausted. This amounted to a storage period of 2 mo in the case of the Ohio tomatoes and about 6 mo in the case of the Florida tomatoes. The tin content was followed during storage periods up to 24 mo at ambient temperatures.

Tin was determined polarographically by the method of Condliffe and Skrimshire (1961). Nitrate was determined by an m-xylenol procedure adapted from A.O.A.C. methods (A.O.A.C. 1965). Mineral analysis was carried out by atomic absorption spectrophotometry using a Jarrell-Ash spectrophotometer.

RESULTS & DISCUSSION

Effect of fertilization on the composition of tomatoes

The Floradel variety of tomatoes grown in the 1965 experiments did not accumulate nitrate. Average nitrate concentrations were less than 5 ppm in most instances. Although the fertilization schedule for this crop included applications considered to be in excess of normal practice, the soil analysis carried out by the Gulf Coast Experiment Station indicated that these plants were actually deficient in nitrogen and potassium as the tomatoes approached maturity. The 1965 Florida experiment was unsuccessful in accumulating nitrate in the fruit, but the tomatoes proved to be useful in serving as a source of nitrate-free samples and in providing detinning histories on tomatoes known to contain little or no nitrate when canned.

In the 1966 Florida experiment the Homestead variety accumulated substantial quantities of nitrate. The nitrate concentrations in the frozen processed controls in the first harvest, tended to be somewhat lower than those obtained in the second harvest. The only real difference among the various fertilization treatments, however, was obtained in the lowest nitrate application rate of 348 lb N/acre. Differences among the other treatment variables were within sample variation. The 348 lb N/acre treatment, however, provided only a single application of nitrogen within a few days after

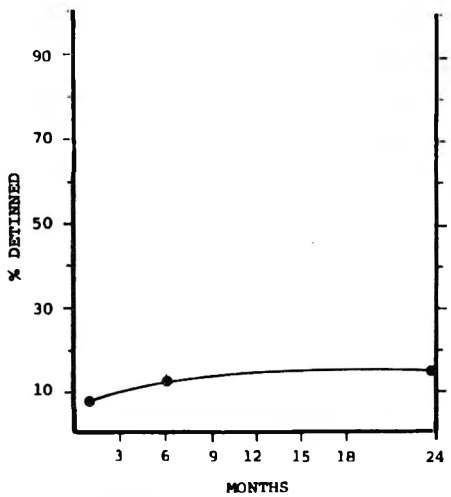

Fig. 1—Detinning in low nitrate tomatoes. Third harvest 1965 Florida packs with initial nitrate near zero.

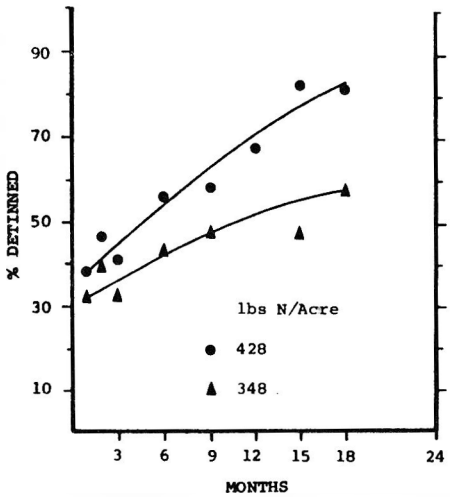

Fig. 2—Detinning in 1966 Florida first harvest tomatoes. Initial nitrate 53 ppm in 348 lb level and 66 ppm in 428 lb level.

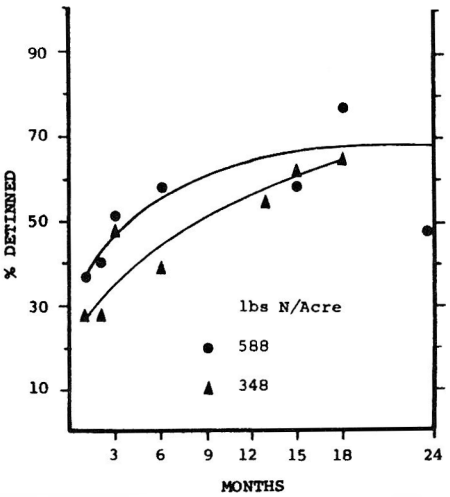

Fig. 3—Tin removal in second harvest 1966 Florida tomatoes.

seeding, and these treatments accumulated smaller quantities of nitrate than all others in the 1966 experiment. The increased fertilization rates used in the other variables resulted in only a slight increase in the amount of nitrate accumulated in the fruit, although nitrate applications at the highest levels were almost double those in the 348 lb N/acre treatment.

In the 1965 Ohio experiment, only modest amounts of nitrate accumulated in the Heinz 1370 tomatoes. In the frozen processed controls nitrate concentrations ranged from 15–37 ppm. The nitrate accumulations in the 1966 tomatoes are of the same order of magnitude, ranging from 6 to about 30 ppm. In both seasons there was a very high can-to-can variation. Great variability in nitrate accumulation characterized all of the studies on tomatoes carried out as a part of this project.

There were few differences among the nitrate concentrations of the various fertilization treatments that could not be accounted for by sample variation. One treatment in the 1966 packs exhibited such a difference. The zero nitrogen variables, 0/0 lb N/acre for each of the three harvests, consistently showed less nitrate in the frozen controls and frozen processed controls than the variables receiving higher levels of nitrate fertilization. Increasingly higher levels of applied nitrogen, however, did not result in an appreciable increase in the amount of nitrate accumulated in the fruit. Thus it would appear that while the availability of nitrate in the immediate environment of the plant is a condition necessary for nitrate accumulation, excessive amounts of nitrogen will not necessarily result in the accumulation of larger quantities in the fruit.

Detinning histories

The detinning histories of the 1965 and 1966 Florida tomatoes are presented in Figures 1 and 2, respectively. Each of the points in the figures represents from 3–8 cans which were individually analyzed and averaged. Tin concentrations are expressed as "percent detinned," the fraction of the available tin coating taken up by the tomatoes. The variability in detinning was much larger in 1966 than in the 1965 packs, due to the fact that the 1966 cans detinned to a much greater extent.

In the 1965 packs only a few ppm of nitrate accumulated in the fruit. There was no difference in detinning behavior among any of the fertilization levels, nor was there any difference in behavior among the three harvests. The single curve in Figure 1 which presents tin removal data from the third harvest of 1965 is representative of the data from all fertilization levels of all three harvests.

After 24 mo of storage the containers were only about 15% detinned in all treatments. Since there was no more than a few ppm of nitrate present initially, the curve in Figure 1 may be regarded as representative of detinning behavior in tomatoes in the absence of corrosive plant constituents.

The Homestead variety of tomatoes utilized in the 1966 experiment did accumulate substantial amounts of nitrate in the fruit. Concentrations in the frozen processed controls ranged from 53–78 ppm. The detinning data in the first harvest 1966 tomatoes have been collected in Figure 2. Five different fertilization treatments were utilized in each harvest. Among these treatments, only the first exhibited detinning behavior significantly different from the others. The lower curve in Figure 2 plots data from the treatment of 348 lb N/acre. This variable received an application of nitrogen a few days after seeding, and no further nitrogen applications were made throughout the growing season. The 428 lb N/acre treatment shown in Figure 2 was identical with the other three treatments in detinning behavior.

The same differentiation among treatments occurred in the second harvest tomatoes whose detinning histories are shown in Figure 3. The 348 lb N/acre treatment, the lowest nitrate application, exhibited a detinning pattern somewhat less than that of the other four, in keeping with its somewhat lower initial nitrate content.

The internal can corrosion of the 1965 Ohio State samples was characterized by great can-to-can variation, as was the nitrate concentrations encountered in the controls. The detinning histories do not show any discernible differences among all of the various nitrogen application variables. In Figure 4 the detinning histories of two of the treatments, 160 and 120/120 lb N/acre, have been plotted for the second harvest samples. These are representative of the medium range of detinning encountered in all levels of both the first and second harvests. Each point in the Figure is the average of 5 cans.

The nitrate content of the frozen processed controls for the 1966 Ohio tomato crop ranged from 6–30 ppm. The zero nitrogen application treatment accumulated less nitrate than the other variables in all three harvests. The zero nitrogen variables also exhibited less detinning. The difference is clearly apparent in the first and second harvests and a smaller difference is found in the third harvest.

Figure 5 compares the detinning history for the zero nitrate treatment in the

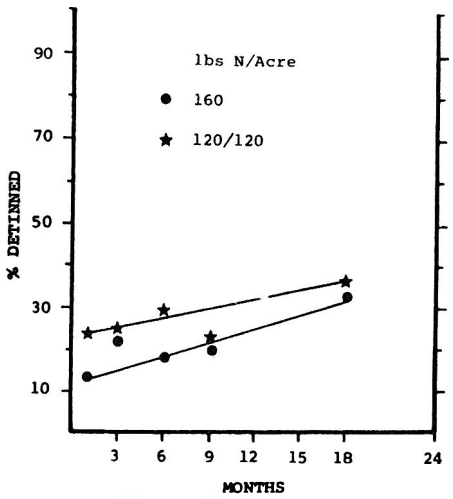

Fig. 4–Detinning histories for second harvest 1965 Ohio tomatoes.

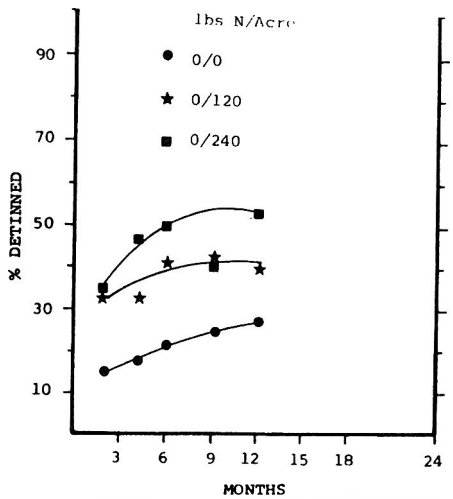

Fig. 5–Representative detinning histories for first harvest 1966 Ohio tomatoes. Minimum detinning occurred in the zero nitrogen variable.

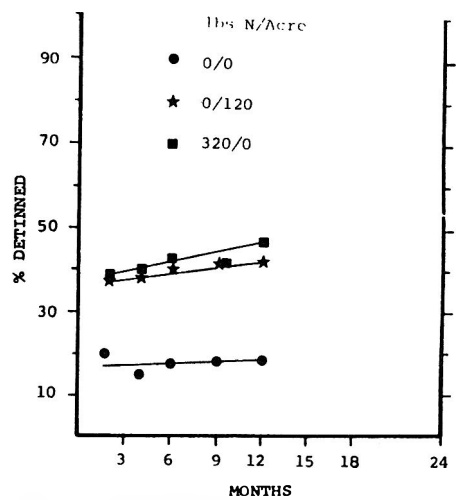

Fig. 6–Representative detinning histories for second harvest 1966 Ohio tomatoes.

first harvest tomatoes with the detinning rate of the remaining first harvest variables. The "late" nitrogen application treatment of 0/120 lb N/acres has been used to represent the detinning histories of the other first harvest variables. The maximum detinning rate exhibited by the first harvest tomatoes was that of the 0/240 lb N/acre treatment, also a late nitrate application variable. The detinning curve for this variable is also shown in Figure 5.

In the second harvest 1966 Ohio State samples the zero nitrogen treatment also exhibited less detinning than the higher nitrate treatments. (Fig. 6.) The zero nitrogen variable has been compared with the treatment of 0/120 lb N/acre, the same nitrogen application variable utilized in Figure 5. Among the second harvest samples, maximum detinning was displayed by an "early" nitrogen application variable treated with 320 lb/acre. In these samples the 0/240 lb N/acre treatment, comparable to the maximum detinning variable displayed in Figure 5, exhibited a detinning rate only slightly greater than the zero nitrogen level, in contrast with the detinning performance displayed by this variable in the first harvest samples.

The difference between the 1966 zero applied nitrate treatments and the detinning in the other treatments is probably real. Differences among the variables with higher nitrate applications, however, did not follow a consistent pattern. They were small in relation to the sample variation, and it is clear that statistically significant differences among different levels of applied nitrogen, other than the zero level, would not be demonstrated in these samples.

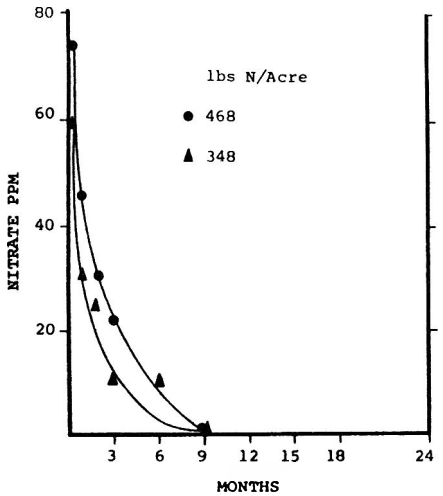

Fig. 7–Loss of nitrate during detinning of second harvest 1966 Florida tomatoes.

Loss of nitrate with storage

In the 1965 Florida tomato packs there was less than 9 ppm of nitrate, expressed as NO_3^-, in the frozen controls. The 1 mo storage examination revealed that these small amounts had completely reacted during processing and storage. No nitrate was detected in any of the cans.

In the 1966 Florida tomato packs between 53 and 78 ppm of nitrate, expressed as NO_3^-, accumulated in the frozen processed controls. At 6½ and 9 mo, for the first and second harvests, respectively, no nitrate remained. Each of the nitrate values for the various examinations are the average of five cans. Figure 7 shows the loss of nitrate with storage for the second harvest samples. In this figure, two nitrate/storage-time curves have been used to illustrate the data from all five of the fertilization levels. The other three curves would fall between the two that are shown. Similar considerations would apply to the first harvest nitrate/storage-time curves. The curves for the second harvest correspond to the levels of 468 and 348 lb N/acre. The time required for exhaustion of the nitrate, about 6½ mo for the first harvest and 9 mo for the second harvest, corresponds roughly with the point at which the detinning rate curves begin to level off in Figures 2 and 3.

The 1965 Ohio State tomato packs contained between 15 and 37 ppm of nitrate in the frozen processed controls. At the 1-month cutting most of these samples had lost roughly half of their initial nitrate content, and at the 3-mo cutting the nitrate was exhausted in nearly every can. A similar situation existed in the samples canned in 1966. The initial nitrate concentration varied from 6–30 ppm. These samples were not examined until 2 mo after canning, and at this cutting most of the cans had no detectable nitrate.

Relation between loss of nitrate and detinning

Utilizing the data from the 1966 Florida tomatoes, it is possible to calculate a regression equation relating the percent detinned to the loss in nitrate. The Florida tomatoes provide 30 paired values of "delta nitrate" (loss during storage) and percent detinned, excluding paired values from cans in which the nitrate had already been exhausted at a previous examination. The values are averages of five cans in nearly all instances. The resulting regression equation and correlation coefficient are given below:

$$\% \text{ DeSn} = 0.214(NO_3^-) + 32.4$$
$$r = 0.41$$

The data are quite scattered, as is indicated by the relatively low correlation coefficient. This value, however, is statis-

tically significant. Snedecor (1937) lists values of 0.36 at the first 5% and 0.46 at the 1% level of significance for correlation coefficients with 28 degrees of freedom. These tomatoes were transported for a distance of about 1,500 mi during the first month of their storage life. This corresponds to the most active period of both nitrate and headspace detinning, and the increased agitation of the canned contents during transportation may have resulted in a substantially increased rate of tin removal during this initial period. This may have contributed to the variation in the data, and to the low slope of the regression equation.

Relation between initial nitrate and detinning

The detinning research program (Farrow, 1970) produced detailed analyses of approximately 2000 individual samples of tomatoes in 303 cans with enameled ends and 1.00 lb electrolytic tin-coating weights. These data may be utilized to calculate regression equations relating the initial nitrate concentration (at zero time) to the extent of tin removal during ambient temperature storage periods of 6, 12 and 18 mo. In addition to the experimental packs from Florida and Ohio we have included results from a number of experimental packs produced at Purdue University in 1965 season. These tomato packs contained no nitrate. The results from these Purdue samples are not otherwise discussed here or elsewhere, but the detinning histories of all variables were virtually identical with those shown in Figure 1.

The following 6, 12, and 18 mo regression equations were calculated for the above sets of tomato packs.

$$\text{At 6 mo \% DeSn} = 0.52(NO_3^-) + 18.1 \qquad r = 0.76$$

$$\text{At 12 mo \% DeSn} = 0.65(NO_3^-) + 22.2 \qquad r = 0.94$$

$$\text{At 18 mo \% DeSn} = 0.79(NO_3^-) + 14.5 \qquad r = 0.95$$

The 6-mo equation implies that nitrate concentrations of about 140 ppm would be required for complete detinning (90% detinned) in 6 mo. The highest nitrate concentrations obtained in the field studies carried in this project did not reach these levels except in a few individual cans.

The 12-mo regression equation predicts essentially complete detinning after 12 mo in tomatoes with an initial nitrate concentration of about 100 ppm. In fact the 1966 Florida tomatoes had removed virtually all of the tin from the cans in this period of time. The 18-mo regression equation is similar since the nitrate had been exhausted in all of the cans prior to the 12-mo examination.

The regression equations from the pooled results of the field studies suggest that an initial nitrate concentration of the order of 100 ppm can constitute a "rapid detinning problem" in tomatoes in a 303 can. Of course the reaction rate depends on a number of factors in addition to the initial nitrate concentration. The detinning rate would be expected to increase with decreasing pH (Farrow et al., 1970). Other obvious rate-influencing factors include storage temperature and the degree of agitation. Transportation of the canned product over an appreciable distance may accelerate the rate of tin removal as long as the active detinning agent is present.

Detinning in tomatoes with added nitrate

Experimental work with buffered nitrated solutions (Farrow et al., 1970) was required to define the pH conditions under which nitrate detinning might be important, and to establish the major reaction products. Experiments with canned products "fortified" with known quantities of nitrate were also needed to provide information on the quantity of nitrate required to cause rapid detinning, and to provide comparisons of detinning rates in buffered nitrate solutions with those in the canned product. This work required experimental material of known history having very low or zero nitrate levels present when canned. Canned samples of the 1965 Florida studies were ideal for this purpose. Since they had no detectable nitrate, part of these samples were utilized to prepare canned tomatoes with known quantities of nitrate added.

Using aseptic techniques, the tomato cans were punctured and sterile sodium nitrate solution was added to each. The opening was then sealed with a drop of solder. Similar aseptic techniques are used routinely in the N.C.A. laboratories for microbiological investigations of spoilage problems by inoculating cans with known numbers of spoilage organisms.

This technique could admit some oxygen to the headspace of the can. To compensate for this, a series of control cans were prepared in an identical manner with distilled water added in a volume equal to that of the nitrate solutions. Subsequent examinations indicated that the quantity of oxygen admitted was quite small. The aseptic technique requires that the top of the can be flamed with a gas burner to prevent the entrance of organisms that might spoil the product. In this process the headspace gases are heated sufficiently to minimize the vacuum inside the can, and as a result very little oxygen is actually admitted.

The quantities of added nitrate were intended to provide final concentrations of 0, 25, 50, 100, and 150 ppm based on a net weight of 450g. The actual net weight of these cans after addition of the nitrate averaged about 467g.

At the time that the fortification experiments were initiated, the 1965 Florida tomatoes had accumulated about 12 mo storage. These tomatoes removed very little tin from the cans throughout the entire storage period. At the time that the aseptic nitrate additions were made, these cans were approximately 13% detinned as a result of normal headspace detinning.

The results of the examination of duplicate cans at the indicated storage intervals are displayed in Figure 8, while Figure 9 shows the loss in nitrate during the same period.

In the control cans to which distilled water had been aseptically added, the small amount of oxygen admitted during the fortification process resulted in only a slight increase in tin removal, indicating the amount of oxygen admitted to the headspace was relatively small.

In the 25 and 50 ppm levels the nitrate

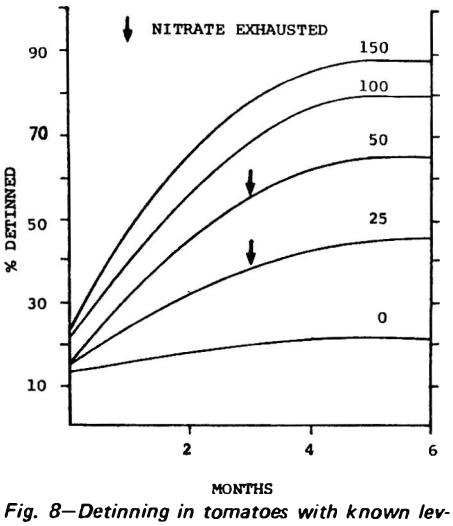

Fig. 8—Detinning in tomatoes with known levels of aseptically added nitrate.

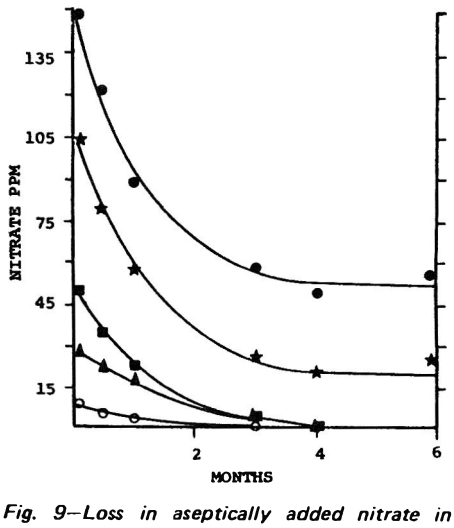

Fig. 9—Loss in aseptically added nitrate in tomatoes.

had been exhausted at the 3-mo cutting, and the tin analyses show that the detinning process leveled off at about that time.

In the 100 and 150 ppm variables, about 25 and 57 ppm of nitrate remained at the 6 mo examination. These cans were completely detinned at this cutting. The detinning rate of the 25 ppm level tapered off after about 50% of the tin had been removed, while the 50 ppm level removed somewhat less than 70% of the tin.

Making due allowance for the headspace detinning in these cans, it would appear that less than 100 ppm of nitrate can constitute a serious detinning problem in the tomatoes in 303 cans having an interior tin coating weight of 1.00 lb/bb. This estimate agrees well with that obtained from field studies. In larger containers, detinning problems could be encountered at substantially lower levels due to the lower surface-to-volume ratio. It is evident that any quantity of nitrate in the tomatoes will act to reduce the shelf life. Quantities in the neighborhood of 50 ppm could result in areas of exposed base plate after approximately 1 yr of storage. It is also evident that in tomatoes, and very probably in other products of a similar pH, detinning will continue until either the nitrate or the tin is exhausted.

The following regression equation relating the percent detinned to the loss in nitrate during storage of the tomatoes with added nitrate was obtained:

$$\% \text{ DeSn} = 0.742(NO_3^-) + 16.7$$
$$r = 0.95$$

It was computed from 17 paired values of delta nitrate and percent detinned, excluding data from cans in which the nitrate had been exhausted at the preceding cutting.

The equation implies that a change in nitrate content of 113 ppm is required for complete tin removal in 303 cans with 1.00 lb/bb interior tin-coating weight. This corresponds to about 25 ppm of nitrate nitrogen. From stoichiometry, approximately 20 ppm of nitrate nitrogen would be sufficient to remove all of the tin from the interior of these cans if the nitrates were reduced completely to ammonia. We have shown (Farrow et al., 1970) that reduction products other than ammonia may be formed as a result of the detinning reaction.

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EVALUATION OF MONOSACCHARIDES, DISACCHARIDES AND CORN SYRUPS AS DISPERSANTS FOR HEAT-PROCESSED DRIED SOY MILK PROTEINS

SUMMARY—Addition of carbohydrates such as dextrose, sucrose or corn syrups to heat-treated soy milk before drying significantly improved the redispersibilities of the resultant dried products, reaching about 100% at 1.0–1.5 times as much sugar as soy milk solids. Among carbohydrates tested, corn syrups, expecially those enzyme-converted in the region of 48.5 D.E., seemed to be suitable materials because of high dispersant efficiency, moderate sweetness and easier drying. One possible reason for the dispersant effects of sugars is suggested to be a physical separation of soy protein molecules. Effects of corn syrups upon the spray-drying of soy milk also are discussed.

INTRODUCTION

SOY MILK has been given considerable attention as an economical nutritive beverage suitable for overcoming protein malnutrition of infants in developing countries. Supplying soy milk as a dry powder might have advantages of convenience and economy because of easier transportation and preservation along with centralized large-scale production.

One of the most important qualities for dried soy milk is a high degree of redispersibility in water. However, when the powdered product is prepared from heated soy milk, large amounts of proteins are insolubilized during the drying process (Van Buren et al., 1964). In a recent paper, Fukushima and Van Buren (1970a) suggested polymerization through disulfide bonds and hydrophobic bonding as mechanisms for this insolubilization during drying. Insolubilization was increased at high concentrations of soy milk solids.

It has been found in many studies that sweetening of soy milk remarkably enhances its organoleptic acceptability. While the level of sugar depends upon the consumer preferences in any particular market area, almost all commercially available bottled soy milks are sweetened with cane sugar nearly equal in concentration to that of the soy milk solids. In some cases, the sugar supplementation may be helpful in overcoming calorie undernutrition.

During the course of an investigation on the effect of sugar on soy milk, our attention was called to the much better redispersibility of powdered soy milk prepared from the sweetened milk. This work was undertaken to clarify the action of sugars on solubility of proteins in dried soy milk, and to investigate the possible application of corn syrups for the preparation of easily soluble and moderately sweetened soy milk powder.

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EXPERIMENTAL

Soy milk

Freeze-dried unheated soy milk prepared from Harosoy 63 variety was provided by Dr. D. Fukushima (Fukushima and Van Buren, 1970a). The material was prepared as follows: Whole soybeans were soaked in water for 1 night, rinsed, ground with Rietz disintegrator (Model No. Ra-4-53) with addition of tap-water so that the total amount of water was 10-fold the original soybean weight and filtered on a filter press. All the processes were carried out at room temperature. The unheated soy milk prepared thus was freeze-dried. The chemical analysis of the material indicated the following composition: moisture 2.79%, crude protein (Kjeldahl N × 5.71) 41.90% and crude fats 24.8%.

Carbohydrates

Corn syrups of different dextrose equivalents (D.E.) were supplied by CPC International Inc., Englewood Cliffs, N.J. Other sugars were purchased commercially.

Heating and drying of soy milk

Heating and drying of soy milks were carried out as follows: An 8.0% (w/v) soy milk solids solution was prepared from the freeze-dried soy milk. Samples (20 ml) of the soy milk were refluxed at 100°C for 10 min or for 90 min, then cooled in running tap-water for 5 min. During the heating process, losses of about 1 and 3% nitrogen for 10 min and 90 min of heating, respectively, were observed, associated with formation of a "skin" on the surface of the soy milk. These corrections were applied to the milk used for drying. The resultant soy milk was agitated vigorously and filtered through Agway milk filter pad No. 87-0440 (Agway Inc., Syracuse, N.Y.). Samples of the filtrate which contained 331 mg of protein were transferred into a 250-ml beaker and dried in a constant-temperature room (50°C, R.H. = 54%) for 20 hr.

Measurement of redispersibility of dried soy milk

The method reported previously (Fukushima and Van Buren, 1970a) was modified as follows: To the 250-ml beaker described above, in which the dried soy milk was contained, distilled water was added to obtain a protein concentration of 0.83%. After shaking for 3 hr at 20°C on a 170-rpm rotary shaker, the suspension was transferred into a volumetric flask and brought up to 50 ml with water. Then it was filtered through the same type of filter pad just described. The nitrogen in the filtrate was determined by the semimicro-Kjeldahl method. The redispersibility of dried soy milk was expressed as percent of the total nitrogen appearing in the filtrate.

RESULTS

Effects of monosaccharides

Effects of addition of glucose, fructose and galactose prior to drying upon the redispersibility of the dried product are summarized in Figure 1. It appears that the protein redispersibility of the 90-min heated soy milk with no sugar added was higher (40 > 17) than the 10-min heated, no-sugar milk. The dried product from unheated soy milk usually indicates about 80% protein dispersibility. At all heating conditions tested, almost all nonnitrogenous substances seemed to be resuspendable in water. As shown in Figure 1, almost no difference in slope is noted between the 2 heat treatments; similarly, no difference due to type of sugar is noted. Also, the amounts of sugar necessary to approach 100% redispersibility were almost the same for all 3, about 12 g sugar per 100 ml of 8.0% (w/v) soy milk solids. No difference in the effects of sugars could be recognized between times of the additions, before or after heating. Additions of these sugars after drying did not show any positive effect on the redispersibility of dried soy milk.

Effects of disaccharides

Effects of sucrose, maltose and lactose were summarized in Figure 2. Sucrose and maltose performed equally well and

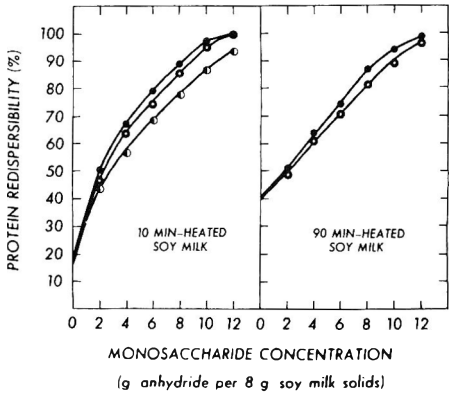

Fig. 1—Effects of some monosaccharides on redispersibility of dried soy milk proteins. ○Glucose, ●fructose, ⊙galactose.

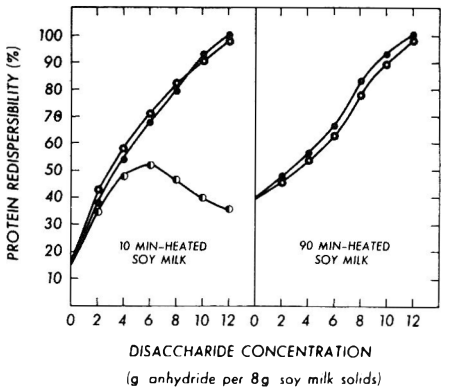

Fig. 2—Effects of some disaccharides on redispersibility of dried soy milk proteins. ○Sucrose, ●maltose, ◐lactose.

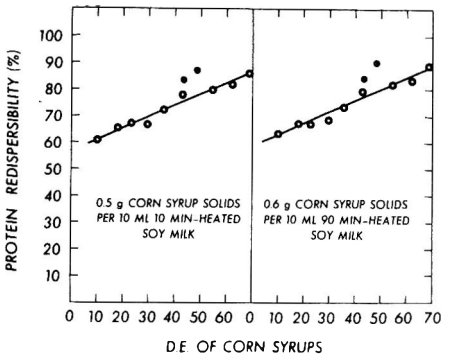

Fig. 3—Relationship between dextrose equivalents (D.E.) of corn syrups and dispersant effects on soy milk proteins. ○Acid conversion, ●enzyme conversion.

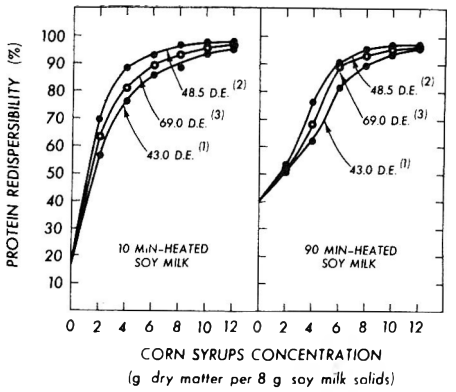

Fig. 4—Effects of some corn syrups on redispersibility of dried soy milk proteins. (1) Mor-Sweet No. 1435 (enzyme conversion). (2) Mor-Sweet No. 1535 (enzyme conversion). (3) Globe No. 1637 (acid conversion).

were comparable to the monosaccharides just described. However, lactose showed a much different behavior and at no concentration did it give anything near 100% redispersibility. Since lactose itself did not show any effect of insolubilization on soy milk proteins before drying of soy milk, this may have been due to the lower solubility of this sugar, leading, perhaps, to sugar crystallization during the drying process.

From the practical viewpoint, maltose, or a mixture of maltose and sucrose, in various ratios might give a suitable organoleptic sweetness and full dispersibility to the dried soy milk at the same time, but experience indicates that some technical difficulties usually accompany the spray drying of these sugars.

Effects of corn syrups

Commercially available corn syrups are economical sweeteners. They have several advantageous properties in connection with the improvement of redispersibility of dried soy milk. First of all, they allow faster spray-drying rates than are possible with other conventional sugars such as dextrose, sucrose or maltose. Actually, lower-conversion syrups have been proven successful as drying aids for other sugars. Secondly, the great variety in their organoleptic sweetness allows arbitrary selection of corn syrups for sweetness, independent of the amounts of sugars required for a full redispersion of soy milk solids.

A clear relationship between the dispersant efficiencies of corn syrups and their D.E. values was demonstrated as shown in Figure 3. In general, as the D.E. increases so does the redispersibility level. Figure 4 shows the relationship between concentrations of 3 types of corn syrups and the redispersibility of dried soy milk proteins. At lower concentrations, the enzyme-converted type seems to be more effective than the acid-converted type; however, for each heat treatment, little difference exists between the corn syrups at 10 and 12% concentration levels.

The pH change in soy milk caused by addition of corn syrups was −0.10 or less at a concentration of 8.0 g corn syrup per 8.0 g soy milk solids.

DISCUSSION

FUKUSHIMA and Van Buren (1970a) reported that the redispersibility of soy milk protein decreased rapidly with the heating time before drying, reaching a minimum at 5–10 min heating at 100°C. Further heating caused a subsequent increase of redispersibility. They (Fukushima and Van Buren, 1970b) explained this as resulting from the oxidative inactivation of exposed −SH groups. Intermolecular polymerization of proteins through hydrophobic interaction was also suggested as a second major cause of soy milk insolubilization, reaching a maximum after 30 min heating at 100°C. In our work, as described above, the degree of improvement in dried soy milk redispersibility was greater for milk heated 10 min than for that heated 90 min. If the suggestion of Fukushima and Van Buren is correct, then it might be suggested that the sugar may act more efficiently to prevent intermolecular −S•S− polymerization than the hydrophobic interaction.

Since no difference in the effects of sugars could be observed between times of additions, before and after heating soy milk, the sugars may have had no great effect in preventing aggregation of proteins during heating, observed with an electron microscope by Kawaguchi and Tsugo (1969). Alternately, such aggregation of proteins may have had little effect upon the redispersibility of the dried product. Addition of sugar after drying was quite ineffective.

The time for heating soy milk is also an important factor in relation to protein efficiency ratio (PER) of soy milk. At 93°C, for instance, heating for more than 60 min was needed to obtain a high PER for soy milk (Hackler et al., 1965). From the nutritional viewpoint, 90 min of heating should be more practical than 10 min of heating.

Sugars which show large solubilities in water and minimum tendencies of crystallization seem to be the most suitable for maintaining high dispersibility for soy milk solids. Judging from another experiment with a highly viscous carbohydrate, crown gall polysaccharide (β-1.2-glucan: Gorin et al., 1961; Sugimoto, 1967), viscosity of sugar solution seemed not to be an important factor for the dispersant efficiency. One possible reason for the dispersant effects of sugars for soy milk solids may be due simply to a physical separation of the protein molecules; in other words, in a reduction of opportunity for contact and polymerization during or after drying. We must leave histological proof of this for a further study.

Among sugars tested, enzyme-converted corn syrups seemed to be the most suitable materials, since it is possible to regulate the organoleptic sweetness of soy milk by selecting an arbitrary D.E. or by mixing them with other sugars such as sucrose or dextrose.

Increase in D.E. of corn syrups tends to decrease spray drying through-put rates. Actually, lower D.E. corn syrups have been proven very successful as a drying aid in spray drying (Anon., 1970). This tendency seems to be particularly disadvantageous, because it is contrary to the order of the dispersant efficiency. The cause may be due mainly to increased hygroscopicity and stickiness of dried products accompanying the increase of D.E. This phenomenon, however, may be significantly reduced by mixing corn

syrups with a nonsticky substance, namely soy milk solids. Recently, soy protein as a drying aid in spray drying of banana puree has been suggested in Israel (Anon., 1968).

A few years ago Shurpalekar et al. (1964) and Chandrasekhara et al. (1966) published recipes for easily soluble infant beverages based partially on soybeans. The good dispersibilities of these spray-dried powders may have been due to their high content of malto-dextrin, malto-extract or dextrose syrup.

The drying conditions in our experiments were substantially different from spray drying and prone to give low redispersibility of dried products. Actually, the dried soy milk tended to form a tough film. For spray drying, therefore, smaller quantities of sugars than 8–12% may be sufficient for high redispersibility.

One disadvantage in applying corn syrups is a possibility of accelerating browning of soy milk powder during long-term storage.

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REFRIGERATED APPLE SLICES: EFFECTS OF pH, SULFITES AND CALCIUM ON TEXTURE

SUMMARY—Apple slices of two varieties were dipped in solutions at various pH values with and without sulfite and with and without calcium. After dipping, the apples were packed in plastic bags and stored at 34°F. At weekly intervals shear strength was measured and related to pH of the dipping bath for the following treatments: 1) unsulfited, 2) sulfited, 3) calcium treated, unsulfited and 4) calcium treated, sulfited. An alkaline sulfite dip resulted in firmer apple slices than an acidic sulfite dip. Addition of calcium to an alkaline sulfite dip resulted in a further increase in firmness, but addition of calcium to an acidic sulfite dip did not. Sulfite was necessary to preserve the light color of apple slices during storage of refrigerated apple slices for several weeks.

INTRODUCTION

REFRIGERATED, unfrozen sliced apples have been used extensively for pies and other bakery products because of convenience, year-round availability and firm texture. Treatments to protect the color of these sliced apples have usually involved a sulfite dip, sometimes with an added treatment, Joslyn and Mrak (1933) and Bolin et al. (1964). Processing of apple slices for refrigerated storage differs from that for freezing, since the latter destroys cell organization and allows enzymes and substrates to mix. In frozen apple tissue there is a considerable amount of oxygen, so that oxidation catalyzed by polyphenol oxidase occurs and rapid browning results, especially during thawing. In refrigerated apples, the cells remain intact and alive except on the surface. Thus, treatment of the surface is all that is necessary, in contrast to penetrating treatments necessary for freezing. For slices to be refrigerated, it is both unnecessary and undesirable to use a penetrating sulfite treatment. Since penetration of sulfites is affected by pH, (Joslyn and Mrak, 1933, and Ponting, 1944), we have investigated the effect of pH on texture (firmness) of apple slices dipped in baths with and without added sulfite. The effect of calcium in firming sulfited and unsulfited apples was also investigated as a function of pH.

MATERIALS & METHODS

VARIETIES of apples studied were Golden Delicious from Washington and Newtown Pippin from both Oregon and California. The apples had been in controlled atmosphere storage for approximately 6 months before use. 3-in.-diameter apples were peeled and cored on a hand-operated machine in the laboratory, then trimmed and sliced longitudinally into twelfths through a stainless steel slicer with radial blades. The slices were not put in a holding solution but were immediately dipped in the solutions being tested. Slices in 1-kg lots were dipped in 2 liters of solution, usually for 3 min, then drained, heat sealed in laminated (polyester-polyethylene) bags and stored at 34°F. At weekly intervals the shear strength was measured as follows: Two apple slices weighing 25g total were placed in the test chamber of a L.E.E.-Kramer shear press (Model Sp-12imp.). The slices were oriented across the multiple grids of the test chamber. Shear strength readings were obtained with a 3,000-lb press ring and recorder calibration at 300; this setting covered the shear strength range of the apple varieties used. The flow control valve was set for maximum rate of travel. Results were recorded as percent of full-scale reading on the recorder chart at peak pressure. Six replicate samples were run at each time and the mean value used.

Experiments were designed to show the effects on apple texture (shear strength) of pH alone and of kind of acid, as well as the effect of pH on sulfite-treated, calcium-treated and combined sulfite-calcium treated apples. Solutions were made as follows:

A) For effect of pH alone. 0.1N Hydrochloric acid was mixed with sodium hydroxide to give a range of pH values at the end of the dipping period, as shown in Table 1.

B) For effect of kind of acid. 0.1N Concentrations of various acids (Table 2) were adjusted to pH 3.0 with sodium hydroxide.

C) For effect of pH on sulfite-treated apples. Solutions containing 0.2% SO_2 were made from gaseous SO_2, sodium bisulfite or sodium sulfite and adjusted to various pH values (Tables 3 and 4) with sodium hydroxide.

D) For effect of pH on calcium-treated apples. Solutions of calcium chloride containing 0.2% calcium were adjusted to various pH values (Table 4) with sodium hydroxide or carbonate-bicarbonate buffers.

E) For effect of pH on calcium-sulfite-treated apples. Solutions containing 0.2% calcium and 0.1% or 0.2% SO_2 were adjusted with sodium hydroxide or carbonate-bicarbonate buffers to various pH values (Table 4).

RESULTS & DISCUSSION

RESULTS are summarized in Tables 1–4. There is a small but definite effect on shear strength from variations in pH, as is evident from Table 1. Acid solutions soften the apples and alkaline solutions harden them, except at a high pH. The maximum firming effect is at about pH 9. The kind of acid used also influences not only the shear strength but the tendency to darken (Table 2). Sulfurous acid is best for maintaining a light color but it softens the fruit severely. Acetic acid both softens and darkens the apple slices.

Softening of sulfited apple slices is more extensive at low than at high pH values (Table 3). The degree of penetration of SO_2 is also greater at low pH values (Joslyn and Mrak, 1933, and Ponting, 1944). However, an alkaline sulfite dip still causes some softening, although it may approach zero. Since refrigerated apple slices are used mainly because their texture is crisp, the process

Table 1—Effect of pH on texture of unsulfited apple slices stored 1 week at 34°F.

	Shear press readings (% of full scale)		
pH of dip	Washington Golden Delicious	California Pippins 1st lot	California Pippins 2nd lot
1–2	23	57	44
(Natural) 3–4	42	70	49
6.5–7.5	45	70	–
8–9.5	48	76	60
10–11.5	40	71	45
Fresh shear =	42	70	49
$S\bar{x}$	2.5	3.2	3.0

Table 2—Effect of kind of acid on texture of unsulfited apple slices[a] stored 10 days at 34°F.

Kind of acid	Shear press reading (% of full scale)	Color
Hydrochloric	68	Light but some gray blotches
Sulfuric	61	Light brown
Phosphoric	59	Light brown
Citric	49	Light brown
Malic	49	Light brown
Ascorbic	42	Light but some brown moldy spots
Acetic	37	Dark brown
Sulfurous	24	Light

[a]California Pippin slices dipped 40 min at pH 3.0. Fresh shear reading = 70. $S\bar{x}$ = 1.7.

Table 3—Effect of pH on texture of sulfited apple slices stored at 34°F.

Variety	pH of dip (0.2% SO_2)	Shear press reading (% of full scale) 1 week	2 weeks	4 weeks
Golden Delicious				
fresh shear = 33	3	10	5	4
	5	7	9	6
$\bar{Sx}$ = 1.5	7	27	18	18
	9	28	29	30
Oregon Pippin				
fresh shear = 46	5	31	24	–
	7	39	34	–
$\bar{Sx}$ = 1.4	9	44	38	–
California Pippin				
fresh shear = 58	5	22	17	15
	7	38	38	18
$\bar{Sx}$ = 1.9	9	53	43	39

Table 4—Effect of calcium[a] and pH on relative shear strength of apple slices stored 1 week at 34°F.

Variety	pH	Relative shear strength: Unsulfited No Ca	Unsulfited + Ca	Sulfited[b] No Ca	Sulfited[b] + Ca
Oregon Pippin	4–5	1.0	1.3	0.7	0.8
$\bar{Sx}$ = .04	9	1.0	1.5	1.0	1.4
California Pippin	4–5	1.0	1.1	0.4	0.5
$\bar{Sx}$ = .05	9–11	0.9	1.2	0.9	1.4

[a]0.2% Ca as $CaCl_2$ in dip.
[b]Oregon apples dipped in 0.1% SO_2; California apples dipped in 0.2% SO_2.

which causes the least penetration of SO_2 but maintains the color is best, provided that flavor is not adversely affected. This would suggest an alkaline rather than an acid sulfite dip and, as Bolin et al. (1964) have pointed out, an alkaline dip does not affect flavor. Our informal taste panel confirmed this. On the other hand, apple slices for freezing should have complete SO_2 penetration. Therefore, an acid sulfite dip should be used for apples to be frozen.

Although either a sulfite dip followed by an alkaline buffer dip or a single alkaline sulfite dip effectively inhibits enzymic browning on the surface, the lack of penetration of SO_2 leaves any residual SO_2 exposed to both autoxidation on the surface and enzymic oxidation at the interface with active polyphenol oxidase, Ponting and Johnson (1945). Thus, the residual SO_2 on or in the apple slices rapidly drops to zero in storage. At this point, browning may occur, especially if the surface is rough (as from dull slicing knives). Bolin et al. (1964) were able to maintain a satisfactory color in apple slices for 3 weeks or longer at 34°F with no residual SO_2 after an alkaline dip, but in our experiments the color was unsatisfactory after 1–2 weeks of refrigerated storage when there was no residual SO_2. Thus, a problem remains in balancing good crispness and flavor, accompanying low residual SO_2, with good color which is provided by high residual SO_2.

Calcium treatment of sulfited apples has been found to be very effective in firming the apples in alkaline solution or suspension but not in acid solution (Table 4). With unsulfited apples there is a firming effect in both acid and alkaline solutions. We have used calcium sulfite solutions buffered with sodium carbonate-bicarbonate in which the calcium should be practically insoluble, but this does not decrease its effectiveness; in fact, 0.2% calcium in such a buffer may make apples too firm and woody. Furthermore, we have found that SO_2 is considerably protected from oxidation in an alkaline calcium sulfite solution, so that SO_2 is retained during storage of apple slices and color is preserved longer. (This will be the subject of another paper.) Thus, by using suitable proportions of calcium and SO_2 in a dip of the proper pH, the qualities of firmness, color and flavor can be balanced to give the most desirable product after storage. Conversely, the storage life can be extended by this means from a maximum of about 3 weeks to as long as 8 weeks at 34°F.

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IMPROVED METHODS FOR DETERMINATION OF CERTAIN ORGANIC ACIDS IN PASTEURIZED AND UNPASTEURIZED LIQUID AND FROZEN WHOLE EGG

SUMMARY—Liquid whole egg to which acetic, butyric, lactic, propionic and succinic acids were added, or which had previously been incubated at room temperature to different microbial populations, was pasteurized at 60.5°C for 3.5 min. A liquid-liquid extraction method, including the centrifugation of the denatured egg, was developed for extraction of all 5 acids. The recovery of the 5 acids was evaluated by an improved gas-liquid chromatographic procedure. Lactic and succinic acids were recovered from the liquid whole egg samples and chromatographed as their butyl ester derivatives and acetic, propionic and butyric acids were recovered and chromatographed as the acids per se. The fresh egg samples containing small amounts of acetic and lactic acid and liquid whole egg product, incubated until a microbial population of 4.5×10^6 was obtained, contained only these 2 acids at a higher concentration. The pasteurization process did not affect concentration of the added short-chain organic acids or those which accumulated during the incubation period; thus, accurate and valid analyses of the short-chain organic acids can be accomplished in pasteurized whole egg products.

INTRODUCTION

THE PRESENCE of certain organic acids such as acetic, lactic and succinic is indicative of bacterial decomposition in liquid and frozen egg products (Hillig et al., 1960). Regulatory agencies, as a part of their inspection programs, now analyze samples of egg products for presence and quantity of these acids. Since pasteurization is now required for all liquid, frozen and dried egg entering interstate commerce, it is essential to know if pasteurization will affect the concentration of these acids.

Several recent studies have reported the use of gas-liquid chromatography (GLC) for determining the concentration of organic acids in biological materials, and in particular egg products (Steinhauer and Dawson, 1969 a; 1969 b; Salwin and Bond, 1969 and Staruszkiewicz, 1969; 1970). These studies have shown that GLC procedures are as accurate as current AOAC (1965) procedures for determining acid concentration.

Since the presence of certain organic acids are important in determining wholesomeness in egg products, an investigation was initiated to improve the GLC procedures for separating and quantitating these acids and to determine the effect of pasteurization on the content of acetic, butyric, lactic, propionic and succinic acids in frozen whole egg.

EXPERIMENTAL

Egg source and preparation

U.S. Grade AA Large eggs were obtained from a commercial source for each phase of this study. The eggs were broken and blended 15 sec at low speed in a 1.5-gal stainless steel Waring Blendor. The blended egg product was strained through a U.S. No. 20 mesh sieve into a 30-gal stainless steel multiple paddle mixer at 3°C and mixed for 20 min.

The desired quantities of acetic, butyric, lactic, propionic and succinic acids were blended 10 sec with 4000-g portions of egg product (Table 1). 6 samples of 225 g each were frozen at −20°C and held at −20°C until analyzed. The remaining acid-egg mixture was pasteurized, divided into 225-g samples and frozen. A series of untreated samples, both pasteurized and nonpasteurized, was frozen and stored for control purposes.

In a second series of experiments, 3, 4000-g portions of blended whole egg were incubated at room temperature for 11, 14 and 17 hr, respectively. 6, 225-g samples of egg product from each incubation time were frozen and held at −20°C for analyses. The remaining egg product was pasteurized, frozen and held for analyses. A nonincubated set of samples, both pasteurized and nonpasteurized, was frozen and stored for control purposes. Microbial total plate counts were determined for each sample before and after pasteurization using trypsone glucose extract agar and incubated for 48 hr at 32°C.

The pasteurizing equipment consisted of 3 units as shown in Figure 1: a preheater water bath maintained at 55°C, a pasteurizing water bath at 60.5°C and a slush ice cooling bath. Glass tubing 5 mm i.d. (7 mm o.d.) was used to conduct the egg product through the preheater and pasteurization sections. A speed-controlled Cole-Palmer "Master Flex" pump (Model 7014 F pump head) was used to pump the egg product through the system at a uniform rate of 100 ml/min, and the product remained at 60–61°C for 3.5 min. Thermocouple wires were glued into place through appropriate holes in the glass tubing. A Honeywell Electronik 16 Multipoint Strip Chart Recorder was used to record temperature.

Extraction of acids from egg products

Acids were extracted with a modified version of the liquid-liquid extractor used in AOAC procedure 15.012 (1965). The sample-holding portion was modified to hold 500 ml of liquid and a 100-ml round-bottomed flask was used instead of a 250-ml flask. The egg samples were prepared for liquid-liquid extraction using the procedure of Steinhauer and Dawson (1969a). Their procedure was modified as follows: The filtration step was replaced by a centrifugation step and the condensation step was eliminated by increasing the sample-holding capacity of the liquid-liquid extractor. Following addition of the water, phosphotungstic and sulfuric acids to the egg products, the resulting mixture was divided into 4, 250-ml portions, transferred to polyethylene centrifuge bottles and placed in a Sorvall RC-2B centrifuge and centrifuged for 13 min at 10,000 rpm. The supernatant from the 4 bottles for each sample was then combined and 450 g of the supernatant placed in the liquid-liquid extractor for 24 hr.

The recovery for each acid was quantitated separately by the procedure of Steinhauer and Dawson (1969a).

Preparation and esterification of the samples for GLC

For determination of the volatile acids as the acids per se, enough ammonium hydroxide was added to make the medium basic after removal of the boiling flask from the extraction unit. Cresol red was used as the indicator. The ether was evaporated from all samples by placing the flask over a 65–70°C water bath and the sample flask removed immediately after the ether was evaporated.

Following ether evaporation from the volatile acid samples, the salts formed from the addition of ammonium hydroxide were liberated by the addition of 1 ml of 12.5% dichloroacetic acid in acetone solution. After ether removal from the samples in which lactic and succinic acids were to be determined, the samples were esterified using a modification of the method reported by Steinhauer and Dawson (1969a). 1 ml 1.25 N HCl in n-butanol (Regis Chemical Company) was used instead of 1 ml n-butanol

Table 1—Quantities of each acid added to egg samples.

Sample No.	Acetic Acid	Butyric Acid	Lactic Acid	Propionic Acid	Succinic Acid
	(mg/100 g egg)				
1	None	None	None	None	None
2	0.510	0.500	0.465	0.506	0.543
3	5.098	4.998	4.651	5.058	5.430
4	20.390	19.990	18.604	20.230	20.634

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and 2 drops conc HCl, and a 50-ml boiling flask was replaced by a 100-ml boiling flask. A powerstat setting of 50/140 was used. After these steps were completed, the quantitative transfer of the sample flask to a 10-ml volumetric flask and determination of the quantity of internal standard to be used were the same procedures as outlined by Steinhauer and Dawson (1969a).

An internal standard was composed of approximately 2.5 and 1.1 g of butyl decanoate (K & K Labs), accurately weighed into separate 100-ml glass-stoppered volumetric flasks and made to volume with acetone. As needed, various dilutions of the 2 stock solutions were made to satisfy the need for a desired concentration of internal standard for a particular sample.

GLC procedures

An F & M Scientific Company Model 810 dual-column-flame-ionization instrument equipped with a Model 9294N Honeywell recorder was used. Each column was 72 by ¼ in. copper tubing; helium carrier gas at 40 psi; flow rate for Column A, 60 ml/min and Column B, 50 ml/min; hydrogen gas at 62 ml/min at 20 psi; air at 625 ml/min at 40 psi; detector temperature at 250°C; injection temperature, 250°C and column temperature 120°C for straight acids. Columns were prepared with high-temperature stabilized diethylene glycol succinate (20%), conditioned at 185°C with a nitrogen flow rate of 60 ml/min. The solid support was 120–130 mesh Anakrom ABS.

The column temperature for the determination of the butyl ester derivatives was programmed. A temperature of 130°C was used until the butyl lactate and the internal standard were eluted (approximately 18 min after injection), then the column temperature was increased at the rate of 6°C/min to 170°C and maintained until the dibutyl succinate was eluted.

The chromatograph was calibrated for both the volatile acids and the butyl ester derivatives as reported by Steinhauer and Dawson (1969a; 1969b) with the exception that butyl decanoate was used as the internal standard for both the volatile acids and butyl ester derivatives.

RESULTS & DISCUSSION

Pasteurization

Bacterial counts of eggs held at 3°C up to 5 hr reached 4.5 x 10^4 bacteria per gram with the product having an initial bacterial count of 4.1 x 10^4 per gram. Those held at 24°C up to 17 hr reached a population level of 4.5 x 10^6 per gram.

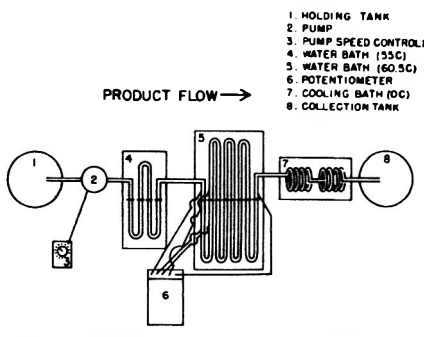

Fig. 1—Diagram of laboratory egg pasteurizer.

Table 2—Efficiency of extraction and esterification of appropriate acids.

Acid	Recovery by liquid-liquid extraction: ml 0.05 N Alcoholic KOH required for 10-ml aliquot[a]	ml 0.05 N Alcoholic KOH required for recovered acid[b]	Average percent recovery
Acetic	20.45	18.83 ± 0.22	92.14
Butyric	20.10	19.79 ± 0.27	98.47
Lactic	19.85	17.88 ± 0.26	90.10
Propionic	21.15	21.08 ± 0.35	99.69
Succinic	20.70	20.04 ± 0.27	96.80

	Recovery by esterification: (mg/ml)	Equivalent as butyl ester (mg/ml)	Recovery from GLC (mg)[b]	Average percent recovery
Lactic	9.67	15.70	13.67 ± 0.41	89.0
Succinic	10.10	19.70	17.53 ± 0.37	89.0

[a]Average of 3 replications.
[b]Average of 10 replications ± standard deviation of the mean.

After pasteurization, the counts were reduced a minimum of 2 log numbers and ranged from 4.0 X 10^2 to 1.4 X 10^3 cells; thus, the heat treatment of 60°C for 3.5 min was effective for the purpose of this study.

Extraction and esterification

1 objective of this study was to develop a common extraction procedure for all the acids studied. The 2 motivating reasons for changing current AOAC extraction procedures were to shorten or dispense with the filtration step and the condensation step. Elimination of the filtration step resulted in larger percentage recoveries of the acids (this method recovered 450 of 700 g, whereas the AOAC (1965) procedures recover 450 of 1000 g).

Elimination of the condensation step saved time

Continuous extraction for 24 hr was required to recover sufficient acids when using a 100-ml boiling flask. A 250-ml flask (or larger) can be used in this procedure and has been shown to reduce the 24-hr extraction time to 3–5 hr. About 85% of the formic acid present can be recovered in a 24-hr extraction period using this procedure.

The percentage recoveries of acetic, butyric, lactic, propionic and succinic acids by this modified liquid-liquid ex-

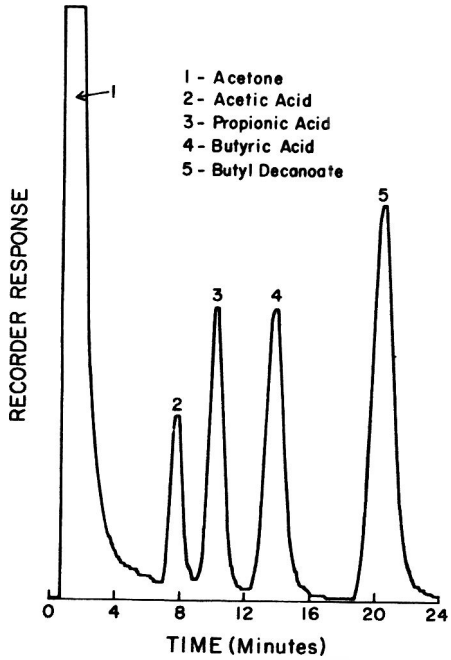

Fig. 2—Gas chromatogram of acetic, propionic and butyric acids and butyl decanoate showing their order of elution and retention times.

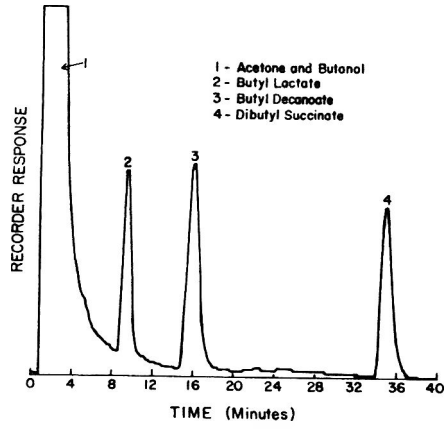

Fig. 3—Gas chromatogram of butyl lactate, butyl decanoate and dibutyl succinate showing their order of elution and retention times.

Table 3–Recovery of acetic, propionic and butyric acids from liquid frozen whole egg using GLC procedures.

Sample	mg/100 g Egg (added)	mg/100 g Egg[a] recovered	
		Nonpasteurized	Pasteurized
Acetic			
1	None	4.27 ± 0.23	4.31 ± 0.25
2	0.51	4.75 ± 0.18	4.82 ± 0.21
3	5.10	9.30 ± 0.29	9.31 ± 0.30
4	20.39	24.40 ± 0.45	24.40 ± 0.39
Propionic			
1	None	None	None
2	0.51	0.52 ± 0.05	0.49 ± 0.04
3	5.10	5.00 ± 0.19	5.08 ± 0.10
4	20.23	20.25 ± 0.31	20.33 ± 0.37
Butyric			
1	None	None	None
2	0.50	0.55 ± 0.10	0.50 ± 0.03
3	5.00	4.90 ± 0.23	5.11 ± 0.17
4	19.99	19.77 ± 0.38	19.93 ± 0.22

[a]Average of 3 samples and 3 injections of each sample ± standard deviation of the mean.

Table 4–Recovery of lactic and succinic acids from liquid frozen whole egg using GLC procedures.

Sample	mg/100 g Egg (added)	mg/100 g Egg[a] recovered	
		Nonpasteurized	Pasteurized
Lactic			
1	None	2.96 ± 0.22	2.78 ± 0.18
2	0.47	3.48 ± 0.19	3.45 ± 0.10
3	4.65	7.65 ± 0.31	7.54 ± 0.23
4	18.60	21.25 ± 0.47	21.74 ± 0.52
Succinic			
1	None	None	None
2	0.54	0.49 ± 0.04	0.55 ± 0.06
3	5.43	5.50 ± 0.18	5.38 ± 0.14
4	20.63	20.37 ± 0.54	20.48 ± 0.47

[a]Average of 3 samples and 3 injections of each sample ± standard deviation of the mean.

Table 5–Recovery of acetic, butyric, lactic, propionic and succinic acids from frozen liquid whole eggs incubated at room temperature and then frozen, using GLC procedures.[a]

Sample	Total plate count before pasteurization[a]	mg/100 g Egg[b] recovered	
		Nonpasteurized	Pasteurized
Acetic			
1	4.0×10^4	4.02 ± 0.27	3.91 ± 0.19
2	1.3×10^5	4.15 ± 0.15	4.20 ± 0.07
3	5.4×10^5	4.33 ± 0.23	4.31 ± 0.20
4	4.5×10^6	5.14 ± 0.31	5.29 ± 0.27
Lactic			
1	4.0×10^4	2.55 ± 0.31	2.65 ± 0.23
2	1.3×10^5	2.69 ± 0.44	2.72 ± 0.18
3	5.4×10^5	3.55 ± 0.44	3.47 ± 0.14
4	4.5×10^6	4.01 ± 0.30	3.92 ± 0.34

[a]No quantities, even in trace amounts, of butyric, propionic or succinic acids were detected.

[b]Average of 3 samples and 3 injections of each sample ± standard deviation of the mean.

traction procedure are shown in Table 2. More than 90% of each acid was recovered by these procedures. This varied from 90% for lactic acid to 99.7% for propionic acid. These are satisfactory recoveries, although slightly lower than those reported by Steinhauer and Dawson (1969a; 1969b).

The esterification efficiencies of lactic and succinic acids are shown in Table 2. Similar recoveries (89%) were obtained for each acid. These rates were slightly higher than reported by Steinhauer and Dawson (1969a).

Salwin and Bond (1969) reported that the propyl ester derivatives of lactic and succinic acids were more satisfactory than the butyl ester derivatives. These authors also report a 10-min esterification method using a BF_3-n-propyl alcohol reagent.

The column temperature program for the butyl ester increased the R value and the sharpness of the dibutyl succinate peak. The R value was 0.90–0.95 compared with 0.30–0.32 as reported by Steinhauer and Dawson (1969a), using a temperature of 130°C.

Internal standard

Since the detector response varied with each compound injected, an internal standard was used in the quantitation procedures. In this study, butyl decanoate was found to be satisfactory for use in both the free acid and butyl ester derivative determinations. The response values, R, of the C_2-C_4 acids, butyl lactate and dibutyl succinate to the internal standard, butyl decanoate, were calculated according to the procedure described by Shelley et al. (1963) and Steinhauer and Dawson (1969a; 1969b).

The elution positions of the internal standard, butyl decanoate, relative to the C_2-C_4 acids and to the butyl ester derivatives of lactic and succinic acids are shown in Figures 2 and 3, respectively.

Recovery of acids

Tables 3 and 4 show the amount of each acid recovered from both pasteurized and nonpasteurized liquid whole egg which had been frozen and thawed and to which known amounts of each acid were added before the pasteurization or freezing step. Pasteurization of the liquid whole egg did not affect recovery of the 5 acids studied. Of these 5 acids, only lactic and acetic were found in detectable amounts in fresh egg. Lactic acid concentration was 2.78–2.96 mg/100 g whole egg and acetic acid concentration was 4.27–4.31 mg/100 g whole egg. The amount of these 2 acids found in fresh liquid whole egg agreed with the unpublished work of Landes and Dawson (1969), who used a silica gel column. The amount of variation in the samples was comparable to that reported by Steinhauer and Dawson (1969a; 1969b).

Table 5 shows the amount of acid recovered from eggs incubated at room temperature to different bacterial population levels. Even at a population of 4.5×10^6 viable cells/g of egg, only acetic and lactic acids were detected. The initial concentration of acetic acid was 3.91–4.02 mg/100 g of egg and after incubation to a bacterial population of 4.5×10^6, the concentration had increased to only 5.14–5.29 mg/100 g egg. Lactic acid was similar, with an initial concentration of 2.55–2.65 mg/100 g egg, which increased to 3.92–4.01 mg/100 g egg with a microbial population of 4.5×10^6.

Steinhauer (1968) reported finding formic, acetic, propionic, lactic and succinic acids in decomposed liquid whole egg samples which had a microbial population of 1.7×10^9 /g egg. Steinhauer et al. (1967) also reported finding small amounts of succinic acid in all samples of egg evaluated. In this study a small peak was observed on the chromatogram in the

vicinity of the dibutyl lactate peak, but when dibutyl lactate was added its retention time was about 2 min after this unknown peak.

Acetic acid was found in measurable quantities in the fresh egg and Landes and Dawson (1969) also reported finding measurable quantities of both acetic and formic acids in fresh egg products.

These results offer an improved GLC method to evaluate liquid or frozen egg products for organic acids indicative of microbial decomposition. They also show that egg products containing these acids can be pasteurized without altering the quantitative recovery of the acids present.

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ANALYSIS OF COFFEE, TEA AND ARTIFICIALLY FLAVORED DRINKS PREPARED FROM MINERALIZED WATERS

SUMMARY—Coffee, black tea and artificially flavored and artificially sweetened orange and grape soft drinks were prepared from solutions of 8 minerals each at 750 ppm. Additionally, coffee and tea were brewed, using 6 natural drinking waters which ranged from 42–1,725 ppm of total dissolved solids. Beverages made from solutions containing carbonates were the least desirable, having flat, insipid characteristics. Although distilled water resulted in an acceptable soft drink, coffee and tea prepared from it were excessively sour and astringent, respectively. The recommended formula of 53.3 g of coffee per liter of water was considered too strong and "burnt" for the panel of trained judges, so that the experiments were repeated at a lower concentration of 47 g coffee/liter water. Large differences in the direction and magnitude of the visual characteristics of the coffee and tea brewed from the various waters also were observed in the marked changes in luminous transreflectance, purity and shifts in dominant wavelengths measured by thin-layer reflectometry.

INTRODUCTION

IT IS widely recognized that the constituents of drinking water greatly influence the composition of beverage coffee and tea which, in turn, alters appearance, flavor and subsequent acceptability. The concentration and species of ions in solution change the rate at which water passes through a bed of ground coffee, thereby causing differential extraction of coffee solids (Gardner, 1958). With tea, Schurer (1960) found that calcium and magnesium bicarbonates, chlorides and sulfates reacted with the tea constituents to produce a turbid infusion with a dull color, flat, insipid taste and an unsightly sediment in the tea pot, the cup and on the beverage surface. No data were found in the literature on influence of water composition on the sensory quality of soft drinks; however, it is well known that individual commercial beverage manufacturers set maximum limits on water hardness in their plants.

According to the Coffee Brewing Center (Lockhart, 1966), complaints about acceptability of beverage coffee frequently implicate water composition as the causative factor. If one or a combination of the following appears in a water analysis, less than optimum beverage quality will ensue: Total dissolved solids (TDS) content above 300 ppm. Total hardness above 150 ppm. Combined calcium and magnesium content above 100 ppm. Carbonate-bicarbonate alkalinity above 100 ppm. Combined sodium-potassium content above 500 ppm. pH above 9.0. Detectable odor.

With the exception of the odor problem, many municipal waters derived from groundwaters in California violate one or more of these limits. The sensory properties of several representative drinking waters, ranging from 22–1,870 ppm TDS, have been studied (Dillehay et al., 1967; Bruvold et al., 1969; Pangborn, 1970), as have solutions of selected minerals in concentrations from 125–2,000 ppm (Bruvold and Gaffey, 1965; Bruvold and Pangborn, 1966; Pangborn et al., 1970). The latter investigation demonstrated that at levels of 750 ppm the 8 minerals employed were readily distinguishable qualitatively and, therefore, were used in the present study to determine their influence on the sensory quality of coffee and tea beverage as well as an artificial soft drink.

METHODS & MATERIALS

General sensory procedures

For the coffee and tea studies, 21 (14 ♂, 7 ♀) building employees participated in 3 screening sessions of 3 triangle tests each, in which they were requested to distinguish between beverages prepared from tap-water (300 ppm T.D.S.) and distilled water (2.5 ppm T.D.S.). Using the criterion of 5 or more correct separations in 9 trials (P = 0.05), 9 people (6 ♂, 3 ♀) qualified for the coffee panel and 10 (8 ♂, 2 ♀) for the tea panel; 6 judges were common to both. Throughout these studies, each judge evaluated each sample an equal number of times, necessitating make-ups on the few occasions judges had to be absent from a regu-

Table 1—Blend and grind analyses for coffee.

Blend			
Country of origin	Grade	Percentage	
Colombia	Armenia, Excelso	40	
Brazil	Santos 4	33	
El Salvador	Central Standard	27	
Grind[a]			
Sieve No. U.S. Standard	Tyler Mesh Equiv.	Percent retention	
12	10	0.2	4.2
16	14	4.0	
20	20	34.5	75.8
30	28	41.3	
Pan	Pan	20.0	20.0

[a]Performed according to U.S. Department of Commerce Simplified Practice Recommendation R 231-48 and conforming to the "drip" category.

Table 2—Terminology developed by the sensory panel to describe flavor quality of coffee and tea beverages.

Term	Description
Sweet	Resembles sucrose; perceived mainly on tip of tongue
Salty	Resembles salt; perceived mainly on anterior sides of tongue
Sour	Resembles acid; perceived mainly on posterior sides of tongue
Bitter	Resembles caffeine or quinine; perceived mainly at base of tongue
Brisk	Sharp, clear, characteristic beverage flavor
Balanced	Well-blended flavor, with no unduly prominent flavor notes
Flat	Dull, weak, flavorless, lacking in characteristic flavor
Stale	Old, unfresh, but nonrancid (distinguish from flat)
Rancid	Old, oxidized-oil flavor
Astringent	Harsh, drying puckery sensation (unripe persimmon)
Metallic	Flavors associated with iron. copper, brass or tarnished silver-plated spoons
Burnt	Overcooked, caramelized, scorched flavors

Table 3–Analysis of distilled and natural waters used to prepare coffee and tea beverage.

Water	T.D.S.[a]	pH	E.C.[b]	Na^+	Ca^{++}	Mg^{++}	B	Cl^-	$Co_3 + HCO_3$	$NO_3 - N$	SO_4
	(ppm)		(millimhos/cm)		(me/liter)		(ppm)	(me/liter)		(ppm)	(me/liter)
Distilled											
1[c]	3.06	7.6	0.0052	0	0	0	<0.10	< 0.03	0.06	–	0
2[d]	0.84	6.5	0.0014	0.003	0.004	0.003	<0.10	0	0	0	0
EBMUD											
1[c]	15	8.0	0.064	0.13	0.1	0.1	0.10	0.28	0.53	–	0
2[d]	42	7.8	0.070	0.08	0.4	0.2	0.28	0.06	0.47	0	0.17
Davis No. 2											
1[c]	377	8.5	0.57	4.06	0.8	1.2	0.57	1.20	4.55	–	0.84
2[d]	411	8.3	0.69	4.35	0.8	1.2	0.98	0.62	4.44	0	1.79
Davis No. 4											
1[c]	720	8.3	1.51	6.74	2.3	10.2	0.88	3.00	13.68	–	2.95
2[d]	888	8.2	1.48	2.10	2.8	10.3	1.46	1.50	12.85	6.8	0.45
Brentwood											
1[c]	701	7.6	1.49	5.98	5.2	4.9	1.17	8.30	4.20	–	6.40
2[d]	927	7.9	1.55	4.10	6.3	5.1	1.29	5.70	4.90	8.4	4.93
Soledad											
1[c]	936	7.6	1.52	2.74	8.8	5.8	0.27	6.50	5.00	–	9.19
2[d]	888	7.7	1.48	0.80	8.6	5.6	0.43	3.57	5.03	3.6	6.20
Coalinga											
1[c]	1711	7.8	3.10	22.8	7.0	8.0	1.68	13.10	2.77	–	26.60
2[d]	1725	7.8	2.88	17.4	7.4	8.6	2.45	7.49	2.93	0.8	25.60

[a]Total dissolved solids.
[b]Electrical conductivity.
[c]Waters collected in May, 1968, and used to prepare tea, and coffee at 4.7% strength.
[d]Waters collected in October, 1968, and used to prepare coffee at 5.33% strength.

larly scheduled session. Test sessions were held Mondays through Fridays between 2 and 3 PM, with samples prepared in 2 batches so that the length of time between brewing and tasting did not exceeed 30 min. Judging was done in individual partitioned booths maintained at 21 ± 1°C, under low, blue illumination to mask visual differences. Coffee and tea were served at 60°C (140°F), with no additives. To maintain the samples at the desired temperature throughout the testing period, approximately 35 ml were poured into 80-ml blue glasses which were immersed in a rectangular, stainless steel water bath kept at 60°C. Aluminum lids containing 2- and 3-digit code numbers covered the glasses. Distilled water at 21°C was provided for oral rinsing, and swallowing was not permitted. Judges were informed of their results immediately after testing and cake or cookies were provided as "rewards."

7 ♀ and 7 ♂ judges qualified for the subsequent panel on artificially flavored drinks served at 11 ± 1°C (52°F). Approximately 25 ml of sample was served in 50-ml beakers. Orange- and grape-flavored drinks were evaluated under red and blue illumination, respectively.

Coffee preparation and evaluation

The coffee beans were roasted and ground on March 19, 1968, and vacuum packed in 1-lb (401) cans by the Coffee Brewing Center, New York. Blend and grind analyses are given in Table 1. Samples were shipped by surface to our laboratory where they were kept at 5°C throughout the 8-month study. Weighed portions of ground coffee were placed in a heavy plastic cone (David Douglas and Co., Inc., Manitowoc, Wis.) lined with a porosity-control filter ("Flav-r-Flo," Boyd Coffee Co., Portland, Oregon), then 500 ml boiling water poured over the grounds and stirred 1 full turn. After 1–1.5 min, when most of the water had passed through the filter into the glass container, a second 500-ml portion of boiling water was poured into the cone and allowed to filter for an additional 3.5–4 min. For waters containing 750 ppm of the minerals, 9 min was allowed for the boiling water to filter through. Aliquots were removed for measurement of pH and extracted solids, with the remainder served immediately into the prewarmed blue glasses.

pH of each sample was measured in duplicate by a Radiometer pH meter, Model 22, on solutions at 22°C. Extracted solids were determined in triplicate on each sample with a hydrometer specifically designed for liquids at 140°F (Rascher & Betzold, Inc., Chicago), using the procedure described in detail by Lockhart (1966). Extracted solids in solution were converted to percentage, using a conversion chart calibrated according to the ratio of volume of water to weight of ground coffee.

Experimental design: coffee

In the first experiment, coffee at 5.33% strength (53.3 g ground coffee/1,000 ml water) was prepared from distilled water and from solutions containing 750 ppm of $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$, NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3. These mineral species occur commonly in ground waters in arid and semiarid sections of the United States. The sensory properties of these solutions at varying concentrations have been quantified by Bruvold and Gaffey (1965), Bruvold and Pangborn (1966) and Pangborn et al. (1970). Using a balanced, incomplete block design (Cochran and Cox, 1960), the 9 coffee samples were evaluated 8 times over 18 test days. Judges were instructed to indicate degree of liking on a 9-point scale where 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely. Additionally, individual quality factors were specifically defined by the judges (Table 2) and used in conjunction with a 13-point intensity scale where 0 = not perceived and 12 = extremely intense.

In the second experiment, beverages were prepared from distilled water and from 6 natural drinking waters collected from municipal wells throughout California, ranging from 15–1,725 ppm T.D.S. (Table 3). Initially, a concentration of 5.33% (53.3 g ground coffee/1,000 ml water) was used, then the experiment was repeated at a coffee concentration of 4.7% (47 g ground coffee/1,000 ml water). The lower coffee strength was that preferred by the sensory panel in the pretesting, while the higher strength was that recommended by the Coffee Brewing Center to give an extracted solids of approximately 1.0%. To determine visual differences, judges evaluated 3 triangle tests of 30-ml portions of coffee at 22°C in 50-ml beakers against a white background under 2, 500-w USDA Sunshine Grading and Inspection Lamps (Leo Kaufmann, San Francisco, Calif., Spec. CBDD). The triangle tests consisted of coffee prepared from distilled water compared with coffee prepared from natural water. Adjectives used to describe the beverage appearance are

Table 4–Terminology used to evaluate visual properties of coffee and tea beverage.

I	Surface phenomena	Iridescence, particles, scum
II	Translucency	Cloudiness, turbidity, particles
III	Hue and intensity of color	Color name and shade
IV	Sediment	Particles in bottom of container

Table 5–Differentiation by triangle test of appearance and flavor of coffee prepared from distilled vs. natural drinking waters.

Natural water	EBMUD	Davis No. 2	Davis No. 4	Brent-wood	Sole-dad	Coalinga
5.33% Coffee						
T.D.S. (ppm)	42	411	888	927	888	1725
% Correct response[a] (n = 66)						
Appearance	45.5*	95.5***	100 ***	92.4***	72.7***	43.9*
Flavor	40.9	72.7***	87.9***	75.8***	81.8***	63.6***
4.7% Coffee						
T.D.S. (ppm)	15	377	720	701	936	1711
% Correct response[a] (n = 54)						
Appearance	75.9***	87.0***	100 ***	87.0***	40.7	57.4***
Flavor	35.2	85.2***	87.0***	81.5***	81.5***	79.6***

*,***Respectively, significant difference at P = 0.05 and 0.001.
[a]Triangle test comparison of distilled water vs. corresponding natural water.

defined in Table 4. After the visual tests, 3 additional triangle tests of the same samples were evaluated at 60°C, followed by a paired presentation of each of the aforementioned coffees, wherein judges indicated the beverage strength on an 8-point scale (1 = extremely weak; 8 = extremely strong). Degree of liking was recorded on the 9-point hedonic scale as before, and the descriptive terms listed in Table 2 used to define qualitative factors.

Tea preparation and evaluation

Orange pekoe and pekoe black tea leaves in 1-lb sealed boxes were obtained from 1 lot from Thomas J. P. Lipton, Inc. and stored at 20°C throughout the 5-month study. The beverage was prepared at a ratio of 1 g tea leaves/100 ml water. 1 liter of water was brought to a boil in a sealed vessel, the tea leaves added, the cover replaced and the tea allowed to steep 3 min. The brew was decanted from the leaves through a strainer, aliquots removed for pH measurement and the remainder immediately poured into the prewarmed blue glasses.

2 consecutive studies were conducted with the brewed tea using the mineralized and natural drinking waters as described under the second experiment for coffee. It was not considered appropriate to conduct visual tests for differences between teas prepared from distilled water and those from the test waters because of obvious differences in colors, which ranged from pale amber to dark mahogany. Consequently, only instrumental color analyses were made on tea.

Instrumental color analysis of brewed coffee and tea

The red (R), green (G) and blue (B) transreflectance values of coffee and tea beverages prepared from the various waters were measured with the Colormaster Differential Colorimeter in 3-mm-depth cells against white and black backgrounds (Little, 1964; Mackinney et al., 1966; Joslyn and Little, 1967). Kubelka-Munk optical constants as well as tristimulus values for the white-background thin-layer mode of sample presentation were calculated for all samples and results compared with those of the distilled water controls as well as with the visual evaluations where such data were obtained.

Artificially flavored drinks

The artificial drinks were prepared from commercial powders (Pillsbury's "Funny Face"). The grape-flavored mix consisted of citric acid, calcium cyclamate (32.1%), sodium citrate, saccharin calcium (2.7%), artificial grape flavoring and artificial coloring. The orange-flavored mix listed the same composition except for "flavoring." The grape and orange beverages were prepared according to the manufacturer's directions, 4.7 g/2 qt (2.5 g/liter) and 5.7 g/2 qt (3.0 g/liter), respectively. 1, 8-day study was conducted, using solutions of the aforementioned 8 minerals at 750 ppm. The pH was measured for each sample. At each session, judges received 9 samples (distilled water plus the 8 minerals) in randomized order and evaluated over-all flavor strength (1 = extremely weak; 8 = extremely strong), degree of liking on a 9-point hedonic scale and intensity (0 = none, 8 = extremely intense) for quality factors labeled sweet, salty, sour, bitter, balanced, flat and astringent.

Analysis of data

Statistical significance of the triangle test data was determined by consulting Table E in Amerine et al. (1965), and analysis of variance applied to the scores and hedonic ratings.

RESULTS & DISCUSSION

Coffee: mineral solutes

Bitterness and sourness were the predominant flavor characteristics ascribed to coffee beverages prepared from distilled water and mineral solutions (Fig. 1). Coffee made from waters containing carbonates was the most bitter, consistent with having the highest percent of extracted solids. These same coffees had the highest pH values (6.34 and 6.92) and, predictably, were the least sour, which probably accounted for the high responses for "flat." Distilled water resulted in a beverage quite bitter and sour and had a hedonic rating of 3.7, corresponding to "dislike slightly" on the rating scale. Beverage made from water with $CaCl_2$ resulted in the most sour coffee, consistent with the lowest pH of 4.9, the lowest extracted solids and the lowest hedonic value of 3.2. Solutions of NaCl produced slightly salty coffee, as expected, but no more so than did the calcium and magnesium chlorides. The low hedonic ratings for all these beverages are due to the panel's dislike for strong coffee in general, as can be seen in comparing hedonic ratings for the distilled water samples in Figure 1 with those in Figure 3, where the lesser amount of ground coffee was used. Analysis of variance of these ratings for degree of liking showed a significant F ratio ($P < .001$) for the 8 minerals and for the judges, as well as for the interaction of the two. The average hedonic scores must differ by 0.4 unit to be significant at P = .05. Corresponding L.S.D. values for averages for bitterness and for sourness were 1.7 and 1.4, respectively.

Coffee: natural waters

Chemical analyses of the 2 sets of natural waters used are given in Table 3. Results from the triangle test comparison of samples prepared from natural waters with distilled water samples showed significant differentiation of appearance in all but one comparison–Soledad water at a brew strength of 4.7% coffee (Table 5). The flavor of all but the samples prepared from EBMUD water was significantly different from the distilled water samples. Percent correct separation was not directly proportional to the amount of dissolved solids in the water, suggesting the importance of the individual ionic species rather than the total solids. The lack of a direct correlation between differentiation of the visual and the flavor characteristics emphasizes the methodological precaution of measuring these attributes separately. Differences between the 2 sets of data in Table 5 could be attributable to slightly different water compositions or to differences in the 2 brew strengths, or both.

Figure 2 gives the distribution of quality responses to coffee at 5.33% strength, prepared from distilled and 5 natural waters, along with values for percent extracted solids, pH, average

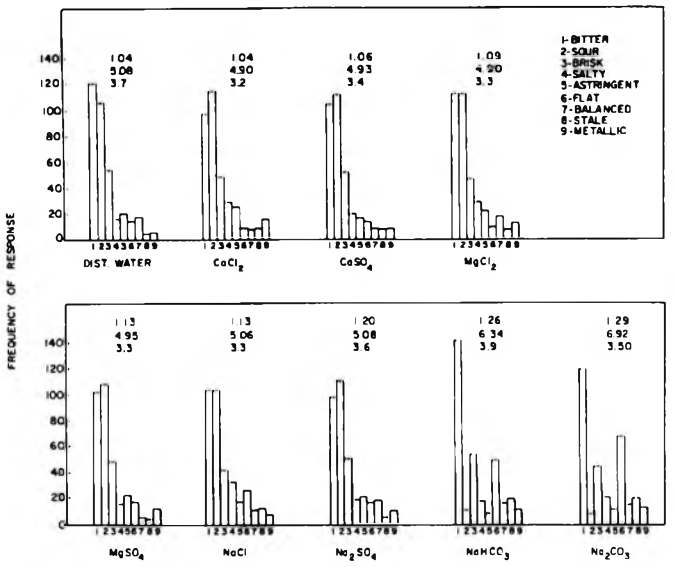

Fig. 1–Distribution of flavor characteristics for coffee at 5.33% strength (53.3 g ground coffee/1,000 ml water) prepared from distilled water and mineral solutions at 750 ppm. Numerical values are percent extracted solids, pH and average hedonic rating, respectively. Frequency of response values and hedonic ratings are based on 176 separate judgments, respectively.

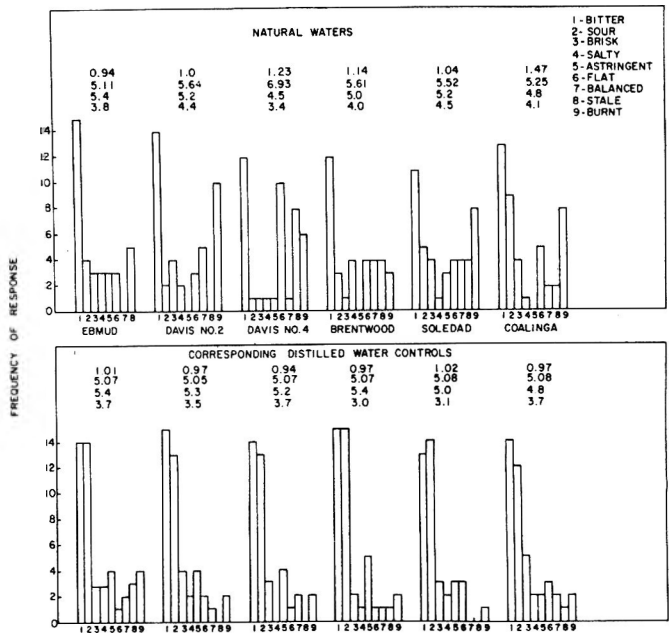

Fig. 2–Distribution of flavor characteristics for coffee at 5.33% strength (53.3 g ground coffee/1,000 ml water) prepared from natural drinking waters (top row) and corresponding distilled water (bottom row). Numerical values are percent extracted solids, pH, average strength and average hedonic ratings, respectively. Frequency of response values, average strength and average hedonic ratings are based on 22 separate judgments, respectively.

strength and average degree of liking. Once again, the predominant tastes were bitterness and sourness for beverages made from distilled water. Bitterness plus variable tastes and flavors were ascribed to coffee made from the natural waters. Sourness was associated with low pH values, but strength was not necessarily related to the amount of extracted solids. Several of the coffees in this series tasted burnt.

Note in Figure 2 that high extracted solids of 1.23 and 1.47% were obtained for coffees prepared from waters from Davis No. 4 and Coalinga, both high in Mg^{++}. It should be pointed out that the hydrometer measurements of solids based on specific gravity include mineral solids as well as solids extracted from the coffee grounds. In subsequent experiments, hydrometer values were supplemented with oven-dehydration measurements of total solids.

Coffees from EBMUD and from distilled water generally were considered to be stronger but were less well-liked than those from other waters. For 2 of the 6 natural waters, Davis No. 4 and Brentwood, there was a significant negative correlation between strength and hedonic score ($P < .001$). Inspection of the 11 individual judges' responses to strength vs. hedonic scores gave 3 negative and 2 positive correlation coefficients ($P < .001$), indicating that some liked increasing and some liked decreasing coffee strengths.

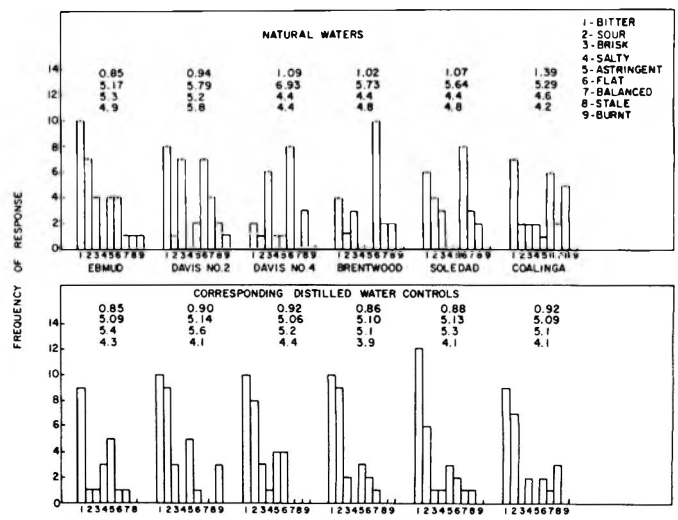

Fig. 3–Distribution of flavor characteristics for coffee at 4.7% strength (47 g ground coffee/1,000 ml water) prepared from natural drinking waters (top row) and corresponding distilled water (bottom row). Numerical values are percent extracted solids, pH, average strength and average hedonic ratings, respectively. Frequency of response values, average strength and average hedonic ratings are based on 18 separate judgments, respectively.

Data in Figure 3 were included for comparison, as 4.7% coffee strength was more to the liking of these judges. The extracted solids ranged considerably below that of the previous study, as expected, and degree of liking was higher. However, scores for coffee strength were quite similar between the 2 studies. pH levels were not altered very much by lowering the amount of coffee, but quality factors of bitterness, sourness and burnt were mentioned less frequently and flat more frequently than shown previously in Figure 2.

Tea: mineral solutes

Astringency and bitterness were the primary qualities observed in tea prepared from distilled water and from 6 of the 8 mineral solutes (Fig. 4). Although tea prepared from distilled water was considered bitter, it was the best-liked, with an average hedonic score of 5.6. Teas brewed from waters containing carbonates were the least bitter and astringent of the series, but were flat and stale, of significantly low tea strength ($P < .01$) and definitely disliked ($P < .01$). The latter samples had pH readings of 7.58 and 8.76 compared to 4.96 for the control made from distilled water.

Tea: natural waters

Natural waters produced tea beverage significantly less bitter and astringent than the distilled water samples (Fig. 5). As the total solids in the water increased, from Davis No. 2 through Coalinga waters, the tea generally was considered more flat, less brisk and less liked. Teas made from EBMUD and Davis No. 2 waters were liked significantly better ($P < .001$) than the corresponding distilled water samples. Analysis of the average strength, determined by Student's "t" test, showed that distilled water produced tea significantly stronger than that produced by the natural waters ($P < .001$). The pH values were higher in tea made from the natural waters, as would be expected due to the mineral composition.

Color of coffee and tea

The luminous reflectance, Y, and chromaticity coordinates, x and y, and differences in Y, x and y (ΔY, Δx, Δy) from the distilled water preparations for the thin-layer white-background mode are summarized in Tables 6 and 7. Included in the tables is a value |H|, calculated from $(\Delta x^{-2} + \Delta y^{-2})^{1/2}$ considered to express the magnitude of differences in chromaticity coordinates from that of the distilled water controls, ignoring direction of difference. Plotting |H| against ΔY showed that the 2 were linearly related. Plotting |H| against the percent correct response from the triangle testing of coffees prepared from natural waters did not result in any clear-cut relationship between the magnitude of |H| and visual evaluation. However, when |H| was given a + or − sign, according to whether the sample was lighter or darker (+ or − ΔY) than the distilled water control, a certain order was imposed upon the pattern, indicating that the sensory panel distinguished the samples with lower Y values more readily than those with higher Y values than the control. The magnitude of ± |H| was directly related to the visual responses. Therefore, the direction of difference in the chromaticity coordinates must be taken into account. Although the perceived color differences

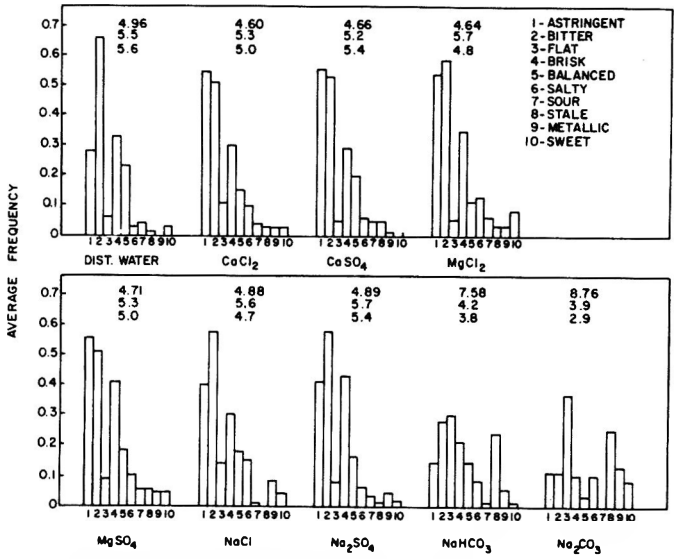

Fig. 4—Distribution of flavor characteristics for tea prepared from distilled water and mineral solutions at 750 ppm. Numerical values are pH, average strength and average hedonic ratings, respectively. Frequency of response values, average strength and average hedonic ratings are based on 80 separate judgments, respectively.

appeared to be 3-dimensional, a 2-dimensional representation proved to be useful. This point warrants further study.

The direction and magnitude of visual difference appeared to be related directly to the presence of a particular ionic constituent. The coffees brewed with waters containing Ca^{++} or Mg^{++} showed decrease in luminous transreflectance (Y value) over the distilled water control (i.e., were lighter) and showed a decrease in purity along with a small decrease in dominant wavelength (i.e., were yellower and less saturated). These changes are consistent with diminished extraction of soluble colorant from the ground coffee. The presence of Na^+ added as NaCl or Na_2SO_4 exerted an effect opposite to Mg^{++} and Ca^{++}; namely, a slight lowering of luminous transreflectance, with a small shift in dominant wavelength toward the red as compared with the control. When

Table 6—Instrumental analysis of color of coffee and tea prepared from distilled water and from 8 mineral solutes (750 ppm). Thin-layer white background reflectance data.

Solute	Y	x	y	ΔY^a	$\Delta x^{(1)}$	$\Delta y^{(1)}$	|H|
			Brewed coffee samples				
$MgCl_2$	37.1	.4616	.4448	+4.6	−232	−53	238
$CaSO_4$	34.5	.4759	.4486	+2.0	−89	−15	90
$MgSO_4$	34.2	.4771	.4482	+1.7	−77	−19	79
$CaCl_7$	33.2	.4764	.4480	+0.7	−84	−21	87
Distilled water control	32.5	.4848	.4501	–	–	–	–
NaCl	31.7	.4866	.4484	−0.8	+18	−17	25
Na_2SO_4	31.3	.4914	.4497	−1.2	+66	−4	66
$NaHCO_3$	19.6	.5398	.4336	−12.1	+550	−165	574
Na_2CO_3	12.4	.5638	.4161	−20.1	+790	−340	860
			Brewed tea samples				
NaCl	53.9	.4020	.4143	+0.1	−1	+1	1
Distilled water control	53.8	.4021	.4142	–	–	–	–
$MgCl_2$	53.4	.4008	.4116	−0.4	−13	−26	29
$MgSO_4$	53.0	.4015	.4113	−0.8	−6	−29	30
Na_2SO_4	52.0	.4214	.4289	−1.8	+193	+147	243
$CaSO_4$	51.8	.3990	.4088	−2.0	−31	−54	62
$CaCl_2$	48.8	.4061	.4128	−5.0	+41	−14	43
$NaHCO_3$	38.9	.4512	.4355	−14.9	+491	+213	535
Na_2CO_3	24.1	.5053	.4324	−29.7	+1,032	+182	1,048

[a]Differences from distilled water controls ($\times 10^{-4}$).

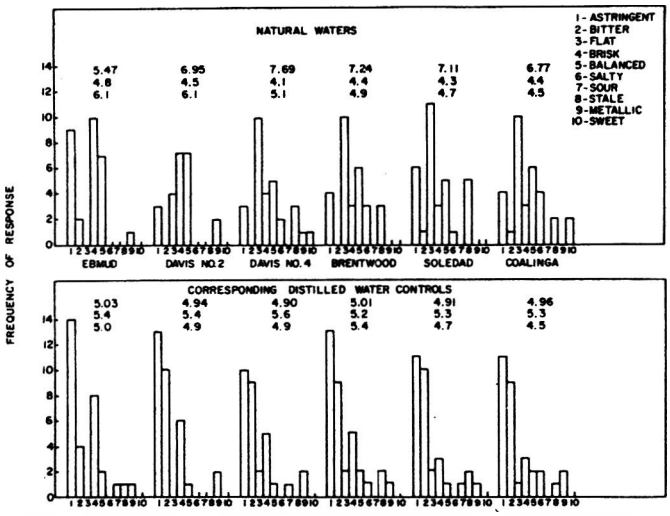

Fig. 5—Distribution of flavor characteristics for tea prepared from natural drinking waters (top row) and corresponding distilled water (bottom row). Numerical values are pH, average strength and average hedonic ratings, respectively. Frequency of response values, average strength and average hedonic ratings are based on 20 separate judgments, respectively.

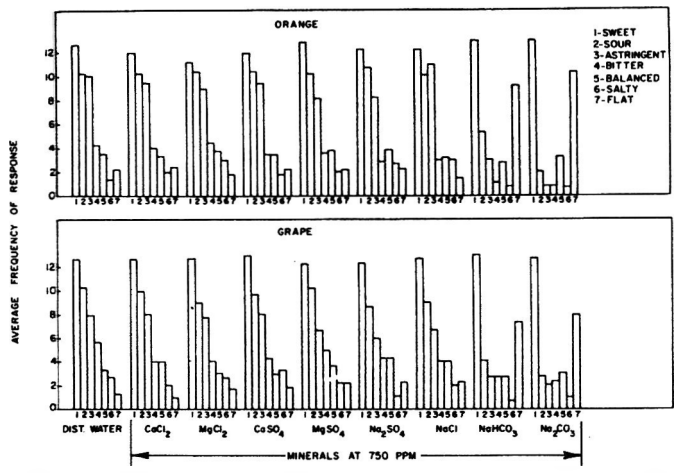

Fig. 6—Distribution of flavor characteristics for orange- and grape-flavored drinks prepared from distilled water and mineral solutions at 750 ppm. Frequency of response values are based on 70 and 42 separate judgments for orange and grape, respectively.

Na^+ was added in the form of $NaHCO_3$ or Na_2CO_3, a profound effect on the physical measurements and the derived psychophysical functions was noted, resulting in a marked decrease in luminous transreflectance, an increase in purity and a shift in dominant wavelength of as much as 10 nm. These changes, immediately apparent on visual examination, cannot be ascribed to the cationic component, but must be considered related to the presence of HCO_3 or CO_3 cations.

The frequency distribution of terminology used to describe the appearance of coffee made from the distilled water and from natural waters showed that lightness-darkness was the most frequently mentioned visual attribute, followed by surface changes (Table 8). An iridescent surface appearance caused by extracted coffee oils was much more evident when distilled water and water with low mineral contents were used. The distilled water produced a significantly lighter brew than did the natural waters (with the exception of EBMUD water, which is almost mineral-free).

It is apparent that the magnitude of the 3-dimensional shift in measured color values was not dependent on the total dissolved solids content but on the distribution and interaction of the various electrolytes present. The CO_3 and HCO_3 effect manifested itself in 1 water (Davis No. 4) containing relatively large amounts of these 2 anions (ca. 13 ppm), despite the presence of Mg^{++}, which might be expected to play an antagonistic role. When CO_3 or HCO_3 or both, were present at lower levels (3–5 ppm) the net effect seemed to depend on whether Ca^{++} or Mg^{++} was present in sufficient quantities to exert its influence on the color characteristics of the brew. (Obviously the extreme insolubility of $CaCO_3$ precludes its presence in appreciable quantity in water.)

Table 7—Instrumental analysis of color of coffee and tea prepared from distilled water and from 6 natural drinking waters. Thin-layer white background reflectance data.

Water	Y	x	y	ΔY^a	Δx^a	Δy^a	|H|	Visual test (% correct)
Coffee at 4.7% strength								
Coalinga	30.9	.4956	.4504	+3.5	−142	+27	144	57.4***
Soledad	29.4	.5000	.4495	+2.0	−98	+18	100	40.7
EBMUD	29.4	.5023	.4488	+2.0	−75	−11	76	75.9***
Distilled	27.4	.5098	.4477	–	–	–	–	
Brentwood	26.4	.5135	.4459	−1.0	+37	−18	41	87.0***
Davis No. 2	24.2	.5220	.4432	−3.2	+122	−45	130	87.0***
Davis No. 4	22.2	.5277	.4387	−5.2	+179	−60	189	100 ***
Coffee at 5.33% strength								
Coalinga	31.6	.4929	.4501	+3.3	−142	+16	143	43.9*
Soledad	29.0	.4960	.4625	+0.7	−111	+140	179	72.7***
EBMUD	29.0	.5023	.4482	+0.7	−48	−3	48	45.5*
Distilled	28.3	.5071	.4485	–	–	–	–	
Brentwood	26.7	.5150	.4465	−1.6	+79	−20	82	92.4***
Davis No. 2	25.8	.5155	.4448	−2.5	+84	−37	92	95.5***
Davis No. 4	18.4	.5457	.4309	−9.9	+386	−176	424	100 ***
Brewed tea samples								
Distilled	51.0	.4258	.4306	–	–	–	–	
EBMUD	46.4	.4316	.4265	−4.6	+58	−41	71	
Davis No. 2	39.6	.4450	.4298	−11.4	+198	−8	198	
Coalinga	37.6	.4329	.4202	−13.4	+71	−104	126	
Brentwood	35.8	.4408	.4235	−15.2	+150	−71	166	
Soledad	34.1	.4402	.4220	−16.9	+144	−86	168	
Davis No. 4	30.1	.4642	.4234	−20.9	+384	−72	391	

*,***Respectively, significant difference at P = 0.05 and 0.001.
[a]Differences from distilled water controls (× 10^4).

Artificially flavored drinks

Sweetness and sourness were the predominant quality characteristics within the orange- and grape-flavored drinks. The 2 water solutes containing carbonates resulted in significantly sweeter and significantly less sour beverages ($P < .001$). These latter data are plotted separately in Figure 7 to illustrate the interdependence

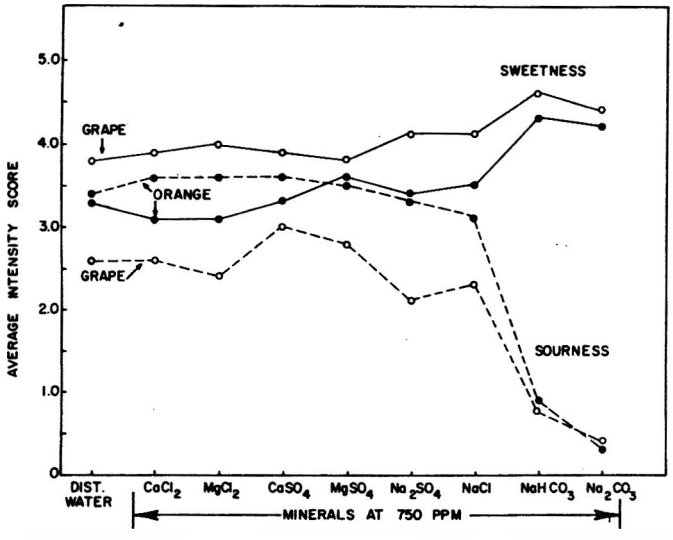

Fig. 7–Average intensity of sweetness (n = 65 and 39) and sourness (n = 60 and 36) in orange- and grape-flavored drinks, respectively, prepared from distilled water and mineral solutions at 750 ppm.

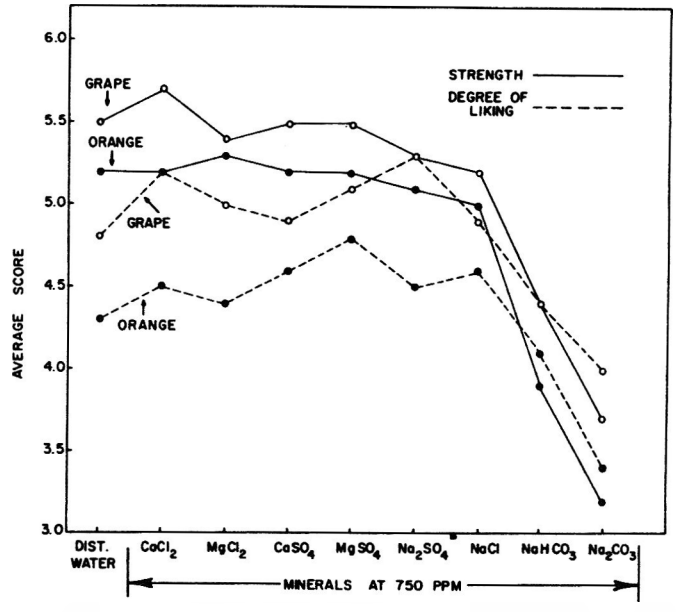

Fig. 8–Average strength and average degree of liking for orange- (n = 70) and grape-flavored (n = 42) drinks prepared from distilled water and mineral solutions at 750 ppm.

of the sweet and sour components. Note that the grape drink is sweeter and less sour throughout, undoubtedly related to its content of citric acid and artificial sweetener. pH values for the 2 sets of beverages do not differ markedly, as shown below:

	Orange	Grape
Distilled water	2.62	2.69
$CaCl_2$	2.61	2.65
$MgCl_2$	2.61	2.67
$CaSO_4$	2.69	2.71
$MgSO_4$	2.69	2.72
Na_2SO_4	2.68	2.71
NaCl	2.64	2.69
$NaHCO_3$	3.76	3.75
Na_2CO_3	4.47	4.28

Note in Figure 6 that the carbonates produce flat beverages in combination with a reduction in sourness, further substantiated by the data in Figure 8, where carbonates significantly reduced flavor strength and degree of liking ($P < .001$).

CONCLUSIONS

IN GENERAL, the electrolytes in both natural drinking waters and in prepared solutes adversely influenced the quality of the resultant beverages. The carbonates were the worst offenders, resulting in a bitter, flat coffee, a flat, stale tea and a flat, insipid soft drink. Although a certain degree of bitterness is characteristic of brewed coffee, the sourness of coffee prepared from distilled water was excessive (Fig. 2). Also, distilled water makes a brewed tea with an excessive degree of astringency (Fig. 5). It appears, therefore, that optimum flavor quality in coffee and tea would be obtained from water containing low levels of dissolved minerals, such as represented by the EBMUD and Davis No. 2 drinking waters used herein (22–411 ppm TDS). Work is under way at present to determine the quantitative and qualitative changes occurring in the volatile constituents of coffee prepared from various waters (Russell and Pangborn, 1970).

The large variations in visual attributes of the coffee and tea, unanticipated at the onset of the flavor study, merit an extensive, systematic investigation. The thin-layer reflectometric method showed promise for measuring the effects of combinations of ions in various concentrations on the light-modifying properties of the brewed beverages. It would be of additional interest to determine the visual modifications induced in coffees prepared from various waters, upon addition of milk, cream and other coffee whiteners.

Table 8–Frequency distribution of descriptive terminology used in triangle-test evaluation of coffee prepared from distilled vs. natural drinking waters. Values represent number of comments in 22 evaluations.

	Visual descriptive terminology							
Comparison	Surface scum or oil	Lighter	Darker	Amber	Brown or light brown	Cloudy	Clearer	Sediment
EBMUD vs. dist. water	13 / 12	8 / 3	2 / 3	2 / 4	0 / 1	5 / 4	2 / 0	0 / 0
Davis No. 2 vs. dist. water	1 / 13	0 / 15	16 / 0	0 / 5	8 / 4	1 / 0	3 / 2	0 / 0
Davis No. 4 vs. dist. water	0 / 11	0 / 18	16 / 0	0 / 5	6 / 4	2 / 0	3 / 1	0 / 0
Brentwood vs. dist. water	2 / 6	0 / 17	14 / 0	0 / 4	3 / 1	0 / 0	0 / 1	1 / 0
Soledad vs. dist. water	2 / 8	0 / 11	11 / 0	2 / 1	0 / 0	2 / 2	1 / 0	1 / 1
Coalinga vs. dist. water	5 / 5	3 / 5	0 / 0	4 / 4	0 / 0	1 / 2	1 / 1	0 / 0
Totals	78	80	62	31	27	19	15	3

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